E X R N A - A G

Bacterial Extracellular RNA-Mediated Reprogramming of Soybean (*Glycine max*) Seed Germination

Target Analysis · Mechanistic Models · Validation Strategy

18 Gene Targets Analyzed

REPORT PREPARED BY

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CONFIDENTIAL

ExRNA Research Report: Soybean

Glycine max — Bacterial Extracellular sRNA Target Analysis

[CONFIDENTIAL]

> **CONFIDENTIAL** — Generated 2026-02-19 **Family**: Fabaceae | **Assembly**: Glycine_max_v4.0 **Treatment**: M-9 bacterial EPS solution **Analysis Status**: targets_identified

Executive Summary [CONFIDENTIAL]

This report presents the analysis of **18 predicted exRNA targets** in Glycine max (soybean). 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	7
Medium	6
Low	5
Total	**18**

Pathway Distribution

PATHWAY	TARGETS
Unknown	5
Hormone Signaling	3

PATHWAY	TARGETS
Defense Immunity	2
Cell Wall	2
Epigenetic Regulation	1
Protein Processing	1
Ros Redox	1
Metabolic Priming	1
Signaling	1
Photosynthesis	1

High-Priority Targets [CONFIDENTIAL]

GENE ID	ANNOTATION	PATHWAY
GLYMA_01G215100	Phospholipase D beta 1 (lipid signaling; ABA/GA pathway)	hormone_signaling
GLYMA_06G259700	TIR-NBS-LRR disease resistance protein	defense_immunity
GLYMA_09G051100	Cellulose synthase A4 (CesA4; primary cell wall biogenesis)	cell_wall
GLYMA_03G196400	Glutaredoxin family protein (thiol-disulfide redox homeostas	ros_redox
GLYMA_09G192700	ROP GTPase (RHO-related protein from plants 9; ABA signal tr	hormone_signaling
GLYMA_09G063900	Cytokinin oxidase 3 (CKX3; hormone degradation)	hormone_signaling
GLYMA_10G242300	LRR receptor-like kinase (ERECTA-like; developmental/defense	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 — Soybean (Glycine max)

Definitive Ranked Target Analysis: Bacterial exRNA-Mediated Germination Improvement in *Spinacia oleracea*

Critical Prefatory Note: This analysis concerns *Spinacia oleracea* (spinach) gene targets, not *Glycine max* (soybean) as stated in the crop header. All gene IDs carry the SOV prefix consistent with the *S. oleracea* v1 genome assembly. The soybean designation in the prompt header appears to be a template error. All mechanistic inferences are drawn from spinach biology and Arabidopsis homologs where appropriate. Confounders including EPS osmopriming effects, polysaccharide elicitor activity, and microbiome-mediated indirect effects are considered throughout.

Executive Summary [CONFIDENTIAL]

This target set of ~110 spinach genes, predicted to be downregulated by bacterial extracellular small RNAs (exRNAs), collectively encodes a coherent dormancy-maintenance program spanning hormone signaling, epigenetic repression, defense-growth tradeoff regulation, redox homeostasis, ion transport, and cell wall reinforcement. The emergent systems-level interpretation is that the bacterial exRNAs act as a multi-target "dormancy dissolution" signal, simultaneously dismantling several independent brakes on germination rather than acting through a single master switch. This multi-target architecture is consistent with cross-kingdom RNA interference mechanisms documented in plant-fungal and plant-bacterial interactions [KNOWN: Cai et al., 2018, *Cell Host Microbe*; Hou et al., 2019, *Nature Plants*], though direct evidence for this specific bacterial-spinach system remains to be established experimentally.

The highest-confidence targets are those whose Arabidopsis homologs have direct, genetically validated roles in dormancy maintenance or germination repression. These include the ethylene receptor (SOV3g000150.1), the AHP-like cytokinin/ABA signaling relay (SOV4g032870.1), the DNA methyltransferase (SOV1g033340.1), the HIRA histone chaperone (SOV6g036290.1), the two EDR2 paralogs (SOV3g043450.1, SOV6g048760.1), the two cation-chloride cotransporters (SOV1g021960.1, SOV2g025380.1), the CNGC channel (SOV1g018480.1), the MYB transcription factor (SOV1g020340.1), the NAC domain protein (SOV2g014810.1), the PP2A regulatory subunit (SOV3g033920.1), the TPS enzyme (SOV2g009230.1), the LOX enzyme (SOV3g035520.1), and the GST/peroxidase redox pair. A second tier of targets with plausible but less directly validated mechanisms includes cell wall remodeling enzymes, additional signaling kinases, and epigenetic readers. A large third tier comprises targets with

weak mechanistic links to germination, including transposon-related genes, housekeeping enzymes, and proteins of unknown function, whose downregulation may be incidental, reflect off-target exRNA activity, or contribute indirectly through resource reallocation.

A critical methodological caveat applies throughout: the exRNA-mediated downregulation of these targets is *predicted*, not experimentally confirmed at the protein or phenotypic level in spinach. Furthermore, the bacterial EPS matrix used for seed priming is itself an osmopriming agent and a known elicitor of plant immune and stress responses, representing a major confounder that must be controlled in any mechanistic attribution experiment. Rankings below reflect mechanistic plausibility and evidence strength, not confirmed causal contribution.

Ranking Methodology [CONFIDENTIAL]

Targets were ranked using a weighted multi-criteria scoring system applied consistently across all 110 genes:

CRITERION	WEIGHT	RATIONALE
Mechanistic directness: Does downregulation of this gene have a clear, documented causal link to germination promotion?	35%	Primary determinant of phenotypic relevance
Arabidopsis homolog genetic evidence: Is there a loss-of-function or overexpression phenotype in *A. thaliana* or another model plant directly affecting germination, dormancy, or early seedling vigor?	25%	Best available proxy for spinach function
Pathway centrality: Is the gene a hub or rate-limiting node in its pathway, or peripheral?	20%	Hub genes have larger network effects
Annotation confidence: Is the gene product identity well-established or merely predicted by domain homology?	10%	Uncertain annotations reduce confidence
Confounder vulnerability: Could the observed effect be explained by EPS osmopriming or immune elicitation independent of this gene?	10%	Reduces ranking if effect is non-specific

Genes were additionally penalized if: (a) their downregulation would be expected to *impair* rather than promote germination based on known biology; (b) their annotation is flagged as likely misannotation or contamination; or (c) they encode housekeeping functions with no plausible germination-specific role.

Tier 1: Critical Targets (Expected Large Phenotypic Effect)

[CONFIDENTIAL]

These targets have strong mechanistic rationale, supported by Arabidopsis genetic data, and occupy central nodes in pathways directly governing the dormancy-to-germination transition.

1. SOV3g000150.1 — Ethylene Receptor

- **Mechanism**: Ethylene receptors in plants (ETR1/ETR2/EIN4 family in Arabidopsis) are **negative regulators** of ethylene signaling; they actively suppress the pathway in the absence of ethylene. Downregulation of the receptor therefore **constitutively activates ethylene signaling** [KNOWN]. Ethylene promotes germination by antagonizing ABA signaling, reducing ABA sensitivity, and promoting endosperm cap weakening [KNOWN: Linkies et al., 2009, *Plant Cell*]. In *Arabidopsis*, loss-of-function *etr1* mutants show enhanced ethylene response and accelerated germination under ABA-inhibitory conditions.
- **Evidence strength**: Strong
- **Key references**: Linkies et al. (2009) *Plant Cell* 21:3803–3822 (ethylene-ABA antagonism in germination); Bleecker & Kende (2000) *Annu Rev Cell Dev Biol* (receptor as negative regulator) [KNOWN]
- **Confidence**: High
- **Confounder note**: EPS itself can trigger ethylene biosynthesis as a MAMP response [INFERRED], potentially confounding attribution to receptor downregulation specifically.

2. SOV4g032870.1 — Histidine-Containing Phosphotransfer Protein 1 (AHP-like)

- **Mechanism**: AHP proteins are central relay components of the plant two-component cytokinin signaling system (AHK → AHP → ARR). Critically, AHPs also relay ABA signals: AHP1 in Arabidopsis interacts with ABA receptors (PYR/PYL) and promotes ABA-responsive gene expression [KNOWN: Punwani et al., 2010, *Plant J*]. Downregulation of an AHP reduces the relay of ABA-promoting signals to type-B ARR transcription factors, thereby **attenuating ABA-mediated dormancy maintenance** [INFERRED]. This is a high-leverage node because ABA is the master dormancy hormone.
- **Evidence strength**: Strong
- **Key references**: Punwani et al. (2010) *Plant J* 62:85–93; Müller & Sheen (2008) *Nature* 453:1094–1097 [KNOWN]
- **Confidence**: High
- **Confounder note**: Cytokinin signaling itself promotes germination; AHP downregulation may have opposing effects on cytokinin vs. ABA arms [INFERRED], introducing uncertainty about net direction.

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

- **Mechanism**: DNA methyltransferases (CMT3/MET1/DRM2 family) maintain cytosine methylation at CG, CHG, and CHH contexts, a primary epigenetic mechanism for silencing germination-promoting genes during dormancy [KNOWN]. In Arabidopsis, *met1* mutants show altered seed dormancy and germination timing [KNOWN: Saze & Kakutani, 2007]. Downregulation during imbibition would allow **demethylation-mediated de-repression** of GA-responsive and growth-promoting loci [INFERRED]. This is particularly impactful because DNA methylation is a stable, self-reinforcing silencing mark; reducing the methyltransferase tips the balance toward active chromatin sta [... truncated]

Synthesis: Causal Models [CONFIDENTIAL]

Causal Models — Soybean (Glycine max)

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

> **Species Note**: As correctly identified in the ranked target analysis, all gene IDs (SOV prefix) correspond to *Spinacia oleracea* (spinach), not *Glycine max*. All models below pertain to spinach seed germination biology. Arabidopsis homologs are used as functional proxies where spinach-specific data are unavailable.

Model 1: The Epigenetic Master Switch — Chromatin De-repression Cascades to Dormancy Dissolution [CONFIDENTIAL]

Core hypothesis: Bacterial exRNAs primarily target the seed's epigenetic silencing machinery, causing a genome-wide de-repression of pro-germination gene programs; all other observed changes (hormonal, metabolic, defense) are downstream consequences of this transcriptional unlocking.

Causal chain:

1. **Entry**: Bacterial exRNAs (likely 20–25 nt sRNAs, potentially stabilized within extracellular vesicles or bound to RNA-binding proteins within the EPS matrix) are taken up by imbibing spinach seed cells through endocytosis or direct membrane penetration facilitated by the hydrated seed coat. [SPECULATIVE — cross-kingdom sRNA uptake is documented in plant-fungal systems (Cai et al., 2018, *Cell Host Microbe*; Weiberg et al., 2013, *Science*), but the delivery mechanism in a bacterial EPS-to-seed context is uncharacterized]
2. **Primary targets — Epigenetic repressors are silenced**:
3. **SOV1g033340.1 (DNA cytosine-5-methyltransferase)** is downregulated → maintenance methylation at CG and CHG contexts fails during early cell divisions post-imbibition → passive demethylation of promoters of GA-responsive genes (e.g., α-amylases, expansins, cell wall hydrolases) [INFERRED — Arabidopsis *met1* and *cmt3* mutants show reduced dormancy; Zheng et al., 2012, *Plant Cell*]
4. **SOV4g015450.1 (SUVR5-like H3K9 methyltransferase)** is downregulated → loss of repressive H3K9me2 marks at heterochromatic loci harboring dormancy-associated genes → chromatin transitions from closed to open state [INFERRED — SUVR5 in Arabidopsis deposits H3K9me2 and H3K27me3; Caro et al., 2012, *PLoS Genetics*]

5. **SOV6g036290.1 (HIRA histone chaperone)** is downregulated → reduced deposition of histone variant H3.3 at specific loci → altered nucleosome dynamics favoring transcriptional activation at germination loci [INFERRED — HIRA deposits H3.3 at genic regions; loss may paradoxically destabilize repressive nucleosome configurations at dormancy loci]
6. **SOV4g038060.1 (GIS2 zinc finger)** is downregulated → de-repression of trichome and growth-related developmental programs; in Arabidopsis, GIS-family members regulate epidermal differentiation downstream of GA/cytokinin [KNOWN for Arabidopsis GIS; INFERRED for spinach ortholog]
7. **SOV4g030590.1 (PHD domain protein)** is downregulated → loss of a "reader" that recruits Polycomb Repressive Complex 2 (PRC2) to H3K4me0 marks → reduced H3K27me3 deposition at pro-germination loci [INFERRED]
8. **Secondary cascade — Chromatin opening enables hormone pathway reconfiguration**:
9. With promoter demethylation and loss of repressive histone marks, GA-biosynthesis genes and GA-signaling components become transcriptionally accessible [INFERRED]
10. Simultaneously, ABA catabolism genes (e.g., *CYP707A* family) are de-repressed, reducing endogenous ABA levels [INFERRED — ABA catabolism genes are known targets of DNA methylation-mediated silencing in Arabidopsis seeds; Nonogaki, 2014, *Annual Review of Plant Biology*]
11. The exRNA-mediated downregulation of **SOV3g000150.1 (ethylene receptor)** now synergizes with the epigenetic de-repression: ethylene receptor is a negative regulator of ethylene signaling [KNOWN], so its loss activates constitutive ethylene responses → ethylene antagonizes ABA signaling and promotes GA sensitivity [KNOWN — Linkies et al., 2009, *Plant Cell*]
12. **SOV4g032870.1 (AHP1-like)** downregulation disrupts a cytokinin-ABA signaling relay that normally reinforces ABA sensitivity [INFERRED]
13. **Tertiary effects — Defense, metabolism, and growth respond to the new hormonal landscape**:
14. GA-dominant state activates endogenous cell wall hydrolases → endosperm weakening → radicle protrusion [KNOWN mechanism]
15. ABA decline releases the defense-growth tradeoff → defense genes (EDR2, MOS1, R-proteins) are no longer maintained at high expression, consistent with their observed downregulation [INFERRED — the exRNA targeting of defense genes may be partially redundant with the hormonal shift, or may serve as a "belt-and-suspenders" reinforcement]
16. Metabolic enzymes shift from stress/storage mode to mobilization mode (TPS downregulation removes T6P-mediated growth inhibition; LOX downregulation reduces JA biosynthesis) [INFERRED]
17. **Net phenotypic outcome**: Faster, more uniform radicle emergence due to genome-wide transcriptional de-repression of germination programs, amplified by a decisive ABA→GA hormonal shift. Seedling vigor is enhanced because the epigenetic reprogramming also unlocks post-germinative growth genes (photomorphogenesis, nutrient mobilization).

Supporting evidence: - DNA methylation and histone modification are established master regulators of seed dormancy/germination transitions in Arabidopsis [KNOWN — Nonogaki, 2014; Footitt et al., 2015, *PNAS*] - Cross-kingdom sRNAs can direct DNA methylation via the RdDM pathway in recipient cells [KNOWN — demonstrated in *Cuscuta*–host interactions; Shahid et al., 2018, *Nature*] - The epigenetic target set in this analysis is unusually coherent (DNA methyltransferase + histone methyltransferase + histone chaperone + chromatin reader + transcriptional repressor), suggesting coordinated pathway targeting rather than random off-target effects [INFERRED] - Passive demethylation during early cell divisions is sufficient to de-repress dormancy genes without requiring active demethylases [KNOWN — main [... truncated]

Synthesis: Confounder Analysis [CONFIDENTIAL]

Confounder Analysis — Soybean (Glycine max)

Critical Analysis of Potential Confounders in the exRNA Germination Improvement System

Preamble [CONFIDENTIAL]

This analysis assumes the experimental system involves treating *Glycine max* seeds with an EPS (exopolysaccharide) solution derived from an M-9 bacterial strain, where the proposed mechanism is cross-kingdom antisense RNA silencing of 18 plant target genes. The central question is: **what fraction of the observed germination/vigor phenotype can be confidently attributed to antisense RNA targeting versus alternative mechanisms inherent to the treatment?**

1. EPS Osmopriming Effect [CONFIDENTIAL]

Mechanism

[KNOWN] Seed priming with osmotic solutions is one of the oldest and most robust methods for improving germination uniformity and speed. EPS solutions are viscous, high-molecular-weight polysaccharide matrices that create a controlled water potential environment around the seed.

- **Hydropriming and osmopriming** allow seeds to undergo Phase I and early Phase II of imbibition (metabolic reactivation, DNA repair, mitochondrial biogenesis, mRNA recruitment to polysomes) without completing germination (radicle emergence). Upon drying-back and re-imbibition, seeds germinate faster and more uniformly. [KNOWN — reviewed in Paparella et al., 2015, *Plant Cell Reports*; Lutts et al., 2016]
- EPS solutions from bacteria such as *Pseudomonas*, *Bacillus*, *Rhizobium*, and others typically have water potentials in the range of **−0.5 to −2.0 MPa** depending on concentration, which falls squarely within the effective osmopriming window (typically −0.5 to −1.5 MPa for soybean). [KNOWN]
- The polysaccharide matrix may also provide a **controlled hydration envelope** around the seed, slowing imbibition and reducing imbibition damage — a known problem in large-seeded legumes like soybean where rapid water uptake causes mechanical damage to cotyledons and embryonic axes. [KNOWN — Powell & Matthews, 1978; Woodstock & Taylorson, 1981]

Expected Magnitude vs. Observed Effect

- [KNOWN] PEG osmopriming of soybean seeds routinely improves germination rate by **10–25%** under optimal conditions and **20–50%** under stress conditions (reviewed in Sadeghi et al., 2011; Ghassemi-Golezani et al., 2008).
- [KNOWN] Hydropriming alone (water, no osmoticum) can improve soybean germination by **5–15%** and seedling vigor indices by **10–30%** (Sadeghi et al., 2011).
- [INFERRED] If the observed germination improvement falls within the **5–25% range**, the entire phenotype could plausibly be explained by osmopriming alone. Only effects substantially exceeding this range, or showing pathway-specific molecular signatures inconsistent with general priming, would require an additional mechanism.
- [INFERRED] Early seedling growth improvement (hypocotyl/radicle length, dry weight accumulation) is a **classic hallmark** of seed priming and is not diagnostic of exRNA activity.

Controls Needed

1. **EPS solution without RNA** — autoclaved or RNase A/III-treated EPS at identical concentration and water potential
2. **PEG 6000/8000 control** — matched to the same water potential as the EPS solution (measured by osmometry)
3. **Water-primed control** — hydropriming for the same duration
4. **Dry seed control** — untreated
5. **Water potential measurement** of the EPS solution itself (critical and often omitted)

Evidence Level

- That EPS acts as an osmopriming agent: **[KNOWN]**
- That this alone could explain the germination phenotype: **[INFERRED — HIGH PROBABILITY]**
- That osmopriming is the *dominant* contributor: **[INFERRED — requires controls to quantify]**

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2. Polysaccharide Elicitor Effects [CONFIDENTIAL]

Known Defense/Growth Priming by Bacterial Polysaccharides

[KNOWN] Bacterial EPS are potent microbe-associated molecular patterns (MAMPs) and damage-associated signals that activate plant innate immunity and growth modulation:

- **β-glucans, lipopolysaccharides (LPS), and EPS fragments** are recogniz [... truncated]

Synthesis: Validation Plan [CONFIDENTIAL]

Validation Plan — Soybean (Glycine max)

Comprehensive 4-Tier Validation Plan: Bacterial exRNA-Mediated Germination Improvement System

Critical Prefatory Notes [CONFIDENTIAL]

Species Reconciliation: The ranked targets and causal models supplied carry SOV-prefix gene IDs consistent with *Spinacia oleracea* (spinach), not *Glycine max* (soybean) as stated in the crop header. This validation plan is written for **soybean** as the experimental crop, with the following adaptations: (1) all SOV target genes are treated as *predicted functional analogs* requiring identification of confirmed *Glycine max* orthologs before molecular validation; (2) soybean-specific germination biology, seed physiology, and genomic resources are used throughout; (3) where spinach-specific mechanisms are invoked, this is flagged explicitly. The plan is designed to be mechanistically agnostic enough to test the exRNA hypothesis regardless of which species' targets are ultimately validated.

Epistemic Framing: The exRNA germination improvement hypothesis rests on a causal chain with multiple unvalidated links: (1) bacterial exRNAs are produced in sufficient quantity and appropriate sequence composition; (2) exRNAs survive the EPS matrix and seed coat barrier; (3) exRNAs enter seed cells and are loaded into RISC or equivalent silencing machinery; (4) exRNAs silence the predicted target genes; (5) target gene silencing causes germination improvement; (6) this effect exceeds or is separable from EPS osmopriming, polysaccharide elicitor effects, and microbiome-mediated indirect effects. **Each link must be validated independently.** The tier structure below is organized to test these links in order of logical priority.

Tier 1: Essential Controls [CONFIDENTIAL]

Purpose: Establish whether the germination phenotype is real, reproducible, and attributable to RNA-mediated mechanisms rather than physicochemical or immune-elicitor properties of the bacterial preparation. These experiments must be completed before any molecular mechanistic work is justified.

Experiment 1.1: Phenotypic Baseline and Dose-Response Characterization

Experiment: Comprehensive germination phenotyping of soybean seeds treated with the full bacterial EPS preparation across a concentration gradient, with rigorous environmental controls.

Hypothesis tested: The bacterial EPS preparation produces a reproducible, dose-dependent improvement in soybean germination rate, germination percentage, and seedling vigor that is distinguishable from untreated controls. This establishes the phenotype that all subsequent experiments must explain.

Method: - Soybean cultivar: Use a single, well-characterized cultivar with published germination kinetics (e.g., Williams 82, the reference genome cultivar [KNOWN — Schmutz et al., 2010, *Nature*]) - Seed lot: Single lot, tested for baseline germination percentage (must be ≥85% for ISTA standards), moisture content, and accelerated aging index - Treatment groups (n=8 replicates of 50 seeds each, conducted across 3 independent seed lots): - T0: Dry seed control (no treatment) - T1: Distilled water soak (matched duration to EPS treatment) - T2: EPS preparation at 0.1× working concentration - T3: EPS preparation at 0.5× working concentration - T4: EPS preparation at 1× working concentration (standard treatment) - T5: EPS preparation at 2× working concentration - T6: EPS preparation at 5× working concentration - Germination conditions: 25°C constant temperature, 16h light/8h dark, on moistened germination paper (standard ISTA protocol) - Measurements at 24h intervals for 7 days: - Germination percentage (radicle ≥2mm) - Mean germination time (MGT): MGT = Σ(n×t)/Σn where n = seeds germinating on day t - Germination uniformity (coefficient of variation of germination time) - Radicle length at 48h, 72h, 96h - Hypocotyl length at 96h - Seedling fresh weight and dry weight at 7 days - Seedling vigor index: SVI = germinatio [... truncated]

Methodology [CONFIDENTIAL]

1. **Target Identification**: Bacterial exRNA sequences aligned against Glycine max 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)