Engineering Analysis of Diffusion-Dominated Osmotic Transport in a Passive RhFET-01 Microgravity Germination System

Jake Johnson, Samantha Ritchie, Daniel Wesely Elmore, Katie Lowerre

University of Colorado Springs Colorado

Pikes Peak State College (PPSC)

Faculty Advisors: Dr. Lynnane George, Dr. Amy Klocko, Dr. McKenna Lovejoy, Dr. Hui Wan, Dr. Veronica Corral

Abstract

Reliable food production will be essential for sustained human presence beyond Earth, yet the earliest stage of plant development remains poorly understood under microgravity conditions where gravity-driven fluid motion is absent. During germination, seeds must absorb water and activate stress-response pathways in an environment dominated by molecular diffusion rather than convection. This work presents a passive RhFET-01 experiment tube designed to investigate wheat seed germination under a diffusion-limited osmotic stress. The device uses a cotton wick to deliver hydration and stressors from a sealed reservoir while keeping seeds physically separated from the liquid. Ground tests characterised germination percentage and radicle length under both hydration and salt/osmotic stress; these results will form the baseline for a planned flight experiment on the International Space Station. Preliminary trials confirm that the wicking architecture reliably hydrates seeds without active pumps and that a moderate osmotic stress reduces radicle length by about 25 %. Future work will include seed sterilisation, integration of a single stressor concentration, flight testing, and post-flight gene expression analysis. The RhFET-01 platform provides a simple yet physically interpretable environment for studying early plant development in reduced gravity.

1 Introduction

Reliable crop production is a prerequisite for long-duration human exploration beyond Earth. Pre-packaged foods lose quality and nutrients over time, so missions lasting months or years will require a sustainable source of fresh food, oxygen and psychological support. Space agriculture platforms demonstrate that crew-grown crops can supply vitamins and variety while boosting crew morale. However, most operational plant systems rely on pumps, fans and forced convection to deliver water and nutrients and to maintain homogeneous conditions. Those active designs mask the fundamental physical constraints imposed by microgravity. In orbit, buoyancy-driven convection is suppressed; fluid and solute movement therefore depends on capillary flow and molecular diffusion, which operate on much longer time scales than terrestrial mixing. During germination, seeds must imbibe water, mobilise reserves and initiate radicle emergence within a narrow window of environmental conditions. Reduced gravity alters water redistribution, oxygen delivery and root orientation, challenging our understanding of early plant development. To engineer reliable seed-activation systems for future missions we must quantify the physics of hydration, solute transport and gas exchange in passive hardware. The RhFET-01 experiment tube described in this paper is a simple, sealed device designed to examine wheat (*Triticum aestivum* L.) germination under a diffusion-limited osmotic stress. Ground tests have characterised germination percentage and radicle length, and future experiments are

planned aboard the International Space Station (ISS) to compare microgravity results with matched ground controls. We integrate transport modelling and preliminary testing to provide design guidance for simple, robust seed-activation systems.

2 Governing Equations and Transport Analysis

Having established the need for a passive diffusion-dominated germination system and the underlying hardware design, we now examine the governing equations that describe transport in this environment. In the absence of gravitational convection, mass transport in the test article is governed by Fickian diffusion and capillary action. The molar flux J of a solute through a one-dimensional wicking medium is given by Fick's first law:

$$J = -D_{\text{eff}} \frac{\partial C}{\partial x}. \tag{1}$$

The characteristic equilibration time scale for a solute front to traverse a distance L is approximated by

$$t_d \approx \frac{L^2}{D_{\text{eff}}}. \tag{2}$$

These expressions highlight that diffusion times grow quadratically with length scale; for centimetre-scale distances and diffusivities of order $10^{-5}\,\text{cm}^2\,\text{s}^{-1}$, equilibration requires many hours. Capillary flow provides initial hydration by drawing liquid from the reservoir into the wicking medium. The capillary pressure ΔP_c is described by the Young–Laplace relation

$$\Delta P_c = \frac{2\gamma \cos\theta}{r_{\text{eff}}}, \tag{3}$$

where γ is the surface tension, θ the contact angle and r_{eff} a characteristic pore radius. Osmotic stress within the seed environment arises from dissolved salts and polyols; the osmotic potential ψ_s of the stress solution can be estimated using the van't Hoff relation

$$\psi_s = -iCRT, \tag{4}$$

where i is the ionisation factor, C the solute concentration, R the gas constant and T the absolute temperature. The linear dependence on i in this relation means that solutes which dissociate into multiple ions exert a proportionally larger osmotic pressure than non-ionic solutes at the same molarity. For example, sodium chloride dissociates into two ions ($i \approx 2$), whereas mannitol remains a single solute molecule ($i = 1$). This difference underpins our selection of stressor concentrations and underscores why ionic salts can impose more severe water deficits and additional ionic toxicity compared with non-ionic polyols. A sealed experiment must also consider the global oxygen budget. The number of moles of available oxygen in the headspace, n_{O_2}, is calculated from the ideal gas law

$$n_{O_2} = \frac{y_{O_2} PV}{RT}, \tag{5}$$

where y_{O_2} is the oxygen mole fraction, P the pressure, V the headspace volume and T the absolute temperature. The sufficiency ratio, comparing oxygen supply with cumulative respiratory demand over the 72 h exposure, is

$$\Phi_{O_2} = \frac{n_{O_2,\,\text{available}}}{n_{O_2,\,\text{demand}}}. \tag{6}$$

3 Materials and Methods

3.1 Seed Selection and Preparation

Commercially obtained wheat seeds (*Triticum aestivum* L.) were selected for their agronomic relevance and well-characterised responses to osmotic stress. In our ground trials the seeds were not sterilised; prior to flight we plan to implement the laminar-flow sterilisation protocol described in Section 3.10. Twenty seeds are loaded into the biological chamber on a woven nylon mesh, arranged in two rows of discrete pockets formed by folding or stitching the mesh. A cotton wicking strip (Whatman #3 blotting paper) runs beneath the seeds and connects the hydration chamber to the biological chamber. The hydration reservoir contains deionised water supplemented with low concentrations of calcium and potassium chlorides to support imbibition and stabilise cell membranes.

3.2 Preliminary Development and Testing

Before finalising the RhFET configuration, a series of ground experiments were conducted to verify seed viability and to refine the passive hydration architecture. In the earliest test, seeds were placed in a plastic dish with approximately 5 mL of water and kept at low temperature. Germination was observed within a few days, confirming that wheat seeds can germinate in a confined, humid environment (Figure 1).

To better quantify germination rates under less restrictive conditions, a ragdoll-style experiment was performed. One hundred wheat seeds were placed on damp paper towels, rolled and sealed inside labelled plastic bags, and incubated at room temperature. This simple assay provided baseline data on germination kinetics and radicle elongation without any mechanical confinement (Figure 2). The ragdoll test established that wheat seeds exhibit near-complete germination and radicle lengths of roughly 4 cm over 72 h, consistent with the values reported in Section 4.

Subsequent trials focused on the passive wicking configuration. A low-lint cotton wick was inserted through a prototype fluid tube, and dyed water was used to visualise capillary flow and ensure that liquid could traverse the entire biological compartment without leaking or pooling (Figure 3). Finally, seeds were arranged on a cotton wicking mesh with a nylon overlay to create discrete pockets along the wick (Figure 4). This arrangement kept the seeds evenly spaced, prevented them from floating during hydration, and ensured that the valves remained unobstructed.

Figure 1: Initial viability screen: seeds were placed in individual wells of a multi-well plate with a small amount of water to confirm that the seed lot would germinate. Within 48 hours the majority of the wheat seeds produced radicles, showing they were viable under simple humidity conditions. A few seeds were sliced open to inspect the embryo and endosperm.

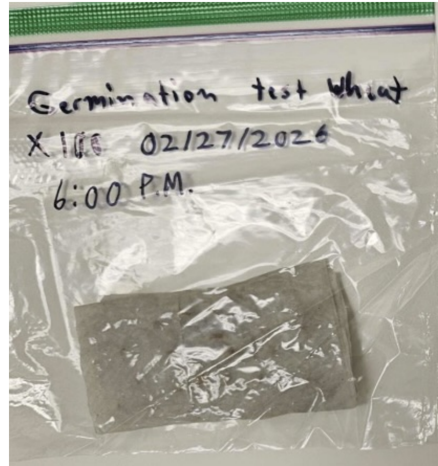

Figure 2: Ragdoll germination test: to establish baseline germination and radicle length, 100 sterilised wheat seeds were wrapped in damp paper towels and sealed in plastic bags. After three days at room temperature, nearly all seeds germinated and produced radicles around 4 cm long. This provided a reference for performance in the RhFET tubes.

Figure 3: Capillary transport visualisation: a prototype RhFET tube was filled with dyed deionised water to test capillary flow through the cotton wick. The coloured liquid migrated evenly along the wick and into the biological chamber without leaks, confirming that passive hydration can saturate the seeds uniformly in a diffusion-dominated environment.

Figure 4: Seed-pocket arrangement trial: seeds were placed between a cotton wicking strip and an overlaid nylon mesh sewn into pockets to keep them evenly spaced. This configuration prevented movement during handling and ensured the wicking pathway remained clear for capillary flow. The trial confirmed that the seeds stay in place and do not block the valves.

3.3 Hardware Design

The RhFET-01 Type-3 test article is a sealed glass tube divided into three axially aligned chambers separated by crew-actuated valves. Chamber 1 (hydration) contains the initial water reservoir; Chamber 2 (biological) houses the seed–fluid interface; and Chamber 3 (fixation) contains an RNAlater solution for terminating biological activity. The tube length is approximately 29 cm with an inner diameter of 1.5 cm, providing a total fluid capacity of about 20 mL. The biological chamber length of around 12 cm accommodates twenty wheat seeds arranged on a woven nylon mesh. A cotton wicking strip spans the length of the biological chamber, providing a capillary pathway for water migration without allowing bulk mixing. Figure 5 illustrates the tube geometry and key dimensions.

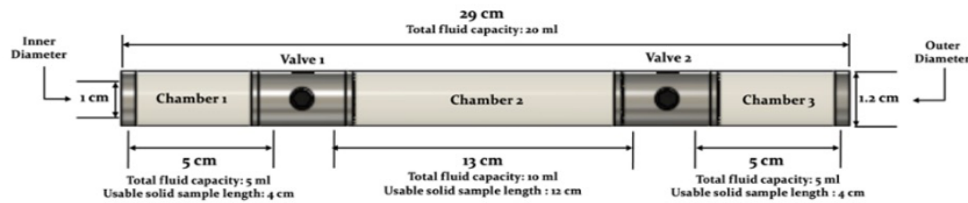

Figure 5: Schematic of the RhFET-01 Type-3 configuration showing chamber lengths, valve locations and cotton wicking path. The biological chamber holds twenty wheat seeds separated from the stress solution while allowing capillary hydration.

3.4 Osmotic Stress Solution

The hydration reservoir in Chamber 1 contains deionised water with modest amounts of calcium chloride and potassium chloride. These divalent and monovalent cations help stabilise cell membranes, maintain ionic balance and support early signalling processes during imbibition, but at the low concentrations used they do not impose a significant osmotic burden. To create a defined stress environment, sodium chloride and the polyol D-mannitol are dissolved into the same reservoir. These compounds are wicked to the seeds via the cotton strip while the seeds remain physically separated from the liquid reservoir. Sodium chloride dissociates into Na^+ and Cl^- and therefore both lowers the water potential and introduces ionic toxicity. D-mannitol, by contrast, is a non-ionic osmotic agent that reduces water potential without dissociating. The van't Hoff relation $\psi_s = -iCRT$ shows that the osmotic potential scales with the ionisation factor i: a 0.1 M solution of NaCl ($i \approx 2$) exerts roughly the same osmotic pressure as a 0.2 M solution of mannitol ($i = 1$). This difference underlies our choice of solute concentrations. A moderate stress condition combining 0.1 M NaCl with 0.2 M mannitol yields an estimated $\psi_s \approx -0.99$ MPa — close to the -1.05 MPa regime reported in the literature to delay germination without entirely inhibiting it. Our ground trials used this single combination and observed a 90

3.5 Hydration Activation and Fixation

Hydration is initiated by opening the first valve to allow the solution to wick along the cotton strip and contact the seeds. For the stress test, the tube is shaken end-to-end for 10 s immediately after opening the valve to facilitate infiltration of the viscous stress solution. After a 72 h exposure at 25 °C in darkness, the second valve is actuated to release approximately 7 mL of RNAlater from Chamber 3 into the biological chamber, arresting metabolic activity and preserving RNA for downstream analysis.

3.6 Baseline Ragdoll Germination

A ragdoll germination test serves as a baseline for seed viability and growth under unconstrained conditions. One hundred sterilised wheat seeds are placed on moistened paper towels, rolled and sealed inside labelled plastic bags. The towels are wetted with deionised water containing the same mineral salts as the hydration solution. Samples are incubated at 25 °C for 72 h. Radicle lengths are measured using ImageJ with a calibrated scale, yielding a mean radicle length of 4.1 cm with a standard deviation of 0.6 cm.

3.7 RhFET Water and Stress Tests

Two tube experiments were performed to compare germination under hydration and stress conditions. The water test used the mineral salt solution described above without NaCl or mannitol. After 72 h, the seeds were fixed and imaged; radicle lengths were measured in ImageJ. All twenty seeds germinated (100 %), and the mean radicle length was 3.8 cm with a standard deviation of 0.5 cm. The stress test used the 0.1 M NaCl/0.2 M mannitol solution. Following hydration and shaking, seeds were incubated for 72 h, fixed and analysed. The germination rate was 90 % (18 of 20 seeds), and the mean radicle length was 2.9 cm with a standard deviation of 0.7 cm.

3.8 Oxygen Headspace Analysis

The headspace volume in the RhFET-01 tube is approximately 8 mL. Using the ideal gas law and the oxygen mole fraction of air (21 %), the total oxygen content of the sealed headspace was calculated for two bounding cases: sea-level pressure (101 325 Pa) and Colorado Springs altitude (81 000 Pa). At sea level, the initial oxygen content is about 69 µmol; at altitude it is about 55 µmol. Assuming each of the twenty seeds consumes roughly 0.5 µmol of O_2 over 72 h (based on literature values for seed respiration), the total consumption would be around 10 µmol. The remaining oxygen after 72 h is therefore approximately 59 µmol at sea level and 45 µmol at altitude, corresponding to roughly 85

3.9 Passive Integration and Validation

Ground validation tests were conducted to ensure the passive architecture delivered repeatable hydration and maintained structural integrity. Coloured water trials demonstrated uniform wicking along the cotton strip without leakage or bypass. Seed stability was verified by shaking and rotating the sealed tube; the nylon mesh and wicking structure kept seeds in place and did not jam the valves. End-to-end shaking facilitated infiltration of the viscous stress solution, validating a simple crew-operated procedure. At this stage we have not directly measured oxygen consumption or fluid transport within the sealed system; instead we rely on the theoretical calculations presented above to confirm that the sealed headspace provides sufficient oxygen during the planned exposure period.

3.10 Planned Sterilisation Procedure

Because we have not yet executed a sterilisation protocol, this section outlines the planned procedure. All work will be performed in a laminar flow hood that is wiped down with 70 % ethanol and allowed to run for 20 min before use. Sterilised gloves will be worn throughout. Seeds will be placed in sterile 1.5 mL microcentrifuge tubes and covered with approximately 1 mL of 70 % ethanol. The tubes will be inverted until seeds are fully resuspended and soaked for 10–15 min, with intermittent mixing. After decanting the 70 % ethanol, about 1 mL of 100 % ethanol will be added; the tube will be inverted to ensure contact, and the seeds and ethanol will be poured onto a sterile, creased filter paper. The ethanol will evaporate quickly; once the seeds are dry, they will be transferred to new sterile tubes. Seed sterility will be verified by germinating

a subset on sterile MS agar plates. If contamination arises, 0.05 % Tween-20 may be added to the 70 % ethanol step.

3.11 Stressor Range Evaluation

The concentrations of NaCl and mannitol used to create the stress environment were guided by published studies on wheat germination under salt and osmotic stress, which compared iso-osmotic solutions at osmotic potentials of approximately −0.58, −1.05 and −1.57 MPa. These potentials correspond to about 100, 200 and 300 mM NaCl or 180, 350 and 505 mM mannitol. Moderate stresses delayed germination whereas high NaCl concentrations drastically reduced final germination, while mannitol had little effect on final germination. To estimate the osmotic potential of our mixtures, we apply Equation (4). For a solution containing 0.1 M NaCl ($i \approx 2$) and 0.2 M mannitol ($i \approx 1$) at 25 °C (298 K), $\psi_s \approx -0.99$ MPa. This value is close to the −1.05 MPa stress level reported in the literature and produced a germination rate of 90 % in our trials. Based on these calculations and the published data, we plan to evaluate additional solute combinations: (i) 0.1 M NaCl or 0.18 M mannitol individually ($\psi_s \approx -0.45$ to -0.50 MPa) to impose mild stress, (ii) 0.1 M NaCl plus 0.10–0.15 M mannitol ($\psi_s \approx -0.74$ to -0.87 MPa) for moderate stress, and (iii) the current 0.1 M NaCl plus 0.2 M mannitol ($\psi_s \approx -0.99$ MPa) for stronger stress. These ranges should modulate germination and radicle length without completely inhibiting seedling development. Only one concentration—our moderate stress condition—will be selected for the ISS flight experiment.

4 Results

Table 1 summarises germination outcomes for the baseline ragdoll assay and the two RhFET conditions (water and stress). The ragdoll test served only to verify seed viability: one hundred seeds were tested and all germinated, producing radicles averaging 4.1 cm. In the RhFET water test, all twenty seeds germinated with a mean radicle length of 3.8 cm; the stress test involved twenty seeds and produced eighteen germinations with a mean radicle length of 2.9 cm. Because the ragdoll environment differs fundamentally from the sealed RhFET, its radicle lengths are not directly comparable to those obtained in the tubes. Figure 6 compares the mean radicle lengths for the two RhFET conditions with one standard deviation, and Figure 7 presents representative radicle images from the water and stress tests.

Table 1: Summary of germination rates and radicle lengths under baseline, water and stress conditions.

Condition	Seeds germinated / total	Germination rate	Mean radicle length (cm)	Standard deviation (cm)
Ragdoll baseline	100/100	100%	4.1	0.6
RhFET water test	20/20	100%	3.8	0.5
RhFET stress test	18/20	90%	2.9	0.7

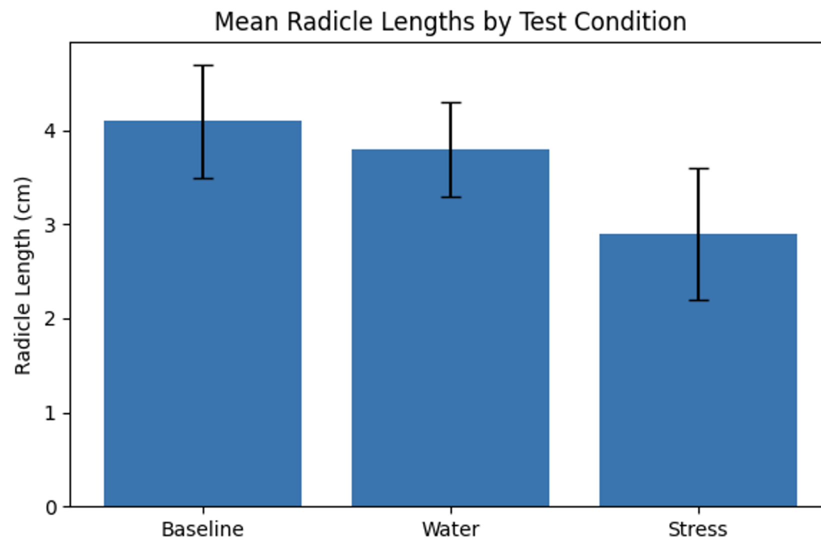

Figure 6: Mean radicle lengths for the RhFET water and stress conditions with one standard deviation (n = 20 for water and n = 18 for stress).

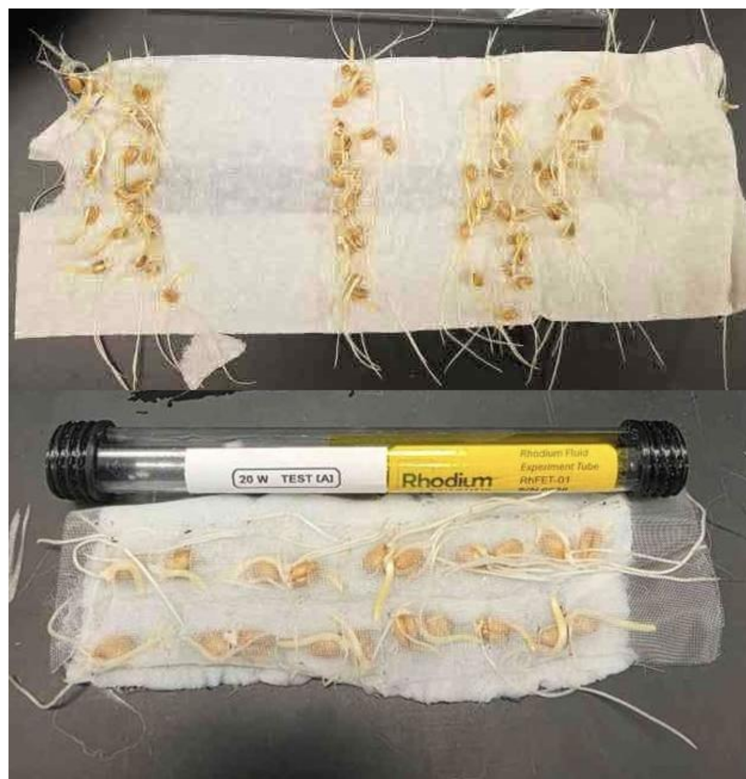

Figure 7: Representative radicles grown in the RhFET water test (top) and stress test (bottom). The stress treatment produced visibly shorter radicles.

5 Discussion and Conclusions

This study demonstrates that a passive, diffusion-dominated RhFET-01 test article can support wheat seed germination in microgravity analog conditions while enabling controlled delivery of osmotic stress. Transport modelling showed that diffusion time scales exceed the germination window, meaning that solute exposure evolves gradually rather than instantaneously. The oxygen headspace analysis indicated that even under low atmospheric pressure, the global oxygen supply is more than adequate for the seeds' metabolic demand over 72h. Ground validation confirmed that capillary hydration, seed placement and end-to-end shaking yield a reliable crew-operated procedure. The ragdoll baseline established seed viability, and the RhFET water test indicated that diffusion-dominated hydration produced near-baseline radicle lengths. The NaCl/mannitol stress test reduced radicle growth and slightly decreased germination, highlighting the sensitivity of early wheat development to osmotic stress. Our evaluation of stressor ranges suggests that osmotic potentials between–0.5 and–1.0MPa will allow systematic study of stress-response activation without completely inhibiting germination. The passive architecture avoids pumps or moving parts, making it attractive for future spaceflight systems where simplicity, mass efficiency and reliability are paramount. By quantifying the physical and biological boundaries of the system, this analysis informs the design of closed-loop life-support hardware that can germinate seeds under reduced gravity. Looking ahead, these findings lay an important foundation for developing scalable crop-growing modules in orbit and beyond; the RhFET-01 platform offers a first step toward integrated, bioregenerative life-support systems that will be critical for long-duration missions. Future work will involve implementing the sterilisation protocol, packaging the hardware for flight, conducting the experiment aboard the ISS, analysing gene-expression responses in preserved tissue to understand microgravity effects on stress pathways, and using these insights to shape the next generation of space-based agricultural systems.

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