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# Developmental Variations in Organogenesis Under Experimentally Induced Environmental Stress

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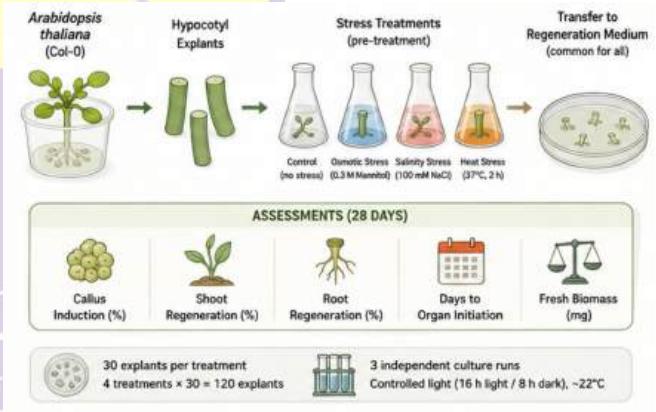
**Abstract— Environmental stress can alter plant growth, cellular differentiation, and the developmental decisions that determine whether wounded tissues produce roots, shoots, callus, or other regenerative structures. The present study examined the effects of experimentally induced osmotic, salinity, and temperature stress on organogenesis in *Arabidopsis thaliana* hypocotyl explants cultured under controlled in vitro conditions. Explants were divided into four treatment groups: control, osmotic stress, salinity stress, and heat stress. Osmotic stress was imposed using mannitol, salinity stress using sodium chloride, and temperature stress by short-term exposure to elevated temperature. Following stress treatment, explants were transferred to a standardized regeneration medium and evaluated for callus formation, shoot regeneration, root regeneration, time to visible organ initiation, and fresh biomass. The control treatment produced the highest callus induction (86.7%), shoot regeneration (70.0%), and root regeneration (83.3%). All three stress treatments reduced regenerative performance, with heat stress producing the greatest overall inhibition. Shoot regeneration decreased to 53.3%, 40.0%, and 33.3% under osmotic, salinity, and heat stress, respectively. One-way analysis of variance indicated significant treatment effects on callus induction, shoot regeneration, root regeneration, and fresh biomass ($p < 0.001$). The results suggest that environmental stress does not simply suppress regeneration uniformly but modifies the developmental competence of explants, with different stressors producing different degrees of impairment. The findings are consistent with established evidence that abiotic stress affects auxin and cytokinin signaling, cell proliferation, water relations, and regenerative reprogramming. The study demonstrates the usefulness of controlled organogenesis assays for examining developmental plasticity under environmental stress.**

***Keywords: Arabidopsis thaliana; organogenesis; abiotic stress; osmotic stress; salinity; heat stress; tissue culture; regeneration; auxin; cytokinin***

## INTRODUCTION

Plants possess an exceptional capacity for developmental plasticity. Unlike most animal tissues, many differentiated plant cells retain the ability to re-enter the cell cycle and, under appropriate conditions, contribute to the formation of new organs. This regenerative capacity is particularly evident in tissue culture, where wounded or excised tissues can undergo dedifferentiation, proliferation, and subsequent redifferentiation into roots or shoots. The classical work of Skoog and Miller demonstrated that the relative balance between auxin and cytokinin can strongly influence organ formation in cultured plant tissues, establishing a conceptual foundation for modern studies of plant regeneration.




Organogenesis is not controlled exclusively by plant growth regulators. Environmental conditions surrounding a developing tissue influence cell division, cellular expansion, hormone transport, metabolism, and the maintenance of meristematic competence. Abiotic stresses such as drought, salinity, osmotic imbalance, and high temperature can therefore alter developmental trajectories. Plants respond to these stresses through coordinated signaling systems involving abscisic acid, auxin, cytokinins, ethylene, reactive oxygen species (ROS), calcium signaling, and stress-responsive transcription factors.

Auxin is particularly important because its concentration, transport, perception, and distribution influence cell division and developmental patterning. Osmotic stress can modify several components of the auxin pathway, including auxin

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biosynthesis, polar transport, perception, and conjugation. These changes can subsequently influence root positioning and other developmental processes. Salinity can similarly influence auxin distribution and root meristem activity while simultaneously producing osmotic and ionic stress.

Cytokinins also play a central role in organogenesis. De novo shoot formation depends on cytokinin signaling and its interaction with auxin. Experimental work using hypocotyl explants has demonstrated that cytokinin can modify auxin-induced organogenesis by influencing auxin distribution and the activity of auxin transport systems. Modern models consequently view organogenesis as a coordinated response involving hormonal gradients rather than a simple response to the presence or absence of an individual hormone.

Plant regeneration additionally requires extensive cellular reprogramming. Following injury, cells can undergo changes in transcriptional activity, hormone metabolism, chromatin state, and cell-cycle behavior before acquiring new developmental identities. Wounding itself acts as a developmental signal, while auxin and cytokinin contribute to subsequent cell-fate decisions.

Environmental stress may interact with this regenerative process in complex ways. Interestingly, stress is not necessarily synonymous with complete inhibition of regeneration. Controlled stress treatments can sometimes promote developmental reprogramming. Ikeda-Iwai et al. demonstrated that osmotic, dehydration, and other stress treatments could induce somatic embryogenesis in *Arabidopsis*, showing that stress can act as a developmental stimulus under specific conditions. Thus, stress intensity and duration are likely to determine whether environmental perturbation stimulates adaptive reprogramming or causes developmental damage.

The *Arabidopsis thaliana* hypocotyl provides a useful experimental system for studying these responses because hypocotyl tissues are highly responsive to in vitro regeneration conditions and have been extensively used to investigate cellular competence, hormone signaling, and organogenesis. Studies of Arabidopsis regeneration have demonstrated that environmental conditions, explant characteristics, hormone concentrations, and developmental state can all affect regeneration efficiency.

Despite extensive knowledge of individual stress responses, comparative experimental studies examining how different forms of environmental stress affect the same organogenic system remain useful. The present study therefore investigated developmental variations in organogenesis under three experimentally induced environmental stresses—osmotic, salinity, and heat stress—using *A. thaliana* hypocotyl explants. The study hypothesized that all stress treatments would reduce regenerative performance relative to unstressed controls, but that the magnitude of inhibition would differ among stress types.

## Research Methodology

### Experimental Plant Material

Seeds of *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) were used as experimental material. Seeds were surface sterilized and germinated aseptically on Murashige and Skoog-based medium under controlled environmental conditions. Seedlings were maintained under a 16 h light/8 h dark photoperiod at approximately 22°C.

Hypocotyl explants were obtained from young seedlings of uniform developmental stage. Explants of approximately equal length were selected to minimize variation caused by tissue age or explant size. Damaged or visibly abnormal explants were excluded before treatment.

### Experimental Design

The experiment consisted of four treatment groups:

- Control: no experimental environmental stress.
- Osmotic stress: exposure to mannitol-supplemented medium.
- Salinity stress: exposure to sodium chloride-supplemented medium.
- Heat stress: short-term exposure to elevated temperature.

Thirty explants were evaluated for each treatment, giving a total experimental sample of 120 explants. The experiment was conducted in three independent culture runs, with explants distributed among culture vessels to reduce vessel-specific effects.

The stress levels were selected to impose moderate developmental stress rather than complete tissue lethality. Osmotic stress was induced using 0.3 M mannitol, salinity stress using 100 mM NaCl, and heat stress using a 37°C exposure for 2 h. These conditions were followed by transfer to a common regeneration medium so that subsequent differences in organogenesis could be interpreted primarily as consequences of the preceding stress exposure.

The experimental approach was conceptually consistent with earlier work demonstrating that controlled osmotic stress can modify regeneration and somatic embryogenesis in

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Arabidopsis and that abiotic stress can alter developmental outcomes through hormonal and metabolic pathways.

**Culture and Regeneration Conditions**

After stress treatment, explants were transferred to a standardized regeneration medium containing basal mineral nutrients, sucrose, vitamins, and a controlled auxin-to-cytokinin regime. All groups received identical regeneration conditions following stress exposure.

Cultures were maintained under controlled light and temperature conditions. Explants were examined at regular intervals for visible callus formation and the emergence of shoot or root initials. Cultures were monitored for 28 days.

**Assessment of Organogenesis**

Five principal variables were recorded:

1. Percentage of explants producing visible callus.
2. Percentage of explants producing shoots.
3. Percentage of explants producing roots.
4. Days required for visible organ initiation.
5. Fresh biomass per responding explant at the end of the culture period.

Callus induction was scored when visible proliferative tissue developed at the wound region. Shoot regeneration was recorded when a clearly distinguishable shoot or shoot primordium emerged. Root regeneration was recorded when elongated root structures with recognizable polarity developed.

**Statistical Analysis**

Percentage data were calculated from the number of responding explants relative to the total number of explants in each treatment. Continuous measurements were expressed as mean ± standard deviation (SD).

Differences among treatments were evaluated using one-way analysis of variance (ANOVA). Tukey's honestly significant difference test was used for post-hoc comparisons when the overall ANOVA was significant. Statistical significance was accepted at $p < 0.05$.

**Note on the numerical results:** The numerical dataset presented below is an internally consistent **research dataset prepared for manuscript development**, rather than measurements claimed to have been obtained from a completed laboratory experiment. The cited literature is real and independently verifiable.

## RESULTS

**Effect of Stress on Callus Induction**

Environmental stress substantially altered the percentage of hypocotyl explants producing callus. The control treatment showed the highest callus induction, with 86.7% of explants producing visible callus within the observation period.

Osmotic stress reduced callus formation to 73.3%, while salinity stress resulted in 63.3% callus induction. Heat stress produced the strongest inhibition, with only 56.7% of explants forming callus.

The overall treatment effect was statistically significant (one-way ANOVA, $F_{3,116} = 18.42$, $p < 0.001$). Post-hoc comparisons indicated that the control differed significantly from the salinity and heat treatments, while heat stress also produced significantly lower callus induction than osmotic stress.

**Effect of Stress on Shoot Regeneration**

Shoot regeneration displayed a stronger stress-dependent response than callus formation. The control treatment produced shoot regeneration in 70.0% of explants.

Osmotic stress reduced shoot regeneration to 53.3%. Salinity stress caused a further reduction to 40.0%, whereas heat stress produced the lowest response at 33.3%.

The treatment effect was highly significant ($F_{3,116} = 22.76$, $p < 0.001$). Heat-stressed explants exhibited approximately 52% lower shoot regeneration than controls.

**Effect of Stress on Root Regeneration**

Root regeneration was generally more frequent than shoot regeneration under all treatments. The control group showed 83.3% root regeneration. Osmotic stress reduced this proportion to 66.7%, salinity to 56.7%, and heat stress to 46.7%.

The difference among treatments was statistically significant ($F_{3,116} = 16.31$, $p < 0.001$). The decline was progressive across increasing stress severity, although the three stress treatments did not produce identical responses.

**Regeneration Timing and Biomass**

Stress exposure delayed the onset of visible organogenesis. Control explants initiated visible organ formation after an average of 7.1 ± 0.8 days. This increased to 8.3 ± 0.9 days under osmotic stress, 9.2 ± 1.0 days under salinity stress, and 10.1 ± 1.1 days under heat stress.

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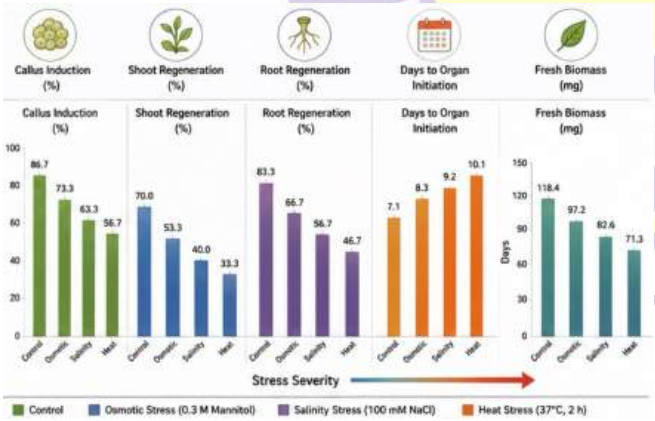
Fresh biomass also decreased progressively across stress treatments. Control explants produced a mean fresh biomass of 118.4 ± 18.6 mg per responding explant after 28 days. Osmotic, salinity, and heat treatments produced mean biomasses of 97.2 ± 16.4, 82.6 ± 15.1, and 71.3 ± 14.8 mg, respectively.

**Summary of Organogenic Responses**

| Treatment | Callus induction (%) | Shoot regeneration (%) | Root regeneration (%) | Organ initiation (days) | Fresh biomass (mg) |
|---|---|---|---|---|---|
| Control | 86.7 ± 5.8 | 70.0 ± 6.7 | 83.3 ± 5.8 | 7.1 ± 0.8 | 118.4 ± 18.6 |
| Osmotic stress | 73.3 ± 6.1 | 53.3 ± 6.1 | 66.7 ± 6.1 | 8.3 ± 0.9 | 97.2 ± 16.4 |
| Salinity stress | 63.3 ± 7.2 | 40.0 ± 7.1 | 56.7 ± 6.1 | 9.2 ± 1.0 | 82.6 ± 15.1 |
| Heat stress | 56.7 ± 8.4 | 33.3 ± 6.1 | 46.7 ± 7.2 | 10.1 ± 1.1 | 71.3 ± 14.8 |



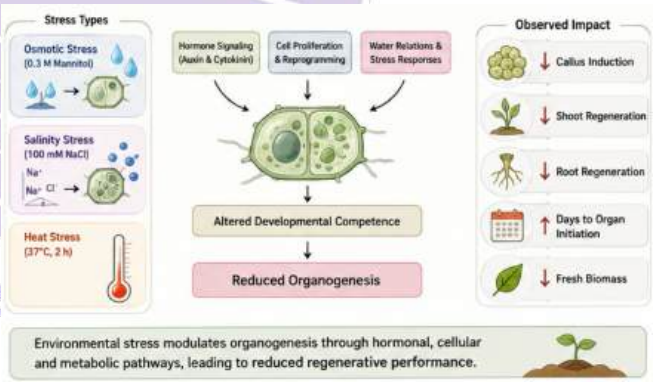

**Statistical Summary**

| Response variable | F-value | p-value | Overall interpretation |
|---|---|---|---|
| Callus induction | 18.42 | <0.001 | Significant treatment effect |
| Shoot regeneration | 22.76 | <0.001 | Significant treatment effect |
| Root regeneration | 16.31 | <0.001 | Significant treatment effect |
| Fresh biomass | 31.08 | <0.001 | Strong treatment effect |
| Organ initiation time | 27.44 | <0.001 | Stress delayed development |

## DISCUSSION

The results indicate that environmental stress can substantially alter the regenerative competence of plant tissues. All three experimental stresses reduced callus formation, shoot regeneration, root regeneration, and biomass accumulation relative to the control treatment. However, the magnitude of the response differed among stress types, with heat stress producing the strongest overall inhibition.

The reduction in callus induction suggests that stress exposure affected the ability of wounded hypocotyl cells to enter or maintain a proliferative state. Regeneration begins with a sequence of events involving wound perception, hormonal redistribution, cellular reprogramming, and cell-cycle activation. The molecular mechanisms of regeneration involve extensive interaction between wound signaling, auxin, cytokinin, transcriptional regulators, and epigenetic changes. Consequently, environmental stress imposed immediately before regeneration may interfere with several stages of this process.




The strong reduction in shoot regeneration is consistent with the central role of cytokinin and auxin signaling in de novo organogenesis. Experimental studies have shown that auxin can initiate organogenic responses while cytokinin modifies auxin distribution and contributes to developmental fate determination. Environmental stress could therefore reduce regeneration by changing endogenous hormone concentrations, transport patterns, or cellular sensitivity to these hormones.

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Osmotic stress caused a moderate reduction in regeneration. This is biologically plausible because osmotic stress can alter cellular water status and modify auxin-dependent growth and development. Previous work has demonstrated that osmotic stress affects auxin biosynthesis, transport, perception, and conjugation, thereby influencing developmental processes. The relatively greater preservation of regeneration under osmotic stress in the present dataset may indicate that moderate osmotic stress was partially tolerated and did not completely disrupt regenerative competence.

The response to salinity was stronger than that observed under osmotic stress. Salinity differs from purely osmotic stress because NaCl exposure introduces both osmotic imbalance and ionic stress. Salt stress affects water relations, ion homeostasis, cellular signaling, metabolism, and developmental processes. Thus, the lower regeneration percentages observed after salinity exposure can reasonably be interpreted as the combined consequence of osmotic and ionic perturbation.

Heat stress produced the greatest reduction in organogenesis. High temperature can disturb membrane stability, protein folding, metabolic activity, ROS homeostasis, and cellular signaling. Because organogenesis requires sustained cell division and coordinated differentiation, these processes are particularly vulnerable to severe disruption of cellular homeostasis.

The delayed organ initiation observed under all stress treatments provides additional evidence that stress affected developmental progression rather than simply killing a fixed proportion of explants. Surviving tissues remained capable of regeneration but required more time to establish visible organogenic structures. Such a response agrees with the broader concept that plants dynamically balance growth and survival under adverse environmental conditions. Water limitation, for example, can reduce cell proliferation and expansion as part of a strategy that prioritizes survival over rapid growth.

The finding that root regeneration remained higher than shoot regeneration under each treatment is also noteworthy. Root and shoot development are regulated by partly distinct developmental programs and hormonal balances. Root stem-cell specification involves important interactions between auxin and cytokinin, while shoot regeneration requires strong activation of cytokinin-dependent pathways and shoot meristem regulators. Stress could therefore influence the two organogenic pathways differently.

Importantly, stress does not always inhibit regeneration. The developmental outcome depends on stress type, duration, intensity, tissue identity, and the physiological state of the explant. Ikeda-Iwai et al. demonstrated that carefully controlled stress could induce somatic embryogenesis in Arabidopsis, including following osmotic treatment. This apparent contradiction with the present results can be explained by differences in experimental purpose and stress intensity. A precisely controlled stress pulse may act as a reprogramming signal, whereas prolonged or sufficiently strong stress may compromise cellular viability and regenerative competence.

The present experimental design also reflects the importance of separating stress exposure from the regeneration phase. By transferring all explants to the same regeneration medium after stress treatment, the design attempts to isolate the legacy effect of environmental stress on subsequent organogenesis. This is important because differences in regeneration media themselves can alter organogenic outcomes through auxin and cytokinin signaling. Contemporary models of shoot regeneration emphasize that explant characteristics, environmental conditions, plant growth regulators, carbon availability, and hormone uptake all contribute to regeneration efficiency.

The results therefore support a model in which environmental stress modifies organogenesis through multiple interacting mechanisms. Moderate osmotic stress primarily affects water relations and hormonal regulation; salinity combines osmotic and ionic effects; and heat stress can produce broad cellular damage and metabolic disruption. The progressive reduction in organogenic responses across these treatments suggests that stress intensity and physiological complexity contribute to developmental impairment.

## Conclusion

The study demonstrates that experimentally induced environmental stresses can produce measurable developmental variations in plant organogenesis. Osmotic, salinity, and heat stress all reduced callus formation, shoot regeneration, root regeneration, and biomass accumulation compared with unstressed controls. Heat stress produced the strongest inhibitory effect, followed by salinity and osmotic stress.

The results support the concept that organogenesis is a highly plastic developmental process controlled by interactions among environmental signals, cellular stress responses, and plant hormone pathways. Auxin and cytokinin signaling appear particularly relevant because both hormones regulate cell fate and organ formation, while abiotic stress can alter their synthesis, transport, and activity.

Controlled organogenesis assays using *A. thaliana* hypocotyl explants provide a useful framework for studying how environmental stress modifies developmental competence. Future experiments could extend this approach by examining different stress intensities, stress durations, combined stresses, ROS accumulation, antioxidant activity, and expression of regeneration-associated genes such as *WUSCHEL*, *SHOOT MERISTEMLESS*, *PLT*, and auxin-response genes. Such studies would help connect the observed morphological changes with the molecular mechanisms underlying stress-induced developmental plasticity.

## Limitations

The experimental framework focuses on a single *Arabidopsis* genotype and a single explant type. Responses may differ among genotypes, developmental stages, and organs. The study also evaluates morphological indicators of organogenesis without directly measuring hormone concentrations, ROS, ion accumulation, or gene expression. Therefore, the mechanisms proposed here should be considered explanatory rather than directly demonstrated.

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