Response surface optimization of kinetin and naphthaleneacetic acid for efficient in vitro shoot regeneration in Thymus daenensis Celak

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Research Article

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Abstract

In vitro regeneration of *Thymus daenensis* Celak., a medicinal and aromatic species endemic to Iran, is often limited by strict hormonal requirements. This makes the development of an efficient and reproducible protocol crucial for large-scale micropropagation and germplasm conservation. In this study, *Response Surface Methodology* (RSM) based on a *Central Composite Design* (CCD) was applied to quantify the individual and interactive effects of naphthaleneacetic acid (NAA) and kinetin (Kin) on shoot regeneration percentage and callus fresh weight.

A second-order polynomial model for regeneration revealed highly significant linear and quadratic effects of Kin ($p < 0.01$) and explained 80.7% of the observed variance. In contrast, the fitted model for callus weight was not statistically significant ($p > 0.05$), suggesting higher biological variability and possible contributions of additional physiological or environmental factors to biomass accumulation.

Multi-response optimization using a composite desirability function identified NAA = 0.99 mg L^{-1} and Kin = 1.41 mg L^{-1} as the optimal concentrations, predicting a regeneration frequency of approximately 93% and a callus weight of 0.23 g. These findings demonstrate that RSM provides a robust framework for defining precise cytokinin–auxin combinations to enhance shoot induction in *T. daenensis*, while highlighting that biomass-related traits in endemic species may require expanded experimental designs incorporating additional culture variables.

Key Message

RSM accurately optimized Kin and NAA for 93% shoot regeneration in , while revealing that biomass-related traits in this endemic species require expanded experimental designs beyond hormone concentrations.

Introduction

Plant tissue culture has become a central tool in plant biotechnology. However, optimizing in vitro culture media remains challenging because multiple factors interact in non-linear ways and jointly determine morphogenic responses. Morphogenesis in plant tissue culture is a multifaceted process governed by the interplay of exogenous plant growth regulators (PGRs), the endogenous hormonal balance of the explant, and the physicochemical properties of the culture environment.

Therefore, empirical trial-and-error approaches are often inefficient and may lead to misleading conclusions (Montgomery, 2017; Myers et al., 1989). Within this context, traditional optimization strategies such as the *One-Variable-at-a-Time* (*OVAT*) method are increasingly criticized because they neglect interaction effects among factors, require large numbers of experiments, and frequently yield suboptimal combinations of medium components (Montgomery, 2017; Myers et al., 1989).

Response Surface Methodology (RSM) has emerged as a powerful interdisciplinary framework that integrates statistically rigorous experimental design with mathematical modeling to describe and optimize complex biological systems (Box & Draper, 1987). By employing designs such as the *Central Composite Design* (CCD), researchers can fit second-order polynomial models that explicitly capture linear, quadratic, and interaction effects while substantially reducing the number of required experimental runs (Box et al., 2005).

In plant tissue culture, RSM has been successfully applied to identify optimal regions for shoot induction, root formation, and biomass accumulation, often revealing non-intuitive factor combinations that would be difficult to detect using OVAT-based screening (Thinakaran et al., 2025). Beyond improving experimental efficiency, these models provide a quantitative response surface that helps interpret how changes in PGR concentrations shift developmental outcomes, thereby strengthening the link between statistical patterns and underlying plant physiology (Box & Draper, 1987; Montgomery, 2017).

Thymus daenensis Celak. is a prominent medicinal and aromatic plant endemic to Iran, valued for its essential oils rich in thymol and carvacrol and for its potential pharmaceutical and nutraceutical applications (Mohebodini et al., 2019). Despite its economic and ecological importance, the establishment of reliable in vitro regeneration protocols for this species remains difficult, largely because of its narrow physiological window for successful organogenesis and the pronounced influence of hormone balance on morphogenic competence. Previous studies have highlighted the pivotal role of auxins and cytokinins, particularly naphthaleneacetic acid (NAA) and kinetin (Kin), in regulating organ formation across a wide range of plant species (Skoog, 1957; George et al., 2008). In *T. daenensis*, Mohebodini et al. (2019) reported that combinations of NAA and Kin can promote shoot regeneration; however, responses were highly dependent on concentration and ratio, suggesting a complex interaction structure that cannot be fully resolved using standard univariate comparisons alone.

To date, no study has systematically modeled the Kin–NAA interaction space in *T. daenensis* using a multivariate RSM framework. Therefore, in the present work, we applied a CCD-based RSM approach to quantify the individual and combined effects of these two PGRs on regeneration efficiency and callus fresh weight. The primary objective was to identify optimized cytokinin–auxin combinations that support reproducible shoot regeneration in this endemic species. By shifting the focus toward predictive multivariate modeling, this study aims to provide a robust quantitative tool for micropropagation of *T. daenensis* while critically evaluating the extent to which a second-order RSM model can capture the inherent variability of distinct morphogenic parameters in a wild medicinal plant system.

Although higher-order designs incorporating additional medium components (e.g., sucrose concentration or gelling agents) represent valuable future expansions, the present two-factor approach enables precise resolution of cytokinin–auxin interactions within physiologically relevant ranges, addressing a primary rate-limiting step in *T. daenensis* micropropagation.

Materials and Methods

Seed Source and Surface Sterilization

Seeds of *Thymus daenensis* were obtained from Pakan Seed Company (Isfahan, Iran). To eliminate surface contaminants while preserving germination viability, seeds were first immersed in 70% (v/v) ethanol for 1 min, followed by three rinses with sterile distilled water. Subsequently, seeds were disinfected with 3% (v/v) sodium hypochlorite for 5 min and again rinsed three times with sterile distilled water to remove residual sterilizing agents (Gamborg & Phillips, 2013; Pérez-Molina et al., 2025). All sterilization procedures were carried out under a laminar flow hood to maintain aseptic conditions throughout handling and transfer.

Preparation of Explants and Culture Conditions

Surface-sterilized seeds were sown on *Murashige and Skoog (MS) basal medium*. The medium was supplemented with 30 g L^{-1} sucrose and solidified with 7 g L^{-1} agar. The pH of the medium was adjusted to 5.8 ± 0.1 prior to autoclaving at 121°C for 15 min (Murashige & Skoog, 1962).

Hypocotyl explants, approximately 2 cm in length, were excised 8 days after germination from uniformly developed seedlings to minimize variation in physiological status (George et al., 2008; Karjoo & Fattahi, 2022). Unless otherwise stated, cultures were incubated at 25 ± 2°C under a 16-h photoperiod provided by cool white fluorescent lamps, with a light intensity of approximately 2000–3000 lux. These conditions were previously reported as suitable for in vitro morphogenesis in aromatic and medicinal species (Thinakaran et al., 2025).

Experimental Design and Statistical Analysis

A *Central Composite Design* (CCD) was employed to investigate the linear, quadratic, and interaction effects of naphthaleneacetic acid (NAA; 0–2 mg L^{-1}) and kinetin (Kin; 0–4 mg L^{-1}) on regeneration percentage and callus fresh weight. The design comprised factorial points, axial points, and replicated center points, enabling the fitting of second-order polynomial models with adequate curvature while keeping the number of experimental runs feasible (Box et al., 2005; Montgomery, 2017). The axial distance (α) was set to 1, resulting in a face-centered CCD in which all factor levels remained within physiologically relevant ranges.

The second-order regression model used for each response was defined as:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \epsilon$$

where *Y* represents the response variable (regeneration percentage or callus fresh weight), β terms denote the regression coefficients for the intercept, linear, quadratic, and interaction effects, and X_i and X are the coded independent variables representing the concentrations of NAA and Kin (Box & Wilson, 1992).

Data analysis, including model fitting, analysis of variance (ANOVA), and generation of response surface and contour plots, was performed using Minitab 22. To identify conditions that jointly optimized regeneration and callus traits, a multi-response desirability function (*D*) was applied, allowing simultaneous maximization of regeneration percentage while constraining callus fresh weight within a biologically relevant range (Derringer & Suich, 1980; Myers et al., 2016).

Explants were evaluated for morphogenic responses 28 days after initiation of culture. Shoot regeneration percentage was calculated as:

$$\text{Regeneration}(\%)=\left(\frac{\text{numberofexplantsproducing} \geq 1\text{shoot}}{\text{total number of explants cultured}}\right)\times 100$$

with 10 explants per Petri dish and three replicate dishes per treatment (n = 30 explants per treatment combination). Callus fresh weight (g per explant) was determined after gently blotting excess moisture on sterile filter paper, using an analytical balance with 0.001 g precision.

Results

Statistical Analysis and Model Fitting

The experimental data for shoot regeneration and callus fresh weight of *Thymus daenensis* were fitted to quadratic models within the *Central Composite Design* framework. The ANOVA for the regeneration model (Table 1) showed that the overall model was significant ($F = 6.71$, $p = 0.010$), with $R^2 = 80.74\%$ and adjusted $R^2 = 63.41\%$, indicating that a substantial proportion of the variability in regeneration percentage was captured by the fitted second-order equation. The lack-of-fit term was not significant ($F = 0.64$, $p = 0.663$), supporting the adequacy of the model for describing the observed regeneration response within the investigated factor space.

Table 1
Analysis of variance (ANOVA) for the quadratic response surface model describing shoot regeneration percentage (%, arcsin-transformed; *n* = 30 explants per treatment) in *Thymus daenensis* hypocotyl explants as affected by NAA (0–2 mg L^{-1}) and Kin (0–4 mg L^{-1}) concentrations.

Source	DF	Sum of Squares	Mean Square	F-value	P-value
Model	5	2.004	0.400	6.71	0.010
Linear	2	1.500	0.750	12.55	0.003
Kin	1	1.500	1.500	25.11	0.001
Square	2	0.504	0.252	4.22	0.056
Kin²	1	0.480	0.480	8.04	0.022
2-Way Interaction	1	0.000	0.000	0.00	1.000
Error	8	0.477	0.059	-	-
Lack-of-Fit	4	0.186	0.046	0.64	0.663
Total	13	2.48214	-	-	-
R^2 = 80.74% R^2-adj = 63.41%					

Partitioning of variance revealed that the linear component of the model was highly significant (F = 12.55, *p* = 0.003), entirely attributable to Kin, whose individual linear term was strongly significant (F = 25.11, *p* = 0.001). In contrast, the linear effect of NAA was not significant, indicating that within the studied range, variation in NAA alone did not markedly affect regeneration percentage. The quadratic component was moderately important (F = 4.22, *p* = 0.056), mainly driven by the significant quadratic term of Kin (F = 8.04, *p* = 0.022), whereas the interaction term (NAA × Kin) was not significant (*p* = 1.000), suggesting that the response surface curvature was dominated by the cytokinin concentration rather than true synergistic interactions between the two regulators.

Effect of Hormonal Treatments on Regeneration

The standardized Pareto chart for regeneration (Fig. 1A) clearly identified Kin as the dominant factor, with both its linear and quadratic effects exceeding the significance threshold at α = 0.05. Neither the linear NAA term nor the interaction term contributed significantly.

Consistent with the ANOVA, the three-dimensional response surface and contour plots for regeneration (Fig. 2A) showed a pronounced curvature along the Kin axis. Regeneration percentage increased from low to intermediate Kin concentrations and declined at higher levels. Maximum predicted regeneration was obtained at intermediate Kin levels in combination with approximately 1.0 mg L^{-1} NAA. Further increases in Kin beyond 1.41 mg L^{-1} led to a gradual reduction in regeneration efficiency, a pattern compatible with a supra-optimal effect of cytokinin, where excessively high concentrations may impair shoot primordia formation and disturb the endogenous hormonal balance of the explant.

Figure 2A. Three-dimensional response surface and contour plots illustrating the effects of NAA and Kin concentrations on shoot regeneration in *Thymus daenensis*.

Analysis of Callus Weight

In contrast to regeneration, the quadratic model fitted for callus weight was not statistically significant ($F = 0.71$, $p = 0.631$), with $R^2 = 47.78\%$, indicating that less than half of the variability in biomass could be explained by the tested NAA and Kin combinations. The ANOVA for callus weight (Table 2) showed that neither linear, quadratic, nor interaction terms reached significance at $\alpha = 0.05$. The lack-of-fit test was also non-significant ($F = 1.32$, $p = 0.396$), implying that although the model did not violate basic assumptions, it lacked sufficient explanatory power for this trait.

Table 2
Analysis of variance (ANOVA) for the quadratic response surface model describing callus fresh weight (g/explant; *n* = 30 explants per treatment) in *Thymus daenensis* hypocotyl explants as affected by NAA (0–2 mg L^{-1}) and Kin (0–4 mg L^{-1}) concentrations.

Source	DF	Sum of Squares	Mean Square	F-value	P-value
Model	5	0.112	0.022	0.71	0.631
Linear	2	0.002	0.001	0.05	0.954
Square	2	0.108	0.054	1.73	0.237
2-Way Interaction	1	0.000	0.000	0.00	0.948
Error	8	0.251	0.031	-	-
Lack-of-Fit	4	0.143	0.035	1.32	0.396
Total	13	0.36336	-	-	-

Despite the lack of statistical significance, the response surface for callus weight (Fig. 2B) revealed biologically meaningful trends. The highest callus biomass occurred at intermediate, balanced levels of NAA and Kin, whereas extreme combinations (very low or very high concentrations of one or both hormones) were associated with reduced callus growth. This weak model fit suggests that callus development in *T. daenensis* is likely influenced by additional factors not included in the present design, such as endogenous auxin status, carbohydrate availability, micro-environmental gradients within the culture vessel, or subtle differences in explant physiological age. These observations indicate that expanded experimental designs incorporating further medium and environmental variables are needed for precise modeling of biomass accumulation.

Optimization and Validation

To integrate the contrasting behaviors of regeneration and callus weight into a single decision framework, a multi-response desirability function was applied. In this optimization, regeneration percentage was set as the primary response to be maximized, while callus weight was constrained to remain within a moderate range to avoid excessive callusing.

The composite desirability analysis identified NAA = 0.99 mg L^{-1} and Kin = 1.41 mg L^{-1} as the optimal hormone concentrations, yielding an overall desirability score (*D*) of 0.84. At this point, the model predicted a high regeneration rate (0.93) accompanied by moderate callus weight (0.23 g), representing a compromise between efficient shoot production and controlled biomass formation. Although experimental validation of the predicted optimum through additional replicated trials is recommended, the non-significant lack-of-fit tests ($p > 0.05$ for both models) and high R^2 for regeneration substantiate the model's predictive reliability within the tested design space, providing a solid foundation for immediate application in *T. daenensis* micropropagation.

Discussion

Kinetin as the Primary Regulator of Shoot Regeneration

The RSM analysis clearly established kinetin (Kin) as the dominant factor controlling shoot regeneration in *Thymus daenensis* hypocotyl explants, with both linear and quadratic effects reaching statistical significance (Table 1). This produced a characteristic parabolic response surface (Fig. 2A), where regeneration efficiency increased with Kin concentration up to an optimum of approximately 1.41 mg L^{-1}, beyond which responses declined—a pattern consistent with the classic cytokinin–auxin balance hypothesis first described by Skoog (1957). Higher cytokinin-to-auxin ratios are well known to favor shoot organogenesis across many species by promoting cell division and inhibiting rooting, and our findings align with this principle while defining a precise threshold for this endemic Lamiaceae species (George et al., 2008).

The absence of a significant NAA main effect or NAA × Kin interaction further suggests that endogenous auxin levels in 8-day-old hypocotyls were sufficient to support initial cell dedifferentiation, rendering exogenous NAA supplementary rather than essential for shoot induction—although it remained important for the overall hormonal equilibrium identified through multi-response optimization.

Contrasting Model Performance for Regeneration and Callus Biomass

A striking contrast emerged between the two response variables: while the regeneration model explained over 80% of variance with high statistical power, the callus weight model lacked significance ($F = 0.71$, $p = 0.631$; $R^2 = 47.78\%$; Table 2), despite displaying a biologically plausible trend toward peak biomass at balanced intermediate hormone levels (Fig. 2B). This discrepancy is unlikely to reflect a simple experimental deficiency, as the lack-of-fit test was non-significant for both traits and the design adequately spanned physiologically relevant concentrations.

Instead, it highlights a fundamental physiological distinction: shoot regeneration appears tightly regulated by exogenous Kin within a narrow, predictable range, whereas callus biomass accumulation involves greater inherent variability driven by unmodeled factors such as explant-specific endogenous hormone gradients, sucrose uptake efficiency, or subtle microenvironmental differences within culture vessels (Fattahi & Karjoo, 2022). Such "noisy" biomass responses are common in wild medicinal plants, where genetic heterogeneity and physiological stochasticity demand either larger sample sizes, broader factor ranges, or additional covariates—like gelling agent type or light quality—to achieve model significance (Thinakaran et al., 2025).

Practical Value of RSM-Defined Optimization for Micropropagation

The multi-response desirability function effectively reconciled these differences by prioritizing regeneration (D = 0.84 at NAA = 0.99 mg L^{-1}, Kin = 1.41 mg L^{-1}) while maintaining moderate callus formation. This yielded predicted outcomes of 93% regeneration and 0.23 g callus weight per explant, balancing propagation efficiency with manageable handling.

Unlike conventional one-factor-at-a-time (OFAT) screening, which might identify a single "best" discrete treatment from grid-based trials, RSM delivers a continuous response landscape (Fig. 2A) that enables precise interpolation of outcomes across the factor space—reducing medium preparation costs and experimental iterations for scale-up (Montgomery, 2017). Relative to prior work on *T. daenensis*, where Mohebodini et al. (2019) established the feasibility of NAA–Kin combinations through preliminary screening, our CCD-based approach provides exact coordinate optimization and quantitative model diagnostics, offering a more robust foundation for industrial micropropagation or germplasm conservation programs targeting this thymol- and carvacrol-rich endemic resource.

Implications and Refinement Opportunities

These results affirm RSM as a mature interdisciplinary tool for cytokinin–auxin optimization in plant tissue culture, particularly when responses exhibit non-linear behavior that evades traditional ANOVA. However, the callus biomass findings caution that not all traits respond equally well to second-order modeling in genetically diverse wild species, suggesting targeted refinements such as incorporating sucrose concentration, pH gradients, or explant age as additional factors in future designs.

Experimental validation of the predicted optimum through replicated trials would further strengthen protocol reliability, potentially enabling *T. daenensis* conservation via high-throughput clonal propagation while minimizing resource inputs in commercial settings. Future studies could extend this framework using three-factor CCD designs incorporating sucrose concentration or culture pH alongside NAA and Kin to enhance model fit for biomass traits, while the current two-factor optimization provides an immediately applicable protocol for shoot regeneration.

Conclusion

This study established an efficient in vitro regeneration protocol for *Thymus daenensis* Celak. through RSM-based optimization of NAA and kinetin concentrations, identifying NAA = 0.99 mg L^{-1} and Kin = 1.41 mg L^{-1} as the optimal combination. This combination predicted 93% shoot regeneration alongside moderate callus biomass (0.23 g).

The CCD analysis confirmed kinetin as the primary driver of organogenesis via significant linear and quadratic effects, whereas callus weight exhibited higher biological variability that exceeded the explanatory capacity of the second-order model within the tested factor ranges.

These findings advance micropropagation of this thymol- and carvacrol-rich endemic medicinal plant by providing precise, quantitatively validated hormone coordinates that outperform traditional screening approaches, with direct applications for germplasm conservation and commercial-scale propagation. Future refinements could incorporate additional medium components, such as sucrose concentration or culture pH, to enhance model fit for biomass traits and further streamline protocol efficiency.

Abbreviations

ANOVA
Analysis of variance
CCD
Central composite design
Kin
Kinetin
MS
Murashige and Skoog medium
NAA
Naphthaleneacetic acid
OVAT
One-variable-at-a-time
PGRs
Plant growth regulators
RSM
Response surface methodology

Declarations

Conflict of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval:

This study did not involve human participants or animals. All plant materials were obtained from commercial sources and used in accordance with relevant institutional guidelines.

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Author Contribution

N. M: Conceptualization, Investigation, Data curation, Writing – original draft.M. M: Supervision, Methodology, Formal analysis, Writing – review & editing.S. P: Formal analysis, Statistical modeling, Writing – review & editing.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Box GEP, Draper NR (1987) Empirical model-building and response surfaces. Wiley
2. Box GEP, Wilson KB (1992) On the experimental attainment of optimum conditions. In *Breakthroughs in statistics: Methodology and distribution* (pp. 270–310). Springer. https://doi.org/10.1007/978-1-4612-0917-7_16
3. Box GEP, Hunter JS, Hunter WG (2005) Statistics for experimenters: Design, innovation, and discovery, 2nd edn. Wiley
4. Derringer G, Suich R (1980) Simultaneous optimization of several response variables. J Qual Technol 12(4):214–219. https://doi.org/10.1080/00224065.1980.11980968
5. Fattahi M, Karjo B (2022) Optimization of the effect of culture medium and sucrose concentration and pH on the production of hairy roots of *Atropa belladonna* L. using response surface

methodology (RSM) under in vitro conditions. Iran J Hortic Sci Technol 23(4):661–674. https://doi.org/10.22059/ijhst.2022.335774.655

6. Gamborg OL, Phillips GC (eds) (2013) Plant cell, tissue and organ culture: Fundamental methods. Springer. https://doi.org/10.1007/978-3-642-77887-2
7. George EF, Hall MA, De Klerk G-J (2008) *Plant propagation by tissue culture: Volume 1. The background* (3rd ed.). Springer. https://doi.org/10.1007/978-1-4020-5005-3
8. Mohebodini M, Mirzadeh Qassabeh R, Chamani E, Fathi R (2019) Investigation of the effect of plant growth regulators on micropropagation and regeneration of *Thymus daenensis* Celak. J Crop Breed 11(31):124–133. https://doi.org/10.18331/jwbt.2019.1.1.7
9. Montgomery DC (2017) Design and analysis of experiments, 10th edn. Wiley
10. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3):473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
11. Myers RH, Khuri AI, Carter WH Jr. (1989) Response surface methodology: 1966–1988. Technometrics 31(2):137–157. https://doi.org/10.1080/00401706.1989.10488522
12. Myers RH, Montgomery DC, Anderson-Cook CM (2016) Response surface methodology: Process and product optimization using designed experiments, 4th edn. Wiley
13. Pérez-Molina JP, Carvajal-Campos P, Jiménez VM (2025) Mapping fifty-five years of somatic embryogenesis research: A bibliometric analysis of trends, global collaborations, and key advances across more than nine thousand articles. Planr Cell Tissue Organ Cult 162(2):44–62. https://doi.org/10.1007/s11240-025-02945-7
14. Skoog F (1957) Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 11:118–131
15. Thinakaran R, Korkmaz E, Ünver B, Ali SA, Iqbal Z, Aasim M (2025) Synergistic application of artificial intelligence and response surface methodology for predicting and enhancing in vitro tuber production of potato (*Solanum tuberosum*). PLoS ONE 20(6) Article e0325754. https://doi.org/10.1371/journal.pone.0325754

Figures

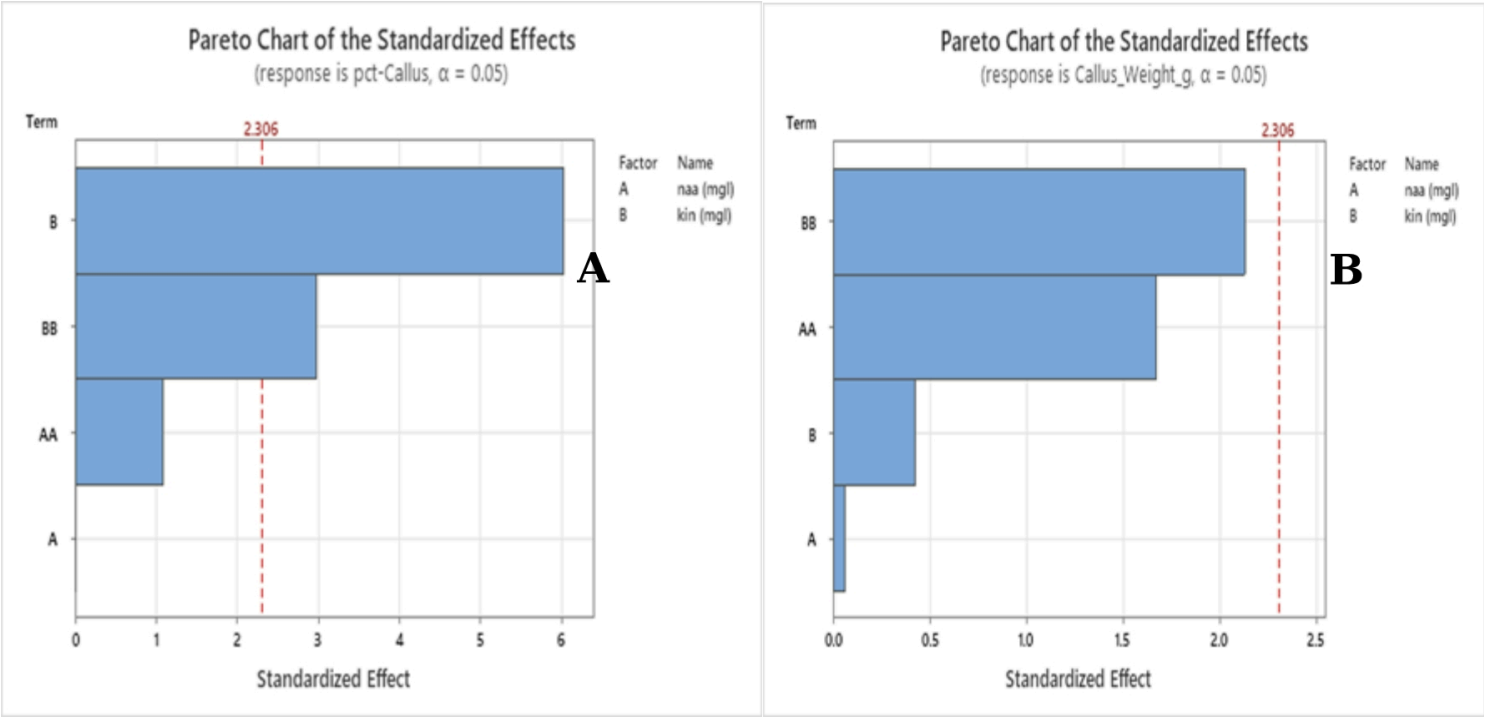

Figure 1

Pareto charts of standardized effects for (A) shoot regeneration percentage and (B) callus fresh weight in *Thymus daenensis* as affected by NAA and Kin concentrations. The vertical dashed line denotes the significance threshold at α = 0.05.

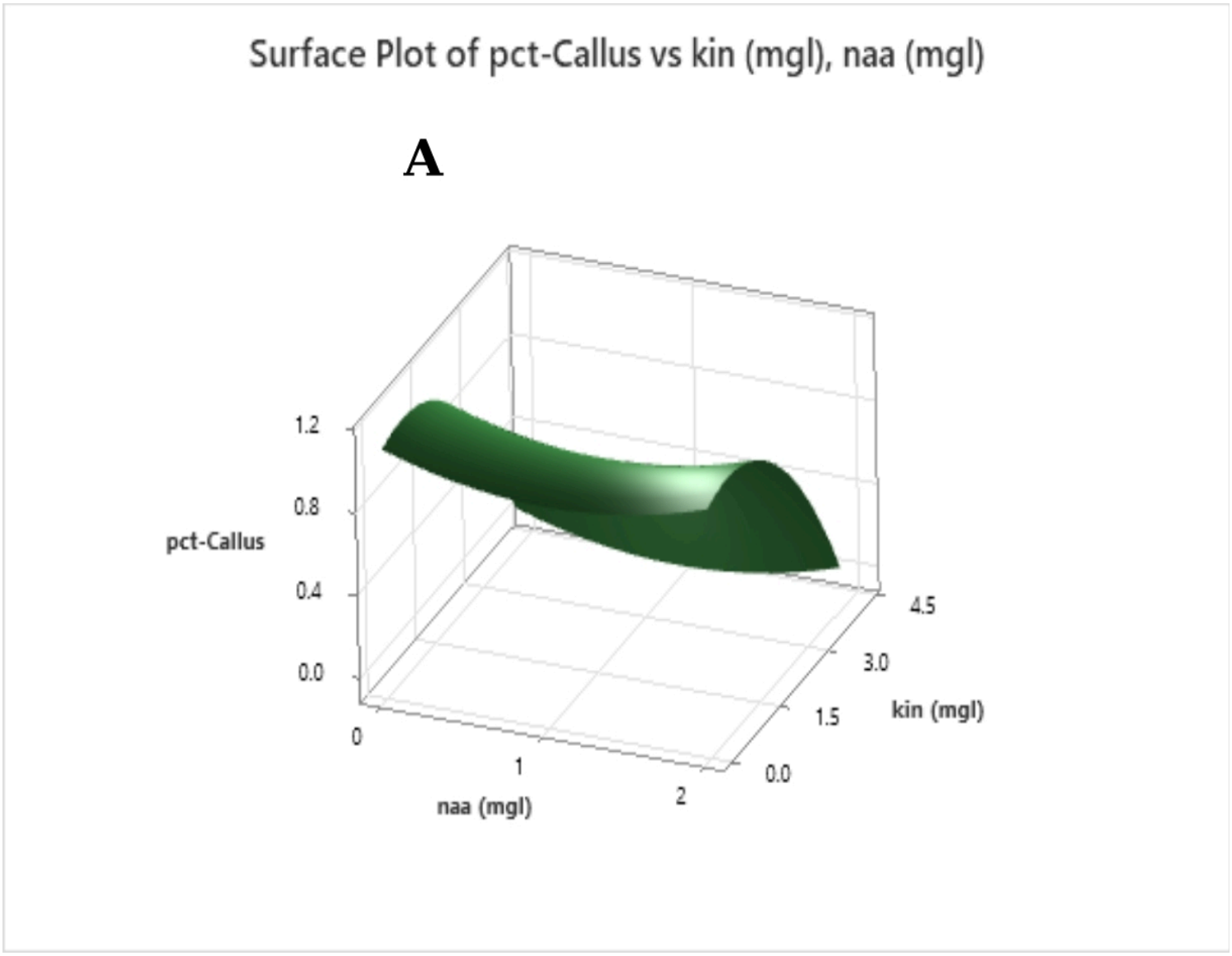

Figure 2

Figure 2A. Three-dimensional response surface and contour plots illustrating the effects of NAA and Kin concentrations on shoot regeneration in *Thymus daenensis*.

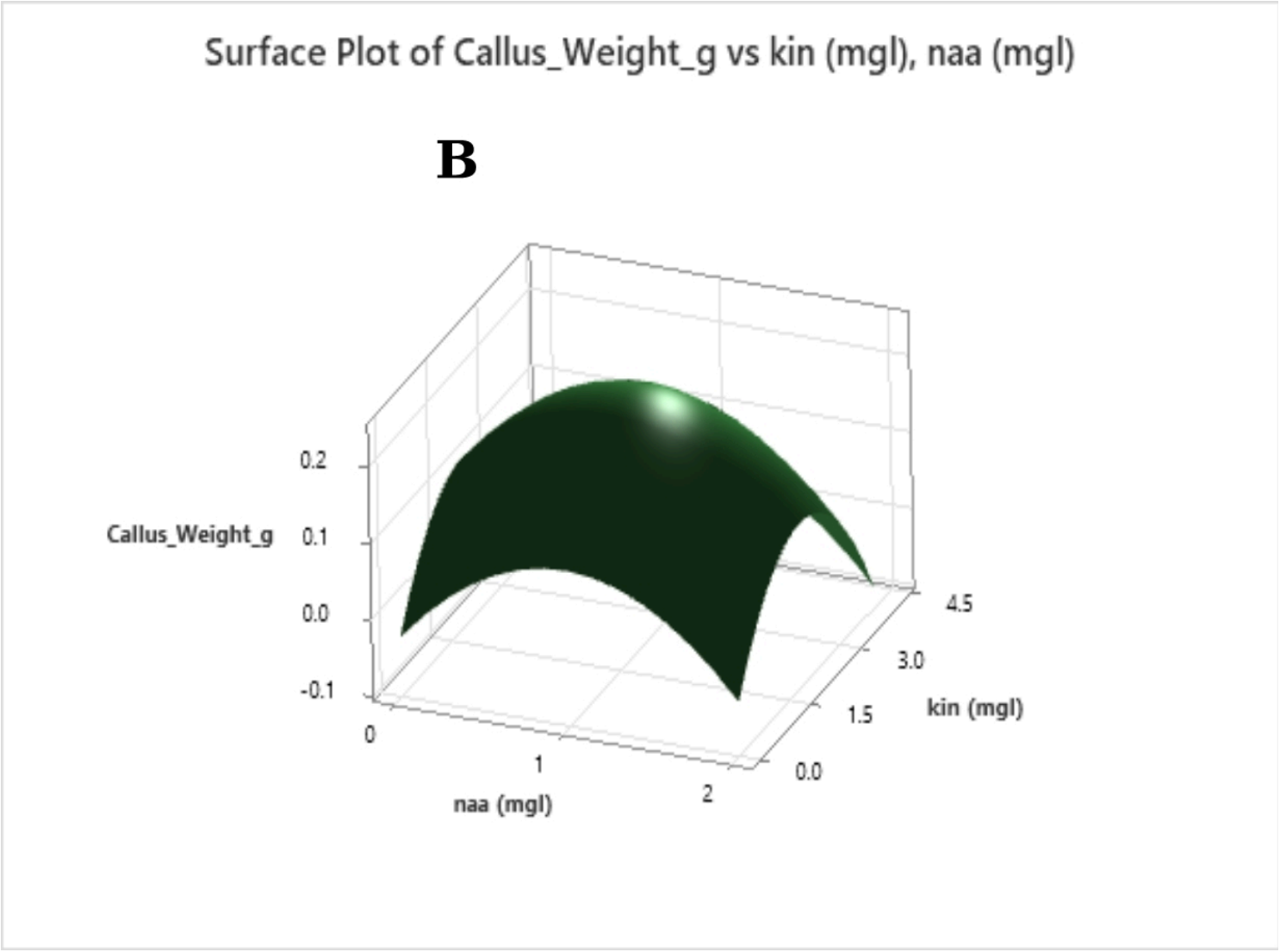

Figure 3

Figure 2B. Three-dimensional response surface and contour plots of callus fresh weight as affected by NAA and Kin concentrations.