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Artificial Intelligence and Machine Learning for Predictive Modeling and Hormonal Optimization in Chamomile (*Matricaria chamomilla* L.) Tissue Culture

Rasoul Golzari[1]* and Bahram Maleki-Zanjani[2]

[1] *Center of Nobonyad Sirjan – University of Applied Science and Technology – Tehran – Iran*
[2] *Department of Agronomy and Plant Breading, Agriculture Faculty, Zanjan University, Zanjan, Iran*

*Corresponding Author: Email: rasoul.golzari@gmail.com

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ABSTRACT
Chamomile (*Matricaria chamomilla* L.) is a high-value medicinal plant with growing pharmaceutical demand, which calls for efficient in vitro propagation systems. This study combined machine learning with classical tissue culture to optimize callogenesis in chamomile explants. Three explant types (cotyledon, root, and node) were cultured on Murashige and Skoog medium supplemented with factorial combinations of indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), and 6-benzylaminopurine (BAP). The experiment used a completely randomized design with three biological replicates per treatment, testing 18 hormone combinations across the three explant types. Cotyledons showed the highest callogenic potential, with the combination of IBA 1 mg/L + NAA 1 mg/L + BAP 0.1 mg/L producing the maximum callus fresh weight (1.68 g). To further optimize hormone balance, three machine learning models—multilayer perceptron (MLP), generalized regression neural network (GRNN), and random forest (RF)—were tested. MLP achieved the best predictive accuracy (R^2 = 0.94; RMSE = 0.16), outperforming GRNN and RF. When integrated with a genetic algorithm (MLP–GA), the model identified an optimized hormone combination (0.38 mg/L IBA, 0.29 mg/L NAA, 0.19 mg/L BAP), predicted to yield 1.77 g callus fresh weight, surpassing experimental treatments. The study demonstrates that hybrid ML–GA frameworks are powerful tools for modeling nonlinear plant growth regulator interactions and can be applied as decision-support systems for scalable, reproducible optimization of chamomile tissue culture.
Keywords: Artificial intelligence, Callogenesis, Plant growth regulators, Predictive modeling, Secondary metabolite

INTRODUCTION

German chamomile (*Matricaria chamomilla* L.), a member of the Asteraceae family, has been widely recognized as one of the most valuable medicinal plants globally due to its diverse pharmacological properties. The essential oil of chamomile, particularly rich in α-bisabolol and chamazulene, exhibits remarkable anti-inflammatory, antimicrobial, and antioxidant activities [1]. With the global chamomile market projected to reach $2.1 billion by 2027, there is an urgent need to develop efficient production systems to meet the increasing demand for standardized chamomile products [2].

Plant tissue culture technology has emerged as a powerful tool for chamomile propagation, offering several advantages over conventional cultivation methods. In vitro culture systems enable year-round production of uniform plant material with consistent phytochemical profiles, less dependent on environmental fluctuations [3]. Moreover, tissue culture techniques facilitate the conservation of elite genotypes and provide a platform for genetic improvement of chamomile [4]. However, the commercial application of chamomile tissue culture has been limited by several challenges, including genotype-dependent responses, low multiplication rates, and variable secondary metabolite production [5]. Climate change's high temps/low rain stress on plants [6]. Tissue culture enables controlled, year-round production, vital for conserving threatened species.

The success of chamomile micropropagation largely depends on the precise manipulation of plant growth regulators (PGRs) in the culture medium. Extensive research has demonstrated the critical role of auxins and cytokinins in regulating various developmental processes in chamomile cultures. For instance, Sayadi *et al*. [7] demonstrated that a combination of 1 mg/L NAA and 1 mg/L kinetin was optimal for callus induction in chamomile, achieving up to 93.26% induction in leaf explants. Similarly, Ahmad *et al.* [8] reported that callogenesis and regeneration responses were genotype-dependent, with the Isfahan genotype producing the highest callus percentage and shoot regeneration on MS medium supplemented with 1 mg/L NAA and 1 mg/L kinetin. Adibian [9] further confirmed that balanced concentrations of NAA and kinetin not only enhanced embryogenic callus formation but also improved rooting, particularly when stem and axillary bud explants were used. In addition, Tai *et al*. [10] established an Agrobacterium-mediated callus transformation system in chamomile, highlighting the importance of optimizing PGR combinations and culture conditions for efficient genetic transformation.

Collectively, these findings emphasize that precise manipulation of auxin and cytokinin concentrations is critical for successful chamomile micropropagation and biotechnological applications.

Recent advances in machine learning (ML) have opened new avenues for optimizing plant tissue culture systems. ML algorithms, particularly artificial neural networks (ANNs) and genetic algorithms (GAs), have shown great potential in modeling the complex relationships between different conditions and plant responses [11, 12]. Aasim *et al.* [13] successfully employed ML techniques to reduce the number of required optimization experiments by 70%, while Yoosefzadeh-Najafabadi *et al.* [14] developed multi-objective optimization frameworks capable of simultaneously improving multiple culture outcomes. Hesami *et al.* [15] demonstrated that a hybrid ANN-GA-PSO model could increase α-bisabolol production by 25% through optimal PGR combination prediction in compared to control.

This study aims to develop an intelligent framework for optimizing chamomile tissue culture by integrating machine learning with traditional experimental approaches. The specific objectives include:

1. To develop predictive models how chamomile grows and responds to different hormone mixtures.
2. Use these models together with optimization tools to identify optimal hormone combinations for maximum callus growth.
3. Create simple and reliable protocols that can be used for large-scale chamomile tissue culture and production.

The proposed framework addresses critical limitations in current chamomile micropropagation systems and provides a robust platform for the sustainable production of high-quality plant material for pharmaceutical applications (Fig. 1).

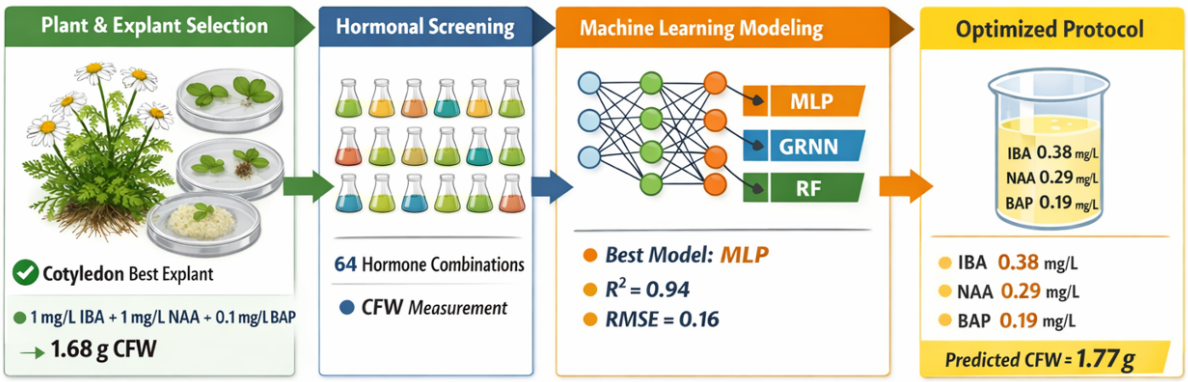

Fig. 1 Schematic representation of the step-by-step workflow of the present study, illustrating the integrated experimental–computational framework used to optimize

MATERIALS AND METHODS

Chamomile seeds were obtained with authorization from the Agricultural Research, Education, and Extension Organization of Iran. A voucher specimen was deposited in the Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran. Plant material was taxonomically verified following the descriptions provided in Flora Iranica [16].

Nodal segments, root pieces, and cotyledons of chamomile were used as explants. The seeds were surface-sterilized with 70% ethanol (30 s) followed by 1.5–2% sodium hypochlorite solution containing a drop of Tween-20 (12–15 min), rinsed three times with sterile distilled water, and treated briefly with an antioxidant solution to reduce browning. The cotyledons were obtained from surface-sterilized, in vitro–germinated seeds. The explants were cultured on Murashige and Skoog (MS) basal medium supplemented with 30 g/L sucrose and solidified with 0.75% agar (pH 5.8 before autoclaving). Cultures were incubated at 24 ± 2 °C under a 16 h photoperiod (40–60 µmol m^{-2} s^{-1}).

To identify the most responsive explant, a factorial experiment was conducted using a completely randomized design (CRD) with three replications. The experiment included three factors: indole-3-butyric acid (IBA; 0, 1, and 2 mg/L), 1-naphthaleneacetic acid (NAA; 0, 1, and 2 mg/L), and 6-benzylaminopurine (BAP; 0 and 0.1 mg/L), resulting in a total of 18 treatment media. Each treatment was applied to cotyledons, roots, and nodes in a completely randomized design with three replicates (five explants per replicate). Responses including survival, contamination, browning, and callus formation were monitored for 6 weeks. The explant type showing the highest callogenic potential was selected for further modeling.

Based on results from Experiment 1, cotyledons were chosen as the most suitable explant. These were cultured on MS medium supplemented with factorial combinations of IBA (0, 0.5, 1, 2 mg/L), NAA (0, 0.5, 1, 2 mg/L), and BAP (0, 0.05, 0.1, 0.2 mg/L), generating 64 treatments. Each treatment included three replicates (one explant per replicate; total 192 cultures).

Based on results from Experiment 1, cotyledons were chosen as the most suitable explant. For Experiment 2, cotyledon explants (approximately 0.5 cm^2) were excised from surface-sterilized chamomile seedlings. They were cultured on Murashige and Skoog (MS) medium supplemented with factorial combinations of indole-3-butyric acid (IBA: 0, 0.5, 1, 2 mg/L), 1-naphthaleneacetic acid (NAA: 0, 0.5, 1, 2 mg/L), and 6-benzylaminopurine (BAP: 0, 0.05, 0.1, 0.2 mg/L), resulting in a total of 64 treatment combinations. Callus fresh weight (CFW; g) was recorded and used as input data for machine learning–based modeling and optimization. In the present study, all methods were performed in accordance with the relevant guidelines and regulations.

Three machine learning models—MLP (Fig. 2), GRNN and RF—were trained on the dataset, which was split into 80% training and 20% testing sets. Hyperparameter optimization was performed using a Genetic Algorithm (population size: 100, 200 generations).

To evaluate and compare the predictive accuracy of the tested models, three key performance metrics were applied: the coefficient of determination (R^2, Equation 1), mean bias error (MBE, Equation 2), and root mean square error (RMSE, Equation 3).

Equ1:

$$R^2 = 1 - \left(\sum_{i=1}^{n}(y_{est} - y_{act})^2 \Big/ \sum_{i=1}^{n}(y_{act} - \bar{y})^2\right)$$

Equ2:

$$MBE = 1/n \sum_{i=1}^{n}(y_{est} - y_{act})$$

Equ3:

$$RMSE = \sqrt{(\sum_{i=1}^{n}(y_{est} - y_{act})^2)/_n}$$

Where "y_{act}" are the actual values, "y_{est}" are the predicted values, and "n" is the number of data points.

The optimized protocol was validated through three independent biological replicates under predicted conditions. All statistical and machine learning analyses were conducted using MATLAB. This integrated approach combines classical tissue culture methods with advanced machine learning for efficient optimization of chamomile micropropagation. In addition, the data of the two experiments were analyzed using SAS software (v. 9.1), and means were compared using Duncan's Multiple Range Test at the 5% significance level.

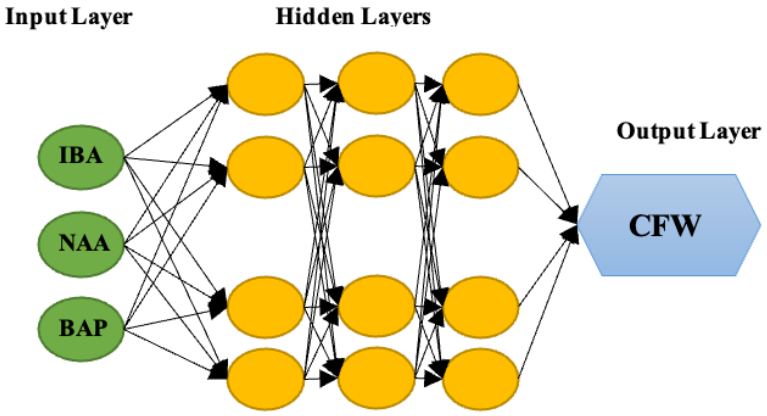

Fig. 2 Diagram illustrating the architecture of a MLP where inputs are IBA, NAA and BAP and output is callus fresh weight.

RESULTS

Selection of the Best Explant for Modeling

Visual observations confirmed the progressive development of callus from early initiation to proliferation, as shown in Figure 3, where *M. chamomilla* explants displayed initial callus formation after one week and substantial growth after one month. The three-way interaction among IBA, NAA, and BAP concentrations significantly affected callus fresh weight (CFW; $p < 0.01$), and this effect was observed across all three explant types (Table 1). No callus formed on hormone-free medium. Auxin-only media (BAP = 0) induced modest callus that depended on IBA–NAA balance: the best auxin-only response occurred at IBA 2 + NAA 1, giving 0.81 g (cotyledon) and 0.77 g (node), while roots peaked at 0.64 g with IBA 1 + NAA 2 (Table 2). Adding 0.1 mg/L BAP markedly enhanced callogenesis across explants and also elicited callus even without auxins (0.52, 0.64, and 0.48 g for cotyledon, root, and node at IBA 0 + NAA 0 + BAP 0.1). A single treatment clearly outperformed all others: IBA 1 mg/L + NAA 1 mg/L + BAP 0.1 mg/L, which produced the maximum callus fresh weight in every explant—1.68 g (cotyledon), 1.32 g (root), and 1.25 g (node)—representing ~107%, 106%, and 62% increases, respectively, over each explant's best auxin-only medium. The next-best combination was IBA 1 + NAA 2 + BAP 0.1 (1.48, 1.16, 0.95 g). An exception to the positive BAP effect occurred at IBA 2 + NAA 1, where adding BAP reduced callus in all explants (e.g., cotyledon 0.81→0.73 g). Across all 18 media, cotyledons were the most responsive explant overall (overall mean 0.70 g) followed by roots (0.61 g) and nodes (0.59 g), and they also achieved the absolute maximum under the optimal triple-hormone treatment.

Based on these findings, cotyledons were selected as the most suitable explant for the subsequent modeling analysis, in which the effects of IBA (0, 0.5, 1, 2 mg/L), NAA (0, 0.5, 1, 2 mg/L), and BAP (0, 0.05, 0.1, 0.2 mg/L) were evaluated using machine learning models (MLP, GRNN, and RF) and optimization approaches to predict and determine the best hormone combination for maximizing callus fresh weight (CFW).

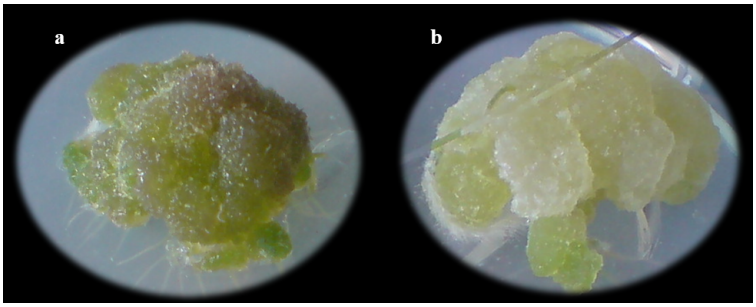

Fig. 3 Callus formation in *M. chamomilla* after (a) one week and (b) one month.

Table 1 Analysis of variance (ANOVA) for the effects of plant growth regulators (PGRs) and explant types on callus fresh weight (CFW) in *M. chamomilla*.

S.O.V	df	Mean Square (CFW)		
		Cotyledons	Root	Node
IBA	2	1.14 **	0.62 **	0.55 **
NAA	2	0.72 **	0.26 **	0.35 **
BAP	1	1.19 **	0.72 **	0.71 **
IBA*NAA	4	0.08 **	0.06 **	0.05 **
IBA*BAP	2	0.48 **	0.24 **	0.15 **
NAA*BAP	2	0.01 **	0.02 **	0.01 **
IBA*NAA*BAP	4	0.22 **	0.16 **	0.11 **
Error	36	0.0037	0.009	0.0014

** Significant at $p < 0.01$

Table 2 Effects of PGRs and the explant types on callus fresh weight in *M. chamomilla*.

IBA (mg/L)	NAA (mg/L)	BAP (mg/L)	Cotyledons CFW (g)	Root CFW (g)	Node CFW (g)
0	0	0	0 ± 0 j	0 ± 0 f	0 ± 0 h
0	1	0	0.52 ± 0.11 f	0.51 ± 0.08 de	0.56 ± 0.06 de
0	2	0	0.51 ± 0.08 f	0.5 ± 0.07 de	0.47 ± 0.06 fj
1	0	0	0.5 ± 0.09 f	0.49 ± 0.06 e	0.47 ± 0.07 fj
1	1	0	0.61 ± 0.07 def	0.6 ± 0.04 bc	0.59 ± 0.08 de
1	2	0	0.75 ± 0.12 bc	0.64 ± 0.05 b	0.66 ± 0.12 cd
2	0	0	0.51 ± 0.05 f	0.47 ± 0.03 e	0.45 ± 0.03 fj
2	1	0	0.81 ± 0.09 b	0.63 ± 0.1 b	0.77 ± 0.14 c
2	2	0	0.78 ± 0.03 bc	0.62 ± 0.08 bc	0.41 ± 0.06 j
0	0	0.1	0.52 ± 0.04 f	0.64 ± 0.05 b	0.48 ± 0.05 fj
0	1	0.1	0.58 ± 0.11 ef	0.57 ± 0.07 cd	0.53 ± 0.06 ef
0	2	0.1	0.59 ± 0.08 def	0.54 ± 0.02 de	0.55 ± 0.08 ef
1	0	0.1	0.63 ± 0.07 cde	0.62 ± 0.04 bc	0.6 ± 0.07 de
1	1	0.1	1.68 ± 0.23 a	1.32 ± 0.34 a	1.25 ± 0.29 a
1	2	0.1	1.48 ± 0.32 a	1.16 ± 0.37 a	0.95 ± 0.13 b
2	0	0.1	0.65 ± 0.07 cde	0.63 ± 0.04 b	0.62 ± 0.06 de
2	1	0.1	0.73 ± 0.06 bcd	0.49 ± 0.08 e	0.63 ± 0.07 cd
2	2	0.1	0.77 ± 0.08 bc	0.59 ± 0.09 cd	0.69 ± 0.09 cd

The values are mean ± SEM. Different letters indicate a statistically significant difference between means at the 5% level,

Modeling

The performance of the three predictive models (MLP, GRNN, and RF) for estimating callus fresh weight (CFW) varied considerably in terms of accuracy and error indices. The fitted models included an optimized multilayer perceptron (MLP), a generalized regression neural network (GRNN), and a random forest (RF) model. Among these fitted models, the MLP provided the most reliable predictions, exhibiting very high coefficients of determination ($R^2 = 0.96$ for training and 0.94 for testing), coupled with the lowest RMSE values (0.12 in training and 0.16 in testing) and minimal mean bias error (MBE = 0.00 and 0.08, respectively), indicating both precision and robustness in generalization (Table 3; Fig. 4).

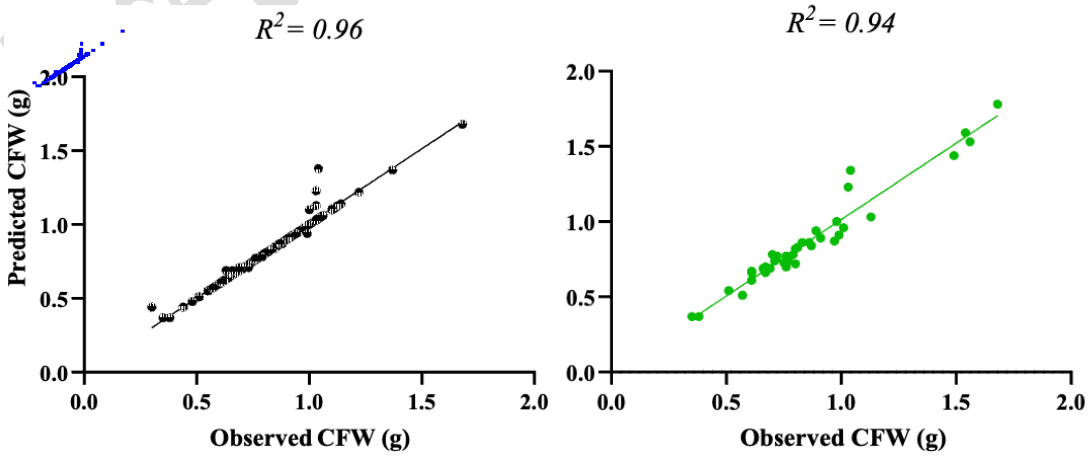

Fig. 4 Observed vs. predicted CFW in callogenesis of *M. chamomilla* using MLP model: Training (blue) and testing (green) results.

Table 3 Performance criteria of generalized regression neural network (GRNN), multilayer perceptron (MLP), and random forest (RF) for callogenesis of *M. chamomilla*.

Model	Processing set	Performance criteria	CFW

MLP	Training	R^2	0.96
		RMSE	0.12
		MBE	0.00
	Testing	R^2	0.94
		RMSE	0.16
		MBE	0.08
GRNN	Training	R^2	0.92
		RMSE	0.36
		MBE	0.02
	Testing	R^2	0.83
		RMSE	1.23
		MBE	0.43
RF	Training	R^2	0.93
		RMSE	0.25
		MBE	0.21
	Testing	R^2	0.90
		RMSE	1.18
		MBE	0.57

R^2: coefficient of determination; MBE: mean bias error; RMSE: root mean square error. CFW: Callus fresh weight.

The generalized regression neural network (GRNN) showed good performance in training (R^2 = 0.92, RMSE = 0.36), but its predictive ability decreased notably in testing (R^2 = 0.83, RMSE = 1.23, MBE = 0.43), reflecting weaker generalization capacity. The random forest (RF) model performed comparably to GRNN, with R^2 = 0.93 (training) and 0.90 (testing), but higher RMSE and MBE values, particularly in testing (RMSE = 1.18, MBE = 0.57), suggesting greater variability and bias in predictions. Overall, the results clearly demonstrate that MLP outperformed both GRNN and RF, making it the most suitable model for accurately simulating and predicting callogenesis in chamomile based on hormone concentrations.

Since the MLP model exhibited the highest predictive accuracy and generalization capacity, it was subsequently integrated with a genetic algorithm (GA) to optimize the concentration of IBA, NAA, and BAP, enabling the identification of the best hormone combination predicted by MLP for achieving the maximum callus fresh weight (CFW).

Optimization

The integration of the MLP model with a genetic algorithm (MLP–GA) successfully identified the optimal concentrations of plant growth regulators for maximizing callogenesis in chamomile cotyledon explants. The optimization results indicated that the best hormonal combination consisted of 0.38 mg/L IBA, 0.29 mg/L NAA, and 0.19 mg/L BAP, which was predicted to yield a maximum callus fresh weight (CFW) of 1.77 g (Table 4). This optimized response was higher than that obtained with any single experimental treatment, confirming the effectiveness of coupling predictive modeling with evolutionary optimization techniques. These findings demonstrate that MLP–GA not only provides accurate simulation of callogenesis but also offers a powerful decision-support tool for fine-tuning PGR levels beyond the limits of tested concentrations to achieve superior callus induction.

Table 4 Optimization of plant growth regulators using genetic algorithm-enhanced MLP to maximize callus fresh weight (CFW).

Data	IBA (mg/l)	NAA (mg/l)	BAP (mg/l)	Predicted CFW (g)
Optimization	0.38	0.29	0.19	1.77

DISCUSSION

The present study demonstrated that callus induction and proliferation in chamomile were highly dependent on the balance between auxins (IBA, NAA) and cytokinin (BAP). In the absence of hormones, no callus was formed, which is consistent with the fundamental role of plant growth regulators (PGRs) in dedifferentiation and callogenesis [7, 8]. Auxin-only treatments supported moderate callus formation, but the addition of low BAP (0.1 mg/L) markedly enhanced callus biomass, indicating a synergistic effect between auxin and cytokinin pathways. Such auxin–cytokinin crosstalk has been well documented, where balanced ratios promote cellular proliferation, while imbalances shift responses toward organogenesis or rooting [17, 18].

Our findings revealed that the combination of IBA 1 mg/L + NAA 1 mg/L + BAP 0.1 mg/L produced the maximum callus fresh weight across all explants, suggesting that a near-equimolar balance of auxins with a low cytokinin level optimally triggers cell proliferation. A similar outcome was reported in chamomile by Sayadi *et al*. [7] and Adibian [9], where the combination of NAA 1 mg/L and kinetin 1 mg/L yielded the highest callus induction percentages and embryogenic callus formation. Ahmad et al. [8] further confirmed that 1 mg/L NAA with kinetin was optimal for callogenesis across several chamomile genotypes, underscoring the general importance of this hormonal ratio for this species.

Interestingly, while BAP generally enhanced callogenesis in our study, a negative interaction was observed at IBA 2 mg/L + NAA 1 mg/L, where the addition of BAP reduced callus biomass. This suggests that supra-optimal auxin levels in combination with cytokinin may

inhibit proliferation, possibly by promoting organogenic signaling over undifferentiated growth [17, 19]. Comparable inhibitory effects of excessive auxin–cytokinin combinations have been observed in *Brassica napus* [20] and *Valeriana jatamansi* [17], where high hormone concentrations resulted in reduced callus growth or abnormal morphogenesis.

Among explants, cotyledons exhibited the highest responsiveness, followed by roots and nodes. The higher callogenic potential of juvenile tissues such as cotyledons is consistent with earlier chamomile studies [8, 9] and in other species such as broccoli [21] and olive [18]. Cotyledons may harbor higher cellular plasticity and endogenous hormone sensitivity, making them suitable explants for callus-based systems. This is further supported by the establishment of efficient transformation and cell suspension cultures from young or meristematic explants in chamomile [10].

The superiority of BAP-supplemented treatments, even at very low concentrations (0.1 mg/L), aligns with reports from both chamomile and other medicinal plants. For instance, Al-Saif *et al.* [22] demonstrated the strong role of BAP in shoot proliferation in pineapple, while Nazir *et al.* [17] reported enhanced shoot and callus induction in Valeriana with combined BAP and NAA. Moreover, Hossain *et al*. [21] highlighted that IBA and BAP combinations were particularly effective for callus proliferation in broccoli, with maximum biomass obtained around 1–1.5 mg/L IBA + 1 mg/L BAP. Taken together, these findings indicate that a balanced auxin–cytokinin ratio not only initiates callus but also sustains vigorous proliferation across species.

In the current study, the best auxin-only combination (IBA 2 + NAA 1) produced substantially less callus than the triple-hormone treatment, highlighting the indispensable role of cytokinin in maximizing proliferation. This agrees with observations in chamomile where NAA alone did not achieve comparable callogenesis without kinetin or BAP supplementation [7, 9]. Furthermore, the effectiveness of low BAP (0.1 mg/L) in eliciting callus, even in the absence of auxin, indicates a basal cytokinin requirement for initiating dedifferentiation, as also noted in olive [18].

The present study highlights the potential of ML–based predictive modeling, particularly the MLP, in capturing the nonlinear and complex interactions among plant growth regulators (PGRs) governing callogenesis in chamomile cotyledon explants. The superior performance of MLP (R^2 = 0.94–0.96; RMSE = 0.12–0.16) compared to GRNN and RF indicates its strong generalization ability and reduced predictive bias, making it a robust decision-support tool for in vitro optimization of CFW. This finding is consistent with recent reports where MLP outperformed other algorithms in modeling plant tissue culture processes, particularly in scenarios where data are highly nonlinear and multidimensional [23, 24].

Interestingly, while GRNN demonstrated strong training performance in this study, it suffered from reduced generalization on unseen data. This pattern contrasts with findings in *Petunia* and *Cannabis sativa*, where GRNN outperformed MLP due to its ability to better interpolate in relatively small datasets [25, 26]. Such discrepancies underscore the fact that algorithmic superiority is highly problem- and dataset-dependent, often dictated by the underlying complexity of biological responses and the distribution of experimental data [27, 28]. The relatively stable performance of RF in our study aligns with its strength in handling high-dimensional data but also with its tendency to overfit when applied to smaller experimental datasets, as noted in *Passiflora caerulea* callogenesis modeling [28].

The integration of MLP with a genetic algorithm (GA) further extended the utility of predictive modeling by identifying an optimal hormone combination (0.38 mg/L IBA, 0.29 mg/L NAA, 0.19 mg/L BAP) predicted to maximize CFW (1.77 g). This hybrid MLP–GA framework mirrors strategies applied across various plant systems, where coupling predictive algorithms with evolutionary optimization reliably identified superior treatment combinations beyond the range tested experimentally [29]. For example, Rezaei *et al.* [25] reported that GRNN–GA optimization significantly improved callogenesis in *Petunia*, while Jafari & Daneshvar [28] showed that GRNN–GA and RF–GA approaches successfully maximized indirect shoot regeneration in *Passiflora caerulea*. Similarly, in *Chrysanthemum*, Hesami et al. [30] found that SVR integrated with NSGA-II provided more precise optimization of somatic embryogenesis than standalone ML models. Collectively, these findings reinforce the strength of hybrid ML–GA pipelines as versatile tools for exploring solution spaces that conventional factorial designs cannot easily cover.

The optimized hormonal balance identified in our study is noteworthy. Auxins (IBA, NAA) and cytokinins (BAP) are well-established as critical regulators of callogenesis, and their synergistic interactions have been shown to influence both callus initiation and subsequent biomass accumulation. Our model-predicted ratio aligns with earlier sensitivity analyses that consistently highlighted the dominance of auxins, particularly IBA, in callus proliferation across multiple species [28]. Comparable optimization outcomes have been observed in *Passiflora*, *Evolvulus*, and *Crocus*, where auxin–cytokinin balance was fine-tuned through ML-guided approaches to achieve higher biomass and regeneration rates [31, 32].

Moreover, the successful demonstration of MLP–GA in chamomile contributes to a growing body of evidence supporting AI-driven strategies for tissue culture optimization. While earlier efforts in tissue culture relied on empirical or response surface methodology (RSM)-based optimization [24], machine learning models provide higher predictive power and adaptability in handling nonlinearity and interaction effects [33]. The advantage of AI-based approaches lies not only in their predictive accuracy but also in their ability to minimize experimental costs and time by reducing the need for exhaustive factorial experiments [34].

The broader implication of this work is that ML–GA hybrid modeling can serve as a reliable decision-support system for advancing precision tissue culture in recalcitrant or economically important species. In the case of chamomile, where hormonal requirements for callogenesis are not well standardized, our results provide a framework for reproducible and efficient callus induction protocols that could accelerate downstream applications such as somatic embryogenesis, genetic transformation, or secondary metabolite production.

The findings demonstrate that MLP–GA not only captures the nonlinear dynamics of PGR interactions but also provides actionable optimization strategies that surpass conventional experimental outcomes. This positions ML–GA hybrid frameworks as powerful and generalizable tools in plant biotechnology, capable of addressing the complexity and unpredictability of in vitro responses across diverse plant taxa. Future studies may extend this approach by integrating additional variables such as light spectra, carbohydrate sources, or explant type, as demonstrated in Cannabis [26], Cotton [32], Banana [35], as well as in *Chrysanthemum indicum* [36], *Crocus sativus* [37], *Cichorium intybus* [38], and *Nigella arvensis* [39] thereby developing holistic predictive systems for plant tissue culture optimization.

CONCLUSION

This study demonstrated that callus induction in chamomile is strongly dependent on auxin–cytokinin balance, with cotyledons proving to be the most responsive explant. Among tested treatments, the combination of IBA 1 mg/L + NAA 1 mg/L + BAP 0.1 mg/L achieved the highest callus biomass, underscoring the importance of near-equimolar auxin ratios with low cytokinin supplementation. Importantly, predictive modeling revealed that the multilayer perceptron (MLP) provided superior accuracy compared to GRNN and RF, making it the most suitable model for simulating callogenesis. Coupling MLP with a genetic algorithm (MLP–GA) enabled precise optimization, identifying an improved hormone combination (0.38 mg/L IBA, 0.29 mg/L NAA, 0.19 mg/L BAP) predicted to yield the maximum fresh weight (1.77 g). These findings highlight the power of ML–GA hybrid approaches to extend beyond conventional experimentation, offering reproducible and efficient strategies for tissue culture optimization and potential downstream applications in chamomile biotechnology.

Consent for Publication

Not Applicable.

Data Availability

Not Applicable.

Conflict of Interests

The authors have not declared any conflict of interests.

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Authors' contributions

RG: Conceptualization, Methodology, writing—original draft preparation; BMZ: Conceptualization, investigation. The authors contributed intellectually to the study, read, and approved the final manuscript.

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