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Non-destructive detection and quantification of embryo presence in *Acer monspessulanum* seeds using neural networks

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Abstract: This study aimed to develop a non-destructive method for predicting embryo presence and quantifying embryo biomass in *Acer monspessulanum* seeds using morphological and physical traits, combined with machine learning and statistical modeling approaches. Seeds were divided into two groups based on the presence or absence of embryos, and 26 morphological and physical traits were measured. Welch's t-tests were used to identify traits significantly different between seeds with and without embryos. A feedforward neural network classifier was trained on these traits to predict embryo presence. Additionally, forward stepwise regression models were constructed to identify key predictors of embryo fresh weight, dry weight, and water content in embryo-containing seeds. Statistically significant differences ($p<0.05$) were observed between embryo-containing and empty seeds in traits such as seed weight, length, roundness, Hue, and floating behavior. The neural network classifier achieved 91.03% accuracy, with strong precision (0.92) and recall (0.94) for identifying embryo-containing seeds. Regression models explained up to 56% of the variation in embryo dry weight and 50% in fresh weight, with seed weight, color parameters, and floatation traits emerging as primary predictors. Embryo water content was primarily predicted by the b* seed coat color parameter (31.6% variance), with additional contributions from buoyancy, perimeter, and post- scarification color traits, yielding a model explaining 45.5% of total variation. The integration of seed phenotyping with neural networks and multiple linear analysis provides a robust, non-destructive method for classifying embryo-containing seeds and predicting embryo development in *Acer monspessulanum*. This approach offers valuable applications in reforestation, seed banking, and ecological restoration initiatives.

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1. Introduction

The genus *Acer* includes numerous woody species distributed across temperate regions of the Northern Hemisphere, many of which are valued for their ecological roles, aesthetic features, and adaptability to diverse environments (van Gelderen *et al.*, 1994). Among them, *Acer monspessulanum* L. (Montpellier maple) is a small to medium-sized, stress-tolerant deciduous tree native to Southern Europe and Western Asia, including parts of Iran. Its compact crown, deeply lobed ornamental leaves, and distinct double samaras contribute to its visual appeal in natural and designed landscapes. In addition, *A. monspessulanum* exhibits high resilience to drought and poor soils (Teimouri *et al*., 2014), making it a suitable candidate for urban greening, reforestation, and restoration projects in arid and semiarid regions.

Despite its promising potential, propagation of *A. monspessulanum* from seed remains problematic due to a combination of physiological dormancy and seed quality heterogeneity. This species often exhibits double dormancy, which includes both an underdeveloped embryo and an inhibitory seed coat, requiring specific pre-treatments to overcome (Baskin and Baskin, 2014). Compounding this issue, a significant proportion of seeds collected from wild populations are often empty, lacking fully developed embryos. These empty seeds cannot be identified visually without destruction, which limits the efficiency of propagation programs.

Importantly, the proportion of empty seeds varies widely between years and populations and is strongly influenced by climatic and nutritional conditions during flowering, pollination, and seed development in the parent tree. Factors such as drought stress, high temperatures during flowering, and poor soil fertility can impair embryo formation, seed filling, and increase the number of non-viable or empty seeds (Fenner, 1992; Wulff, 1995; Gutterman, 2000; Adams, 2014; Mácová *et al.,* 2022; Dadlani and Yadava, 2023). These challenges underline the need for accurate, non-destructive methods to evaluate seed viability before germination trials or nursery sowing (Xu *et al*., 2024).

While classical methods such as X-ray radiography and tetrazolium (TTC) staining provide reliable embryo viability assessment (Ahmed *et al.,* 2018), they are either destructive, costly, or time-consuming. Building upon RGB-based software tools for external phenotyping (e.g., SmartGrain by Tanabata *et al.,* 2012; SeedExtractor by Zhu *et al.,* 2021), X-ray imaging methods (Gagliardi and Marcos-Filho, 2011; Gomes-Junior *et al.,* 2012), and infrared imaging (Agelet *et al.*, 2012) with chemometrics (Wakholi *et al.*, 2018) offer a complementary perspective by revealing internal seed structure and germination capacity. While advanced modalities like X-ray and NMR provide superior insight into internal anatomy, RGB imaging remains the more practical and accessible choice for high-throughput phenotyping due to its low cost, operational simplicity, and rapid data capture. This accessibility makes it ideally suited for large-scale studies focused on external morphological traits (Halcro *et al.*, 2020). Consequently, recent advances in digital phenotyping and artificial intelligence (AI) offer alternative approaches to evaluate internal seed quality based on external visual traits (Nguyen *et al.,* 2015; De Medeiros *et al.,* 2020 a, b). The application of machine learning techniques, such as Artificial Neural Networks (ANN), Random Forest (RF), Support Vector Machines (SVM), and k-means clustering, to agricultural problems is now widespread (Rehman *et al.,* 2019; Saha and Manickavasagan, 2021). By extracting features such as RGB color intensity, projected area, and seed shape from digital images, and combining them with machine learning tools like artificial neural networks (ANNs), researchers have successfully predicted seed viability in various species (Fraas *et al.,* 2014; Rousseau *et al.,* 2015; De Medeiros *et al.,* 2020 a, b).

In this study, we developed and tested a non-destructive method for detecting empty seeds in *Acer monspessulanum* using image-based features and a neural network classification model. Our aim was to determine whether morphological and colorimetric traits derived from 2D images could be used to predict the presence or absence of embryos with high reliability. This approach may enhance seed quality screening in undomesticated tree species with complex dormancy and contribute to the propagation and conservation of drought-tolerant ornamental species.

2. Materials and Methods

Seed collection and preparation

Mature seeds of *Acer monspessulanum* were obtained from the Natural Resources and Watershed Management Organization of North Khorasan

Province, Iran. The seeds were collected from naturally growing trees in their native habitat. A total of 200 seeds were randomly selected, manually dewinged, and labeled sequentially from 1 to 100 in duplicate.

Acid scarification and soaking

To facilitate seed coat removal and improve embryo extraction, seeds were immersed in 65% nitric acid (HNO_3) for 6 hours at room temperature. Following acid treatment, seeds were rinsed three times with distilled water (15 minutes per rinse) to remove residual acid. The seeds were then soaked in distilled water at 24±1°C on a rotary shaker. Floating and non-floating seeds were recorded after 24 and 48 hours of soaking. Embryos were extracted manually after 48 hours of soaking.

Embryo extraction and viability testing

Although nitric acid treatment improved seed coat removal, the viability of embryos were lost during extraction. Attempts to assess embryo viability using 2,3,5-triphenyltetrazolium chloride (TTC) staining failed during the main experiment. However, a separate viability test showed that 70% of the seeds contained embryos, while the remaining 30% were empty. The 200 seeds used in the main experiment followed approximately the same proportions of embryo-containing versus empty seeds, ensuring that the dataset accurately reflected embryo presence in the population.

Image-based morphological data collection

Before and after seed treatment and after embryo extraction, all 200 seeds and their respective embryos were placed on gridded paper and photographed under standardized lighting conditions (Fig. 1). Quantitative image-based traits of seeds and embryos - including projected area, perimeter, length, width, roundness, intensity, Hue, and RGB color intensity values (red, green, and blue channels) - were extracted using Digimizer software. RGB color intensity values were converted to CIELAB color space parameters (L*, a*, and b*) using Python (v 3.11) and the skimage library, after normalization of RGB values to the 0-1 range.

Embryo weight measurements

The fresh weight of the embryos was measured immediately after extraction. To determine dry

Fig. 1 - Seeds of *Acer monspessulanum* prior to treatment (left), following acid scarification and 48-hour water soaking (middle), and the corresponding extracted embryos (right).

weight, embryos were placed in a drying oven at 70°C for 48 hours, then weighed using a precision analytical balance.

Data analysis

Statistical analysis of seed traits based on embryo presence. To investigate relationships between the recorded traits, data were analyzed by grouping seeds according to embryo presence. Before selecting the t-test (Welch's t-test), we examined the homogeneity of variances. Statistical comparisons were performed in R using a two-sample Welch's t-test due to significant unequal variances in some traits and unequal sample sizes, with significance assessed at $p<0.05$. In addition, Fisher's Exact Test was used for the Seed Floating24 and Seed Floating48 traits because of their binary distribution.

Artificial neural network (ANN). Following the initial statistical analyses, the easily observable and well-defined traits that showed significant differences with in the t-test were selected for further modeling. To investigate feed-forward neural network potential of these traits in predicting embryo presence, a feedforward ANN model was developed using Python 3.11 with Keras (TensorFlow backend) libraries. The input layer consisted of seed length, seed roundness, seed weight, and seed floating after 24 and 48 hours, and the output layer was binary (1= embryo present, 0= empty seed). The dataset was randomly split into training (60%) and testing (40%) subsets. Prior to model training, all features were standardized to zero mean and unit variance using StandardScaler in order to improve convergence during neural network optimization. The architecture consisted of: An input layer with dimensionality equal to the number of features in X. A first hidden layer of 96 neurons with ReLU activation. A second hidden layer of 128 neurons with ReLU activation. A final output layer with a single neuron and sigmoid activation for binary classification. Alternative hyperparameter configurations were explored with Keras Tuner (Random Search), but the final reported model used fixed parameters based on preliminary results. The network was compiled using the Adam optimizer, with binary cross-entropy as the loss function and accuracy as the evaluation metric. Training was performed for 10 epochs with a batch size of 32, using 40% of the training data as an internal validation set. Model performance was assessed on both training and test sets. Metrics included: Accuracy, Confusion matrix AND Precision, recall, and F1-score (via classification_report). In addition, loss curves (training *vs* validation) were plotted across epochs using Matplotlib to monitor convergence.

Multiple linear regression. At the final stage, for seeds confirmed to contain an embryo, embryo fresh weight, embryo dry weight, and embryo water content were used as quantitative indices of embryo vigor. It should be noted that these indices provide information on embryo development but, as indirect measures, are most informative when interpreted together with physiological or performance-based vigor traits, such as germination rate, seedling growth, or TTC staining results. The processes related to dormancy breaking were not studied here, as the current work focuses on non-destructive embryo detection and phenotypic trait analysis. To evaluate how these vigor indices were explained by seed phenotypic features, multiple linear regression with stepwise (forward) selection was applied, using image-based traits and seed weight as explanatory variables (entry criterion: $p<0.05$; removal criterion: $p>0.10$). Statistical significance was accepted at $p\leq 0.05$. All analyses were performed using SAS (version 9.4) software.

3. Results

Trait differences by embryo presence

To assess whether specific morphological and physical traits are related to embryo presence, seeds were divided into two groups: those containing an embryo and those without. A two-sample t-test assuming unequal variances (Welch's t-test) was performed to compare the mean values of the measured traits between the two groups (Table 1).

The analysis revealed statistically significant differences ($p<0.05$) in several traits, including perimeter (before scarification), color intensity (before scarification), length (before scarification), roundness (before scarification), Hue (after scarification), seed weight, and seed floating at 24 h and 48 h. Specifically, seeds containing embryos were significantly longer, rounder, heavier, and exhibited distinct Hue and intensity values, indicating morphological differentiation linked to embryo present. In addition, a significantly lower proportion of embryo-containing seeds remained afloat after 24 h and 48 h of soaking, indicating lower buoyancy and higher density, traits commonly of embryo-

Table 1 - Comparison of seed traits of *Acer monspessulanum* based on embryo presence using two-sample t-test (Unequal Variances)

Time	Seed Traits	With embryo		Without embryo		T-test
		Mean	SE	Mean	SE	(p-Value)
Before acid scarification	Seed weight	48.475	10.361	32.716	11.844	0
	Area	0.24	0.048	0.251	0.057	0.2
	Perimeter	1.782	0.172	1.845	0.208	0.047
	Intensity	0.24	0.094	0.273	0.107	0.046
	L*	27.432	11.276	31.239	12.471	0.051
	a*	14.55	6.362	15.883	6.356	0.189
	b*	2.728	2.759	3.159	3.135	0.371
	Hue	37.248	6.913	36.85	6.625	0.708
	Length	0.668	0.073	0.713	0.089	0.001
	Width	0.455	0.061	0.445	0.065	0.331
	Roundness	0.94	0.041	0.916	0.046	0.001
After acid scarification	Seed Floating24	0.362	0.482	1	0	0
	Seed Floating48	0.188	0.392	0.946	0.227	0
	Area	0.347	0.049	0.356	0.064	0.324
	Perimeter	2.113	0.153	2.144	0.2	0.298
	Intensity	0.275	0.055	0.271	0.055	0.584
	L*	33.596	6.746	32.916	6.914	0.533
	a*	34.418	5.466	33.9	6.126	0.584
	b*	24.992	3.038	26.03	4.245	0.1
	Length	0.754	0.074	0.773	0.089	0.162
	Width	0.584	0.051	0.584	0.064	0.955
	Hue	21.231	2.711	20.027	2.924	0.009
	Roundness	0.971	0.025	0.966	0.027	0.186

containing seeds. Conversely, no significant differences were detected in traits such as projected area, width, and several color parameters (e.g., b values), indicating that these characteristics may be less influenced by embryo presence. A marginally significant difference was observed in L* (before scarification; *p = 0.051*), suggesting a possible trend that may warrant further investigation. These findings demonstrate that a combination of morphological and physical parameters can serve as useful non-destructive indicators for identifying embryo-containing seeds of *Acer monspessulanum*.

Machine learning classification

In addition to statistical comparisons, a supervised machine learning approach was applied to classify *Acer monspessulanum* seeds based on embryo presence, using the same morphological and physical traits analyzed in the Welch's t-test and Fisher's Exact Test. A fully connected feedforward neural network was implemented using a sequential architecture composed of three dense layers: an input layer with 96 units, a hidden layer with 128 units, and an output layer with a single neuron for binary classification. The network architecture was selected based on iterative testing to maximize prediction accuracy while minimizing over fitting (Eryigit and Tugrul, 2021). The model included 13,121 trainable parameters.

The model achieved high predictive performance. On the test dataset, the model yielded an accuracy of 91.03% and a mean absolute error (MAE) of 0.91, with a minimum and maximum target value of 0.0 and 1.0, respectively. Training accuracy reached 96.55%, indicating strong learning capacity with minimal overfitting. Evaluation using a confusion matrix showed robust classification results, with 24 true negatives, 47 true positives, 4 false positives, and only 3 false negatives. The overall precision, recall, and F1-score were 0.91, 0.90, and 0.90, respectively, indicating balanced performance across both classes. Notably, seeds with embryos were predicted with high precision (0.92) and recall (0.94), reinforcing the model's reliability in detecting embryo presence (Fig. 2).

These results confirm that the integration of morphological and physical seed traits - particularly those identified as statistically significant in the t-test analysis - can effectively support machine learning-based classification of embryo-containing seed. The deep learning model provides a promising non-destructive tool for embryo detection in *Acer monspessulanum* seeds.

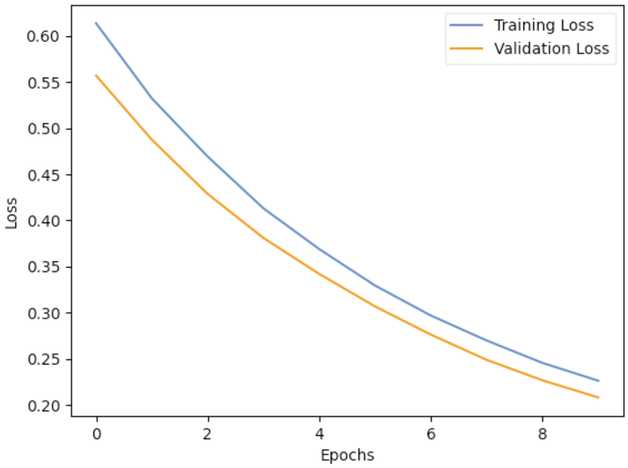

Fig. 2 - Training and validation loss curve of the Neural Network Model for embryo classification in *Acer monspessulanum* seeds.

Multiple linear regression models for predicting embryo biomass and water content

Stepwise forward multiple linear regression was conducted separately for embryo fresh weight, dry weight, and water content using only seeds confirmed to contain embryos, to identify the most predictive traits.

The predictive models showed moderate to strong agreement between observed and estimated embryo traits in *Acer monspessulanum* seeds (Fig. 3). In all three cases, data points (open blue circles) cluster around the 1:1 reference line (grey diagonal), indicating good agreement between predicted and measured values. For embryo fresh weight (left panel), the majority of values are concentrated between 10-25 mg, with only a few deviations above 30 mg. embryo dry weight (middle panel) exhibits a similar pattern, with predictions tightly grouped between 5-15 mg, though some scatter is evident at higher values. Embryo water content (right panel) shows a broader range (20-70%), with predictions capturing the general trend but with greater dispersion compared to embryo fresh weight and embryo dry weight. Overall, the models demonstrate strong predictive accuracy, particularly for embryo fresh weight and embryo dry weight, whereas embryo water content shows slightly higher

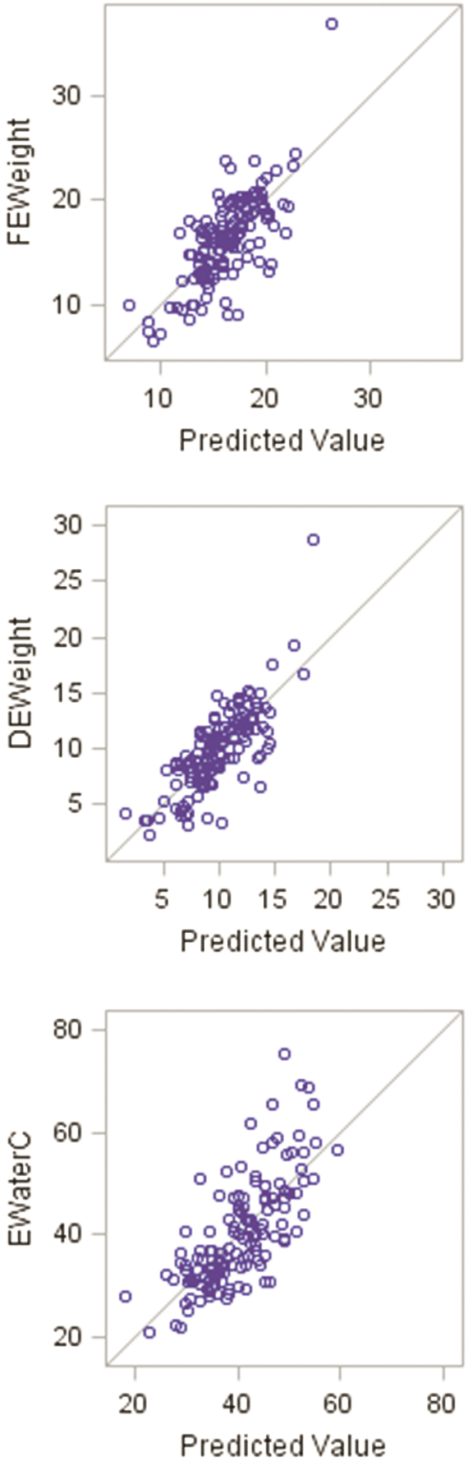

Fig. 3 - Relationship between observed and predicted values of embryo fresh weight (left, FEWeight), embryo dry weight (middle, DEWeight), and embryo water content (right, EWaterC) in *Acer monspessulanum* seeds. Each point represents and individual seed measurement, while the solid line denotes the 1:1 relationship.

variability. These results highlight the reliability of the predictive approach in estimating embryo traits critical for seed physiology studies.

Stepwise regression model for predicting embryo fresh weight

To identify the most influential predictors of embryo fresh weight, a forward stepwise selection procedure was applied using a multiple linear regression model. The results are summarized in Table 2. The model successively introduced variables that contributed the most to explaining variation in embryo fresh weight among seeds with embryos, based on partial R^2, F-value, and Mallows' C(p) criterion.

Seed weight was identified as the most significant predictor of embryo fresh weight, explaining 27.5% of the variation in the model (Partial $R^2 = 0.2752$). The inclusion of seed weight alone produced a strong and highly significant model ($F = 51.65$, $p < 0.0001$), indicating its primary role in determining embryo fresh weight in *Acer monspessulanum* seeds. The second variable included was the a* color parameter measured before acid scarification, which significantly improved the model by explaining an additional 13.5% of the variance (Partial $R^2 = 0.1349$). With this addition, the model R^2 increased to 0.4102 ($F = 30.88$, $p < 0.0001$). The intensity parameter, also measured before acid scarification, further enhanced the model fit, contributing an additional 6.9% to the explained variance (Partial $R^2 = 0.0690$), resulting in a cumulative R^2 of 0.4792 ($F = 17.75$, $p < 0.0001$). Seed floating after 48 hours of water immersion (Seed Floating48) was added at the fourth step and explained an additional 1.9% of the variance in embryo fresh weight (Partial $R^2 = 0.0189$), bringing the model R^2 close to 50% (0.4981). This variable showed a significant but smaller effect compared to the earlier entered predictors ($F = 5.01$, $p = 0.0269$). Subsequent variables, including intensity and a* color after acid scarification, width, roundness, Hue, and b* color parameter, contributed minimally to the model (each partial $R^2 < 0.004$), with none reaching statistical significance (all $p > 0.05$). These variables produced negligible improvements in the overall explained variance, indicating that the first four variables sufficiently capture the major variation in embryo fresh weight.

Stepwise regression model for predicting embryo dry weight

A forward stepwise regression analysis was conducted to identify the most influential predictors of embryo dry weight in *Acer monspessulanum*. Variables were sequentially added to the model based on their contribution to explained variance and statistical significance, as indicated by changes in partial R^2, model R^2, Mallows' C(p), and F-tests (Table 3).

Seed weight was the strongest single predictor of embryo dry weight, explaining 20.8% of the variance on its own (Partial $R^2 = 0.2081$). This variable produced a highly significant model ($F = 35.74$, $p < 0.0001$), underscoring its importance in determining embryo dry weight in *Acer monspessulanum*. The b* color parameter measured before acid scarification was the second variable selected and contributed an additional 22.3% to the explained variance (Partial $R^2 = 0.2225$). This substantially increased the model fit to an R^2 of 0.4306 ($F = 52.76$, $p < 0.0001$), highlighting the relevance of seed coat coloration in predicting dry weight. Adding the a* color parameter before acid scarification explained a further 4.1% of the

Table 2 - Summary of forward selection for predicting embryo fresh weight in *Acer monspessulanum*

Step	Variable entered	Number Vars In	Partial R^2	Model R^2	C(p)	F Value	Pr > F
1	Seed Weight	1	0.2752	0.2752	456.687	51.65	<.0001
1	a* (Before acid scarification)	2	0.1349	0.4102	142.181	30.88	<.0001
2	Intensity (Before acid scarification)	3	0.069	0.4792	-0.8864	17.75	<.0001
3	Seed Floating48	4	0.0189	0.4981	-35.691	5.01	0.0269
4	Intensity (After acid scarification)	5	0.0074	0.5055	-34.145	1.99	0.161
5	Width (Before acid scarification)	6	0.0037	0.5092	-2.342	1	0.3195
6	a* (After acid scarification)	7	0.0039	0.5131	-13.016	1.03	0.3112
7	Roundness (After acid scarifica-	8	0.0024	0.5156	0.0916	0.65	0.421
8	Hue (Before acid scarification)	9	0.0022	0.5178	15.458	0.58	0.446
9	b* (Before acid scarification)	10	0.0039	0.5216	2.587	1.03	0.3128

Table 3 - Summary of forward selection for predicting embryo dry weight in *Acer monspessulanum*

Step	Variable Entered	Number Vars In	Partial R^2	Model R^2	C(p)	F Value	Pr > F
1	Seed Weight	1	0.2081	0.2081	110.12	35.74	<.0001
2	b* (Before acid scarification)	2	0.2225	0.4306	43.52	52.76	<.0001
3	a* (Before acid scarification)	3	0.0406	0.4712	33.01	10.29	0.0017
4	Intensity (Before acid scarification)	4	0.0815	0.5527	9.9	24.22	<.0001
5	Seed Floating24	5	0.0197	0.5723	5.84	6.07	0.0151
6	Intensity (After acid scarification)	6	0.0102	0.5826	4.69	3.21	0.0755
7	a* (After acid scarification)	7	0.0066	0.5891	4.66	2.08	0.1512
8	Area (Before acid scarification)	8	0.0056	0.5947	4.94	1.77	0.186
9	Roundness (After acid scarifica-	9	0.0039	0.5986	5.75	1.24	0.268
10	Hue (Before acid scarification)	10	0.0023	0.6009	7.03	0.74	0.3911

variance (Partial $R^2 = 0.0406$), leading to a cumulative R^2 of 0.4712. This variable was also statistically significant ($F = 10.29$, $p = 0.0017$), indicating its complementary role alongside b* in characterizing seed coat traits linked to embryo dry weight. The intensity parameter measured before acid scarification added 8.1% to the model's explanatory power (Partial $R^2 = 0.0815$), bringing the total R^2 to 0.5527 ($F = 24.22$, $p < 0.0001$). This suggests that brightness or darkness of the seed coat further refines predictions of embryo dry weight. Seed buoyancy after 24 hours of immersion (Seed Floating24) contributed an additional 2.0% to the model (Partial $R^2 = 0.0197$), with the overall model R^2 reaching 0.5723 ($F = 6.07$, $p = 0.0151$). This variable showed a modest but statistically significant effect on embryo dry weight. Subsequent variables including intensity and a* after acid scarification, seed area, roundness after acid scarification, and Hue before acid scarification provided minor increments in explained variance (each partial $R^2 < 0.011$), none of which were statistically significant ($p > 0.05$), except for intensity after acid scarification, which approached marginal significance (*p = 0.0755*). This indicates that the major variation in embryo dry weight is explained predominantly by the first five variables included in the model.

Stepwise regression model for predicting embryo water content

To identify key variables influencing embryo water content in *Acer monspessulanum*, a forward stepwise regression analysis was performed. Variables were introduced into the model sequentially based on their individual contributions to explained variance, assessed through partial and cumulative R^2, Mallows' C(p), F-values, and associated p-values (Table 4).

The b* color parameter measured before acid scarification was the strongest predictor of embryo water content, explaining 31.6% of the variance (Partial $R^2 = 0.3159$). This variable alone resulted in a highly significant model ($F= 62.79$, $p<0.0001$), demonstrating the importance of seed coat color characteristics in determining embryo hydration levels. Seed buoyancy after 24 hours (Seed Floating24) contributed an additional 6.1% of explained variance (Partial $R^2 = 0.0606$), increasing the total model R^2 to 0.3765 ($F= 13.12$, $p= 0.0004$). This highlights seed floating capacity as a meaningful indicator of embryo water content. Perimeter measured before acid scarification added 3.7% to the explained variance (Partial $R^2 = 0.0370$), resulting in a model R^2 of 0.4134 ($F= 8.45$, $p= 0.0043$). This suggests seed size and shape features contribute to variations in embryo water content. Intensity after acid scarification increased the model fit by 1.4% (Partial $R^2 = 0.0138$), with a marginal significance ($F= 3.20$, $p= 0.0758$). Although this effect was weak, it indicates some influence of seed coat brightness after treatment on embryo hydration. The a* color parameter after acid scarification further explained 2.8% of variance (Partial $R^2 = 0.0280$), significantly improving the model to an R^2 of 0.4552 ($F= 6.78$, $p= 0.0103$). This highlights the role of seed color changes post-treatment in relation to embryo water content. Additional variables including seed weight, Hue before acid scarification, intensity before acid scarification, a* before acid scarification, L before acid scarification, area before acid scarification, and seed floating after 48 hours contributed small

Table 4 - Summary of forward selection for predicting embryo water content in *Acer monspessulanum*

Step	Variable entered	Number Vars In	Partial R^2	Model R^2	C(p)	F Value	Pr > F
1	b* (Before acid scarification)	1	0.3159	0.3159	379.098	62.79	<.0001
2	Seed Floating24	2	0.0606	0.3765	246.838	13.12	0.0004
3	Perimeter (Before acid scarifica-	3	0.037	0.4134	173.896	8.45	0.0043
4	Intensity (After acid scarification)	4	0.0138	0.4272	159.229	3.2	0.0758
5	a* (After acid scarification)	5	0.028	0.4552	108.876	6.78	0.0103
6	Seed Weight	6	0.0081	0.4634	108.429	1.99	0.1611
7	Hue (Before acid scarification)	7	0.0086	0.472	106.757	2.12	0.1475
8	Intensity (Before acid scarification)	8	0.01	0.482	101.634	2.49	0.117
9	a* (Before acid scarification)	9	0.0275	0.5095	52.557	7.17	0.0084
10	L (Before acid scarification)	10	0.0023	0.5118	66.874	0.59	0.4445
11	Area (Before acid scarification)	11	0.0029	0.5147	7.949	0.76	0.3841
12	Seed Floating48	12	0.0034	0.5181	90.934	0.88	0.3491

increments (each partial R^2 <0.028), with varying degrees of significance. Notably, a* before acid scarification was significant (*p= 0.0084*), whereas seed weight and several others were not statistically significant (*p>0.10*). These findings suggest that most variation in embryo water content is captured by the first five to nine variables entered into the model.

4. Discussion and Conclusions

This study demonstrates that a combination of morphological and physical traits can effectively distinguish embryo-containing seeds from empty ones in *Acer monspessulanum*. Using a two-sample t-test, several traits were found to be significant distinguishing traits for the presence of an embryo, including seed weight, length, roundness, Hue values, and soaking behavior (floating after 24 and 48 hours). This aligns with established seed physiology: filled seeds often exhibit greater tissue density, developmental maturity, and compositional differences compared to empty seeds (Bewley *et al.,* 2013), reflecting fundamental physiological distinctions.

Among the most discriminative features, seed weight emerged as a key indicator of embryo presence. This is consistent with studies demonstrating that seeds are significantly heavier than non-viable ones and are more likely to contain well-developed embryos (Daneshvar *et al.,* 2017; Domic *et al.,* 2020). Size-related traits are strongly positively correlated with seed weight, indicating that physical dimensions have a greater influence on weight than shape does (Duc *et al.,* 2023). Additionally, seed buoyancy, assessed through floating tests, proved to be a strong physiological marker. Techniques like incubation-drying-separation (IDS) and modified specific gravity (MSG) separation are widely used to remove empty seeds. These methods exploit the principle that viable seeds absorb and metabolically bind more water during soaking than dead seeds. This differential water uptake allows for highly effective sorting based on the viscosity of the flotation medium (Daneshvar *et al.,* 2017). Embryos are more prevalent in sunken seeds due to their higher density and structural integrity, both strong indicators of viability (Sautu *et al.,* 2006). The significant role of tissue density metrics as predictors in seed quality classification establishes a direct correlation between structural integrity and viability. This principle is operationalized in flotation tests, where the higher density of viable seeds causes them to consistently sediment (Daneshvar *et al.,* 2017). Consistent with research on hardwoods, flotation behavior serves as a standard proxy for seed viability (Schmidt, 2000; Bonner, 2008). This method's efficacy is confirmed in *Juniperus polycarpos*, where the sunken fraction contains a significantly higher proportion of viable seeds, solidifying its role as a reliable preliminary sorting technique. (Daneshvar *et al.,* 2017).

Significant associations were identified between embryo presence and colorimetric variables, specifically Hue (post-scarification) and color intensity (pre-scarification). These optical traits may reflect underlying biochemical changes in the seed coat during embryogenesis, such as lignification and the accumulation of pigments (e.g., phenolic compounds), which alter light interaction. Consequently, spectral imaging emerges as a powerful tool for non-destructively quantifying these chemical compositions. The distinct spectral signatures of viable seeds highlight the potential of spectral and hyperspectral imaging and digital color analysis as rapid, non-destructive methods for assessing seed viability (Boelt *et al.,* 2018; ElMasry *et al.,* 2019; Wang *et al.,* 2022; Fan *et al.,* 2023).

Building upon our robust statistical findings, we implemented a neural network (NN) classifier to predict embryo presence, which achieved a high overall accuracy of 91.03%. The model demonstrated balanced performance, excelling particularly in the identification of embryo-containing seeds, as evidenced by strong precision (0.92) and recall (0.94) scores. This high predictive power indicates that the integration of key morphological and physical traits into a machine learning (ML) framework is a practical strategy for developing rapid, automated, and non-invasive seed sorting systems. Our results corroborate a growing body of research demonstrating the efficacy of artificial intelligence (AI) for seed quality assessment, with successful applications already established for plant species. The consistently high performance of various architectures - from CNN (92.06%; Xu *et al.,* 2024), 3DCNN (92.00%; Fan *et al.,* 2023), and LDA (95.8%; Jeong *et al.,* 2024) to exceptionally accurate 2DCNN models (99.96%; Fan *et al.,* 2023) across studies on maize and soybean - demonstrates the robustness of this approach. Notably, Artificial Neural Networks (ANNs) are repeatedly validated as a particularly effective algorithm for deciphering the complex spectral patterns indicative of seed physiological quality (De Medeiros *et al.,* 2020 a; Xu *et al.,* 2024; Jeong *et al.,* 2024). This convergence of evidence from independent research groups confirms that the integration of HSI with machine learning is a transformative methodology for rapid and precise seed classification.

The regression analyses for embryo biomass components further validated the predictive value of specific traits. Seed weight consistently emerged as the strongest predictor across models for both embryo fresh and dry weights, underscoring its fundamental role in determining embryo biomass in *Acer monspessulanum*. This finding aligns with the well-established link between seed size and embryo development, where heavier seeds tend to harbor more substantial embryo reserves, contributing to seedling vigor and establishment potential.

For embryo fresh weight, seed weight alone explained 27.5% of the observed variation, with the model's explanatory power substantially improved by the inclusion of seed coat color traits, notably the a* parameter measured before acid scarification. The positive contribution of color parameters (a* and intensity) suggests that seed coat pigmentation and brightness are closely related to embryo development. These traits may reflect underlying biochemical or structural properties of the seed coat that influence nutrient allocation or protection of the embryo. Furthermore, seed buoyancy after 48 hours (Seed Floating48) contributed a smaller yet significant additional variance, potentially indicating seed coat permeability or structural integrity as factors modulating embryo fresh weight. Similarly, in predicting embryo dry weight, seed weight remained the dominant predictor, accounting for 20.8% of variance.

The b* color parameter, indicative of seed coat color along the yellow-blue spectrum, added substantial predictive power, raising the model R^2 to over 43%. This highlights the complementary role of seed coat color characteristics in predicting embryo dry mass, potentially reflecting biochemical composition linked to desiccation tolerance or storage compound accumulation. Other seed coat traits such as a* and intensity before acid scarification further improved the model, with seed buoyancy after 24 hours also contributing significantly, again pointing to physical seed coat properties affecting embryo biomass accumulation. In contrast to embryo biomass, embryo water content was most strongly predicted by the b* color parameter before acid scarification, explaining over 31% of variance, suggesting that seed coat coloration plays a crucial role in regulating embryo hydration. Seed buoyancy after 24 hours also contributed notably, supporting the hypothesis that seed coat permeability or water absorption capacity affects embryo water status. The influence of seed perimeter further implicates seed size and shape as factors in water retention. While seed weight was less predictive of water content, color parameters both before and after acid scarification remained significant, indicating that dynamic changes in seed coat coloration and properties during acid treatment may reflect or influence water relations within the embryo.

Overall, the forward stepwise regression approach demonstrated that a relatively small set of seed traits - primarily seed weight, seed coat color parameters (a* and b*), seed coat brightness (intensity), and buoyancy measures - capture most of the variation in embryo fresh weight, dry weight, and water content. These findings emphasize the multifaceted role of seed morphological and physical traits in embryo development and hydration, which are critical for understanding seed quality and potential germination success in *Acer monspessulanum*. Future studies should investigate the mechanistic basis of these correlations, particularly the biochemical and structural seed coat changes linked to color variation and buoyancy, to better elucidate their impact on embryo growth and water regulation.

Collectively, these findings contribute to the growing body of literature advocating for non-destructive, image-based, and AI-driven methods in seed technology. While traditional x-ray or dissection methods remain reliable, they are labor-intensive and not scalable for large-scale screening in forestry or conservation programs (ISTA, 2020). By contrast, the approach demonstrated here offers a cost-effective and high-throughput alternative for selecting embryo-containing *Acer monspessulanum* seeds, with potential applications in other hard-seeded or recalcitrant species.

The non-destructive imaging-based approach combined with neural network analysis developed in this study is not limited to *Acer monspessulanum*. Given its reliance on general morphological and physical traits, this method is scalable and potentially transferable to seeds of other species, offering a cost-effective tool for seed quality assessment, reforestation programs, and conservation initiatives. Future studies could apply this framework to a wider range of species to evaluate its broader applicability and optimize species-specific prediction models.

This study demonstrates that a combination of morphological and physical seed traits can serve as reliable, non-destructive indicators of embryo presence and developmental status in *Acer monspessulanum*. Key traits such as seed weight, floating behavior, and colorimetric indices were significantly predictive of embryo viability and biomass. The successful application of a neural network classifier further highlights the potential of machine learning tools to accurately and efficiently differentiate embryo-containing from empty seeds based on external features. Additionally, multiple linear regression models provided insight into the predictive relationships between seed traits and embryo biomass components, enabling the estimation of internal seed quality without destructive testing. These findings support the development of scalable, cost-effective screening methods for assessing embryo presence in seeds in forestry, ecological restoration, and conservation programs, with potential applicability across a wide range of species.

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