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THE INFLUENCE OF SEED PRE-TREATMENTS AND SUBSTRATE TYPES ON SEEDLING EMERGENCE OF ACER PALMATUM 'ATROPURPUREUM'

SUMMARY

Acer palmatum Thunb. is an ornamental shrub native to Japan, Korea, and China, widely cultivated in green areas due to its diverse leaf shapes and colors. This study was conducted in the greenhouse at the Didactic and Research Base of "King Mihai I" University of Life Sciences from Timișoara, Romania. The main objective of this paper was to evaluate seedling emergence and growth performance of *Acer palmatum* 'Atropurpureum' under the influence of two pre-germination treatments (90-day cold stratification and hydrothermal treatment by 50 °C water immersion for 24 hours) and three commercial substrates were tested: TS 3 peat, TS 4 medium peat combined with Greenfibre, and Pro Line Tray Substrate peat. Biometrical analyses were performed, there being characterized the influence of the analysed factors solely and combined on seedling height, girth diameter, root number, main root length, number of leaves, and leaf surface area. The stratified seeds grown in Pro Line Tray Substrate peat exhibited the highest values and the most favorable results for all biometrical parameters measured.

Keywords: Japanese maple, seedling, seed pre-treatment, substrate, ornamental shrub

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INTRODUCTION

Acer palmatum Thunb., commonly known as Japanese maple, is a deciduous ornamental species native to East Asia, particularly Japan, Korea, and China. Valued for its aesthetic diversity, the species encompasses a wide range of cultivars and is extensively used in landscape design due to its vibrant foliage, variable form, and adaptability (Dirr, 2009). Among its many cultivars, 'Atropurpureum' is the most appreciated for its deep red foliage and aesthetic appeal, making it a popular choice in temperate climate gardens and urban green areas.

Despite its horticultural value, propagation of *A. palmatum* by seed remains challenging due to pronounced seed dormancy, which limits germination and uniform seedling emergence (Hartmann *et al*., 2011). To overcome this dormancy, pre-sowing treatments such as cold stratification or hydrothermal stimulation are commonly applied (Bewley *et al.,* 2013). Cold stratification, which mimics natural winter conditions, is widely used to break physiological dormancy in temperate woody species, including maples (Bonner and Karrfalt, 2008). Alternatively, hydrothermal treatments have been investigated as a faster method to enhance seed germination by softening the seed coat and activating enzymatic processes (Baskin and Baskin, 2014).

In addition to dormancy-breaking methods, the successful establishment of seedlings highly depends on the proper selection of the growing substrate, which influences water retention, aeration, nutrient availability, and root development (Raviv *et al.,* 2004). Peat-based substrates remain common in containerized seedling production, often supplemented with materials such as wood fibre to enhance structural stability and reduce environmental impact (Gruda, 2012).

The use in the seedlings data analysis of the phenotypic plasticity index (PPI) is relevant for nursery seedlings, because it can predict the potential response of the seedlings to different environmental factors, in practice helping to the optimization of nursery management (dos Santos *et al*., 2023; das Chagas Mendonca *et al*., 2025) by analysing the biometrical parameters of seedlings (Pardos and Calama, 2022) or developed trees (Kurjak *et al*., 2024).

Therefore, this research aims to evaluate the combined effects of two dormancy-breaking pre-treatments (cold stratification and hydrothermal stimulation) and three different commercial substrates, on the early growth of *Acer palmatum* 'Atropurpureum' seedlings. By examining the interaction between pre-sowing treatments and substrate composition, the study seeks to enhance propagation efficiency and promote sustainable practices in the production of this high-value ornamental species, by reducing the use of chemical inputs during the early stages of production of planting material.

MATERIAL AND METHODS

Seed harvest and materials used

Fruits of *Acer palmatum* Thunb. were collected on October 16, 2023, from a private garden in Dumbrăvița, Timiș County (45°48′22″N, 21°14′45″E). Post-harvest conditioning was conducted at the Arboriculture Laboratory, University of Life Sciences "King Mihai I" from Timișoara (Romania). Seeds sampled from healthy, mature trees were air-dried for one week, dewinged, and cleaned of damaged or empty seeds prior to germination (Figure 1).

A polyfactorial experimental design was used to assess the germination stimulation methods and substrate suitability. Two pre-treatments were applied: (1) Cold stratification (**a1**) – 100 seeds were mixed with moist sand and stored at 4 °C and 80% RH for 90 days (Chen *et al*., 2015); and (2) Hydrothermal treatment (**a2**) – 100 seeds were soaked in 50 °C water for 24 hours before sowing (Alipor *et al*., 2019).

From the pre-treated seeds, a total of 180 seeds (90 per treatment) was sown on the 16th of January, 2024, in seed trays, with 10 seeds per cell in three replicates (Šerá, 2023). For seed germination, three commercial substrates were used as presented and described in Table 1: Klasmann TS 3 (**b1**), Klasmann TS 4 medium + clay (**b2**) and Pro Line Bio Tray Substrate (**b3**).

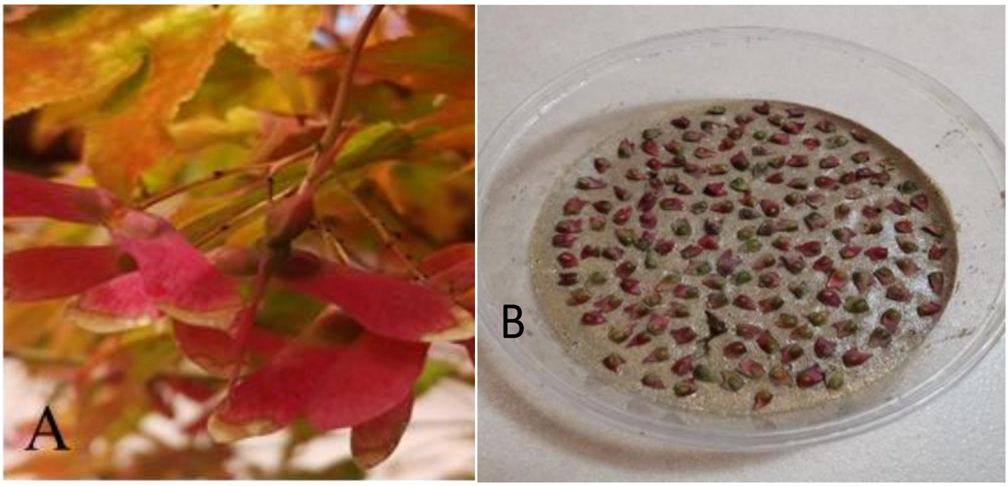


Figure 1. Fruits of *A. palmatum* ‘Atropurpureum’ before harvest (A) and seeds after samara removal (B) (photo: Poșta D.S.)

Seeds were germinated in seed trays and grown in a climate chamber under controlled conditions (20 °C and 70% humidity level). On the 17th of March, 2024, seedlings were transplanted into 13×13×13 cm pots and transferred into the greenhouse. Manual air ventilation and watering were carried out to maintain air temperature below 27 °C, and soil at field capacity. The greenhouse was covered with a 70% UV protection sunshade.

Table 1. Type of substrates used for seedling emergence

No. of substrates	**1**	**2**	**3**
Substrate type	**Klasmann TS 3**	**Klasmann TS 4 medium + clay**	**Pro Line Bio Tray Substrate**
			
Composition	White peat moderately decomposed (0-25 mm),	White peat 0-25 mm White sod peat 10-25 mm White peat fibre 0-30 mm 20 kg/cbm clay granules	White peat (0-5 mm) Raw materials: TerrAktiv FT Raw materials TerrAktiv Frozen through black peat
Structure	medium 0-25 mm	medium 0-30 mm	extra fine (0-5 mm)
Chemical data	pH value: 6 Fertilizer level (g/l): 1.0 + extra trace element fertilizer	pH value: 6 Fertilizer level (g/l): 1.0 + extra trace element fertilizer	pH value: 6 Salt level (g/l): 0.7-1.3 Horn shaves fertilizer (kg/m^3): 2.0
Nutrients added	Nitrogen (mg N/l): 140 Phosphorus (mg P_2O_5/l): 100 Potassium (mg K_2O/l): 180 Magnesium (mg Mg/l): 100 + all necessary trace elements Iron added as EDTA chelate	Nitrogen (mg N/l): 140 Phosphorus (mg P_2O_5/l): 100 Potassium (mg K_2O/l): 180 Magnesium (mg Mg/l): 100 + all necessary trace elements Iron added as EDTA chelate	Nitrogen (mg N/l): 300-400 Mineralizes N (mg N/l): 50-80 Phosphorus (mg P_2O_5/l): 200-300 Potassium (mg K_2O/l): 300-500 Magnesium (mg Mg/l): 100-200
Physical data	Dry matter: <10 % Water capacity: 75-80% Air capacity: 10-15%	Dry matter: <10 % Water capacity: 70-75% Air capacity: 15-20%	Dry matter: <10 % Water capacity: 78-82% Air capacity: 8-12%

Experimental design and data collection

The experimental factors used were pre-treatment (factor a) and substrate (factor b). The experiment was organized with six variants (2 pre-treatments * 3 substrates), each variant having three replicates. The biometrical data were collected from five plants from each replicate and from all considered variants, the total number of analysed seedlings from biometrical point of view from the experiment was 90.

Seedling parameters analysis

Seedling emergence was monitored daily over 45 days until on 3rd of March, 2024. After 60 days from seedling emergence (figure 2), the seedlings were transplanted and biometric measurements were carried out including seedling height, girth diameter, root number, main root length, number of leaves, and total leaf area. In addition, for all the quantitative variables studied, phenotypic plasticity index (PPI) was calculated according to Valladares *et al.* (2006) applying the following formula:

PPI=(maximum mean value - minimum mean value)/(mean maximum value).

The index varies from zero to one and allows comparisons among traits measured with different units (Valladares *et al*., 2000).

Figure 2. Seedlings before (C) and after (D) transplanting (photo: Poșta D.S.)

Statistical analyses

The primary data were compiled and subjected to statistical analyses to determine the effectiveness of seed pre-treatments, substrate type used and the combined effect of both factors ANOVA analysis was performed first to assess statistically significant differences between the means followed by Tukey's test at $p<0.05$. The software used for ANOVA was JASP 0.19.3 (JASP, 2025).

RESULTS AND DISCUSSION

Seedling growth performance was assessed using several biometric including the root system, where both the number of roots and the length of the primary root were measured to evaluate early development and establishment potential.

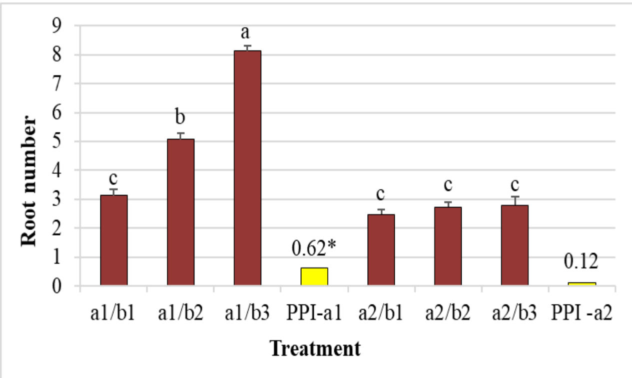
ANOVA regarding the influence of the pre-treatment, substrate and pre-treatment * substrate on the number of roots on the early seedlings of *A. palmatum* is presented in Table 1. The results highlight the highly significant influence of each of the two analysed factors applied and their combined effect on the number of roots per seedling ($p<0.001$).

Table 1. ANOVA – roots number per seedling

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	173.611	1	173.611	245.235	< 0.001
substrate	104.689	2	52.344	73.939	< 0.001
pre-sowing treatment ✻ substrate	86.956	2	43.478	61.415	< 0.001
Residuals	59.467	84	0.708		

Note. Type III Sum of Squares

As illustrated in Figure 3, the number of roots varied according to the applied germination stimulation method and substrate type used. Seeds subjected to hydrothermal treatment displayed relatively uniform root development across all substrate types, with a mean root number of 2.66, showing no statistically significant differences between substrate types.



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS4
a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 3. Root number of *A. palmatum* 'Atropurpureum' seedlings. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at $p<0.05$ according to Tukey's HSD test. Asterisk indicate significant differences within phenotypic plasticity index (PPI) of the two seed pre-treatments;

In contrast, due to cold stratification statistically significant differences were observed in terms of root number across the two experimental combinations: a1/b3 (cold stratification with Pro Line Tray Substrate) and a1/b2 (cold stratification with TS 4 + Greenfibre), which yielded in an average root number of 8.13 and 5.07, respectively.

These values suggest a synergistic effect between cold stratification and specific substrate compositions on root development. Such results are consistent with findings from previous studies emphasizing the role of prolonged stratification and substrate aeration in promoting root system differentiation in temperate woody species (Hartmann *et al.*, 2011; Baskin and Baskin, 2014; Poșta *et al.* 2024).

With regard to the PPI calculated for root number, the results showed that cold stratified seeds resulted in seedlings with high phenotypic plasticity (0.62) in root development, suggesting that these seedlings are more responsive to changes in substrate conditions. On the contrary, hydrothermal treatment produced seedlings with low plasticity (0.12), showing consistent root number regardless of substrate type. The significantly higher PPI in cold-stratified seeds suggests that this treatment may enhance seedling's ability to adjust root traits based on substrate conditions-an important consideration for nursery propagation and substrate selection. These outcomes support the hypothesis that stratification not only enhances germination rates but also promotes more vigorous root architecture, particularly when paired with substrates optimized for moisture retention and air porosity (Criscione, 2021).

Similar results were reported by Fetouh and Hassan (2014) in Magnolia grandiflora seedlings where stratification significantly enhanced root-related traits. Other studies (e.g., on Korean pine and Saskatoon berry) demonstrated that cold stratification improves germination metrics including germination rate, uniformity, and speed and generally enhances early seedling vigour and growth traits (Górnik *et al.,* 2023; Song *et al.,* 2023).

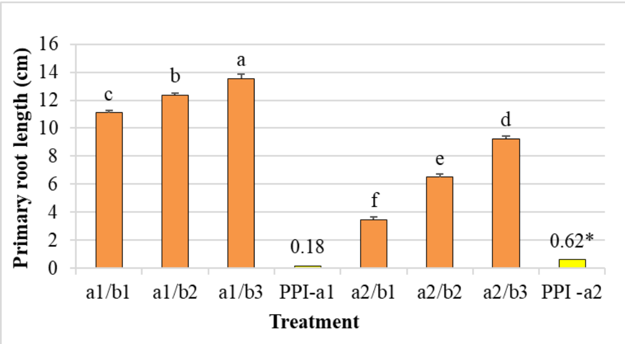
ANOVA analysis of primary root length under the influence of the pre-treatment, substrate and pre-treatment * substrate in the early seedlings of *A. palmatum* is presented in Table 2. As in the case of the number of roots per seedling, the results obtained highlight the highly significant influence of the two analysed factors applied in the experiment and their combined effect on the length of the primary root ($p < 0.001$).

Table 2. ANOVA – *A. palmatum* primary root length (cm)

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	790.914	1	790.914	1113.664	< 0.001
substrate	252.324	2	126.162	177.645	< 0.001
pre-sowing treatment ✻ substrate	41.731	2	20.865	29.380	< 0.001
Residuals	59.656	84	0.710		

Note. Type III Sum of Squares

The length of the primary root also exhibited significant variations across the experimental treatments (figure 4). Seeds subjected to cold stratification demonstrated a considerable improvement in primary root development compared to those treated hydrothermally (50 °C for 24 hours).



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate;
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS4
a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 4. Primary root length of *A. palmatum* 'Atropurpureum' seedlings. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at p<0.05 according to Tukey's HSD test. Asterisk indicate significant differences within phenotypic plasticity index (PPI) of the two seed pre-treatments.

Seed stratification appeared to positively influence root elongation, promoting deeper and more robust root systems as a crucial factor for early seedling vigor and adaptation in nursery conditions. These findings are in accordance with previous results, which reported enhanced root growth in stratified seeds of temperate woody species due to dormancy release and favourable metabolic activation (Bonner and Karrfalt, 2008; Bewley *et al*.,2013; Poșta, 2015).

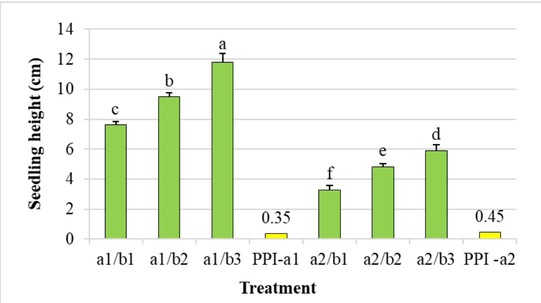
ANOVA analysis of the seedling height of *A. palmatum* under the influence of the pre-treatment, substrate and pre-treatment * substrate is presented in Table 3. The results obtained are evidencing the highly significant influence of the two analysed factors applied in the experiment and their combined effect on the seedling height ($p < 0.001$).

Table 3. ANOVA – *A. palmatum* seedling height (cm)

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	553.040	1	553.040	1625.830	< 0.001
substrate	173.061	2	86.530	254.382	< 0.001
pre-sowing treatment * substrate	9.631	2	4.815	14.156	< 0.001
Residuals	28.573	84	0.340		

Note. Type III Sum of Squares

Seedling height varied significantly across treatments, with average values ranging from 3.27±0.28 cm to 11.78 ± 0.30 cm. The tallest seedlings were recorded in the treatment combining stratified seeds with the Pro Line Tray Substrate, while the shortest seedlings were observed as a result of the hydrothermal treatment (50 °C for 24 hours) on TS 3 peat. This trend is clearly depicted in figure 5, showing statistically significant differences between treatments. PPI were not significantly different between the treatments. PPI-a2 showed slightly higher plasticity than cold stratification, suggesting that hydrothermally treated seedlings show relatively more variability in height in response to substrate, despite their lower seedling height.



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate;
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS4
a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 5. Seedling height of *A. palmatum* 'Atropurpureum'. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at $p<0.05$ according to Tukey's HSD test. PPI - phenotypic plasticity index of the two seed pre-treatments

The enhanced seedling growth in stratified seeds might be attributed to a more balanced germination process, leading to better use of substrate nutrients and moisture. Conversely, reduced seedling height in hydrothermally treated seeds suggests limited physiological processes, possibly due to insufficient dormancy release or thermal stress. These results reinforce the critical role of proper dormancy-breaking methods and optimized substrate selection in maximizing early growth potential in nursery production systems (Ranal and Santana, 2006; Hartmann *et al*., 2011).

In Table 4 is presented ANOVA analysis regarding the influence of the pre-treatment, substrate and pre-treatment * substrate on the girth diameter on the early seedlings of *A. palmatum*. The results obtained show the highly significant influence of the two analysed factors applied ($p < 0.001$). In the case of the combined effect of the factors, the influence on the girth diameter is not significant from statistical point of view.

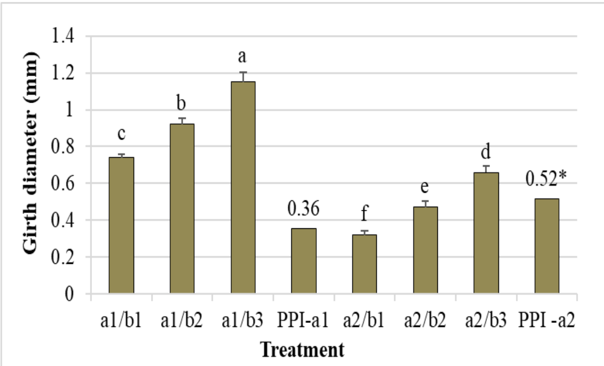
Table 4. ANOVA – *A. palmatum* seedling girth diameter (mm)

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	4.597	1	4.597	1097.846	< 0.001
substrate	2.131	2	1.065	254.424	< 0.001
pre-sowing treatment * substrate	0.015	2	0.007	1.768	0.177
Residuals	0.352	84	0.004		

Note. Type III Sum of Squares

Girth diameter is a key morphological indicator of seedling vigour and structural stability, correlating with both nutrient uptake capacity and transplanting success (Mattsson, 1997). The girth diameter of the seedlings followed a similar variation pattern to that observed in shoot height, with significant differences between substrate types and pre-germination treatments (figure 6).

Seedlings emerged from stratified seeds exhibited larger girth diameters across all substrates tested. Cold stratification led to thicker seedlings, with the best result recorded in Pro Line Bio Tray substrate (a1/b3), confirming its superior performance for overall seedling vigour. While hydrothermal treatment resulted in thinner seedlings overall, it showed higher phenotypic plasticity (PPI=0.52), suggesting that girth in this group is more influenced by substrate quality. The significant PPI difference (0.52 vs 0.36) indicates that although cold-stratified seedlings grow better in absolute terms, hydrothermally treated seedlings are more sensitive to substrate when it comes to girth development.



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate;
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS4
a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 6. Girth diameter of *A. palmatum* 'Atropurpureum' seedlings. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at $p<0.05$ according to Tukey's HSD test. Asterisk indicate significant differences within phenotypic plasticity index (PPI) of the two seed pre-treatments.

In addition to root and stem parameters, the number of leaves was also evaluated as an indicator of photosynthetic potential and seedling vitality.

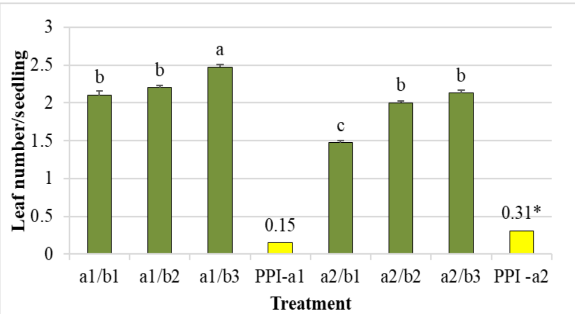
In Table 5 is presented ANOVA analysis regarding the influence of the pre-treatment, substrate and pre-treatment * substrate on the number of leaves per seedling. The results obtained for the leaves number per seedling proved highly significant influence of both analysed factors applied ($p < 0.001$). Also, the combined effect of the factors wasn't statistically significant for the number of leaves per seedling.

Table 5. ANOVA – *A. palmatum* number of leaves per seedling

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	3.600	1	3.600	19.722	< 0.001
substrate	3.800	2	1.900	10.409	< 0.001
pre-sowing treatment * substrate	0.867	2	0.433	2.374	0.099
Residuals	15.333	84	0.183		

Note. Type III Sum of Squares

As shown in figure 7, the leaf number/seedling ranged from 2.00 ± 0.33 to 2.47 ± 0.36, with the highest values observed in stratified seeds, particularly in the a1/b3 combination. Although differences were relatively small, they remain biologically relevant, especially when seedlings are intended for ornamental purposes where early foliage development is desirable. Cold-stratified seedlings produced higher number of leaves, especially in b3 (Pro Line Bio Tray) substrate. However, their PPI was low (0.15), suggesting they maintain stable leaf development regardless of the substrate.



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate;
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 – hydrothermal treatment of seeds, 50 °C - 24 hours/TS4
a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 7. Leaf number/seedling in *A. palmatum* ‘Atropurpureum’. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at $p<0.05$ according to Tukey’s HSD test. Asterisk indicate significant differences within phenotypic plasticity index (PPI) of the two seed pre-treatments.

Hydrothermally treated seedlings had greater variability in leaf number across substrates, showing reduced performance in b1. Their PPI was higher (0.31), meaning they are more responsive to substrate differences, even though their average leaf number was lower than cold-stratified seedlings. Previous studies have suggested that substrates rich in organic matter and well-drained structure tend to enhance leaf emergence, particularly in species sensitive to waterlogging and poor aeration (Gruda, 2012).

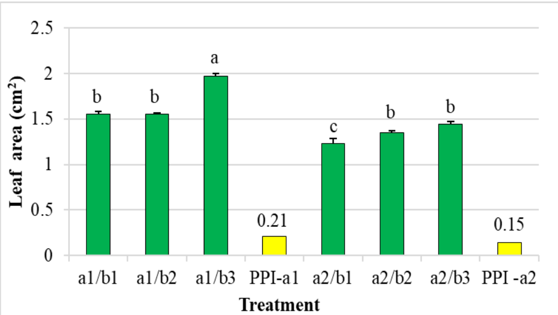
In the case of foliar surface ANOVA analysis (Table 6) the results obtained evidenced similar behaviour with the number of leaves and the girth diameter. Thus, the influence of the pre-treatment, substrate and pre-treatment * substrate on the foliar surface per seedling was highly significant for both analysed factors applied ($p<0.001$); and the combined effect of the factors wasn't significant for this parameter.

Table 6. ANOVA – *A. palmatum* foliar surface per seedling (sq mm)

Cases	Sum of Squares	df	Mean Square	F	p
pre-sowing treatment	2.798	1	2.798	66.407	< 0.001
substrate	1.665	2	0.833	19.756	< 0.001
pre-sowing treatment ✻ substrate	0.431	2	0.216	5.118	0.008
Residuals	3.540	84	0.042		

Note. Type III Sum of Squares

Regarding the leaf area, a strong positive correlation (r=0.87) was noted between leaf number and total leaf surface area according to Pearson' correlation. The highest leaf area was recorded in a1/b3 variant (stratified seeds grown in Pro Line Tray Substrate peat), reinforcing the conclusion that this treatment combination supports optimal early growth conditions.



a1/b1 – cold stratification/TS3
a1/b2 – cold stratification/TS4
a1/b3 - cold stratification/Pro Line Bio Tray Substrate;
a2/b1 - hydrothermal treatment of seeds, 50 °C - 24 hours/TS3
a2/b2 – hydrothermal treatment of seeds, 50 °C - 24 hours/TS4 a2/b3 - hydrothermal treatment of seeds, 50 °C - 24 hours/ Pro Line Bio Tray Substrate

Figure 8. Total leaf area/seedling in *A. palmatum* 'Atropurpureum'. The data presented are means±st. error. Different lowercase letters above the bars indicate significant differences between the means at $p<0.05$ according to Tukey's HSD test. PPI - phenotypic plasticity index of the two seed pre-treatments.

In literature, the application of different temperature ranges for the dormancy-release of the seeds combined with different substrates used have an influence the seedling growth, including directly the leaf number and indirect the foliar surface (Costa *et al.*, 2021).

The overall results obtained show the statistically significant impact of the pretreatment and substrate considered separately, on all the biometric features analysed on the seedlings of *A. palmatum* 'Atropurpureum'. The combined effect of the applied factors was significant only the root features and seedling height.

The obtained results align with findings from ornamental seedling production, where adequate root and shoot development under stratification and nutrient-balanced substrates enhances establishment success (Raviv *et al*., 2004).

CONCLUSIONS

The findings suggest that both seed pre-treatment and substrate type significantly affected the germination and early growth of *A. palmatum* 'Atropurpureum'. Cold stratification with Pro Line Bio Tray substrate produced the best overall performance, promoting vigorous, stable growth with low PPI values. Hydrothermal treatment yielded lower growth but higher PPI for some traits, reflecting greater sensitivity to substrate composition.

The overall results obtained suggest the development of future research for the obtaining of vigorous planting material of *A. palmatum* in a sustainable manner. The next researches should be focused on the analysis of other developmental stages of the Japanese maple.

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