

Improving Germination Performance of Pumpkin (*Cucurbita pepo* L.) under Water Deficit through Selenium and Calcium Seed Priming

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Abstract

Seed priming is an effective pre-sowing strategy for improving germination under water-limited conditions. This study evaluated the effects of selenium (Se, 1.5 mg L⁻¹) and calcium (Ca²⁺, 5 mg L⁻¹) nutrimpriming on germination performance, seed imbibition dynamics, and early seedling development of a local pumpkin (*Cucurbita pepo* L.) cultivar under limited moisture and polyethylene glycol (PEG 8000)-induced osmotic stress (5% and 10%). Under controlled moisture (6 mL), Se-primed seeds showed significantly higher germination percentage, germination index, and seedling vigor index, along with reduced mean germination time and increased dry weight, compared with non-primed seeds. Calcium priming produced the highest relative chlorophyll content and fresh biomass, though these increases were not statistically significant relative to Se or the control. PEG treatments significantly reduced germination traits, seedling growth, and biomass overall. Both Se and Ca²⁺ priming partially alleviated these osmotic stress effects, significantly improving germination percentage and radicle length under PEG-induced conditions, with numerically higher chlorophyll content and biomass accumulation. Imbibition analysis showed that PEG restricted water uptake, whereas Se- and Ca²⁺-primed seeds maintained imbibition dynamics comparable to the hydropriming control. Morphometric evaluation during imbibition revealed treatment-dependent changes in seed size parameters, with Ca²⁺ increasing seed length and Se increasing length-to-width ratio. Trichome density on the adaxial cotyledon surface was numerically higher in Se- and Ca²⁺-primed seedlings, while increasing PEG concentration significantly reduced trichome number. These findings demonstrate that low-dose Se and Ca²⁺ nutrimpriming enhances germination performance and early seedling establishment of pumpkin under water-deficit conditions, supporting its potential as a practical pre-sowing strategy for drought-prone growing environments.

Introduction

Seed germination is a complex biochemical and biomechanical process regulated by environmental factors. Among these, water potential is the most influential, governing imbibition, metabolic activation, and cell elongation during the early stage of germination. Over decades of practice, researchers and farmers have developed various seed priming techniques to improve germination under suboptimal conditions. Nutrimpriming with essential elements such as Se and Ca²⁺ has proven particularly effective for enhancing water uptake during germination (García-Locascio et al., 2025; Pan et al., 2025).

Pumpkin (*Cucurbita pepo*) is a nutrient-rich, economically important horticultural crop widely cultivated in arid and semi-arid regions. Pumpkin seeds and their oil are rich natural source of phytosterols, proteins, polyunsaturated fatty acids, antioxidant vitamins, carotenoids, tocopherols, and essential minerals (Ratnam et al., 2017). According to the USDA United States Department of Agriculture National Nutrient Database, pumpkin seeds contain approximately 46 mg of Ca²⁺ and 9.4 µg of Se per 100 g (Patel & Rauf, 2017). The recommended dietary allowances (RDA) for these elements vary by age; for adults, the current RDA is 55 mcg for Se and 1,300 mg for Ca²⁺ (National Institutes of Health (NIH), 2024a, 2024b). Despite this nutritional value, pumpkin seeds remain highly susceptible to water deficit during germination (Irik & Bikmaz, 2024; Jahed et al., 2022). yet little is known about how water uptake and germination performance in pumpkin respond to nutrimpriming with Se and Ca²⁺.

Selenium (Se) is well documented as a trace element that helps plants cope with abiotic stress (Rasool et al., 2022). When applied as a foliar treatment during early growth, Se has been shown to lower reactive oxygen species (ROS; O₂^{•-} and H₂O₂) and sodium ion (Na⁺) accumulation, while improving leaf integrity, nutrient homeostasis, photosynthetic capacity, osmoregulator content, and antioxidant enzyme activity, alongside favorable shifts in stress-related gene expression. In pumpkin specifically, priming seeds with 8 mg L⁻¹ selenium dioxide (SeO₂) markedly increased germination percentage and germination rate (Zhang HuiJun et al., 2017). Combining selenate (SeO₄²⁻, 10 mg L⁻¹) with iodide (I⁻, 1000 mg L⁻¹) produced even greater improvements in germination rate than selenate alone or the untreated control (Golob et al., 2020).

Calcium (Ca²⁺) plays a complementary role, acting as a secondary messenger in stress signaling while helping to maintain membrane integrity and support water uptake under osmotic stress (Su et al., 2025; Wei et al., 2025). In cucumber, hydropriming seeds for 6 h followed by germination in 5 mM calcium nitrate [Ca(NO₃)₂] increased germinated seed weight relative to both the untreated control and a higher 40 mM dose. At higher concentrations (10–40 mM), Ca(NO₃)₂ instead boosted the activity of several protective enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR)) (Fan et al., 2017). Osmoprimering, especially with calcium chloride CaCl₂ for 3 days, was effective in improving (GP) and reduce mean germination time(MGT) on melon (Castañares & Bouzo, 2018). In melon, osmoprimering with calcium chloride (CaCl₂) for three days improved germination percentage and shortened mean germination time (Castañares & Bouzo, 2018). More recently, foliar application of sodium selenate (Na₂SeO₄) and calcium nitrate [Ca(NO₃)₂·4H₂O] during the flowering stage of pumpkin was found to significantly raise seed Ca²⁺ content under 50% field capacity, with calcium treatment also producing the highest CAT and APX activity under the same water-limited conditions(Al-Bajalan & Kka, 2025). Together, these findings suggest that Se and Ca²⁺ support plant growth under drought stress primarily by strengthening antioxidant defenses and limiting oxidative damage.

Water status also leaves its mark on the plant surface. Trichome density and activity typically increase under stress, particularly in non-glandular forms, as part of the plant's broader resistance response (Karabourniotis et al., 2020). Cucurbitaceae species display considerable diversity in trichome structure – unicellular or multicellular, branched or unbranched, glandular or nonglandular (Xiao & Loy, 2007). and under drought, more active glandular trichomes in some cucurbits release defensive compounds that can help reduce both transpiration and herbivory (Kaur, 2021). Because trichome development is itself sensitive to water status, it may serve as a useful early, visible marker of stress response that complements standard germination metrics. Yet this link has received little attention: most seed-priming studies under abiotic stress focus on final germination outcomes rather than on how seeds regulate hydration during imbibition or how that regulation connects to early structural changes. In pumpkin, the combined effects of selenium and calcium priming on imbibition dynamics, seed morphometrics, and cotyledon trichome development under osmotic stress remain essentially unexplored. This study therefore examined how low-dose Se and Ca²⁺ nutrimpriming influences germination performance, water uptake kinetics, and early epidermal responses in pumpkin seeds under PEG-induced osmotic stress.

Materials and Methods

Plant material

Cucurbita pepo L. seeds from a local cultivar were obtained from two sources: (F₀ generation) a local farmer in Kalar City. And (F₁ generation) newly harvested seeds from mature fruits produced by the F₀ plants, grown during the cultivation season (May to September 2024) as the main biological material.

Seed surface sterilization (applies to all experiments below)

Prior to each experiment, seeds were rinsed with water and surface-sterilized by immersion in 0.1% sodium hypochlorite for 2 min, followed by three rinses in distilled water (Darshani et al., 2021). Seeds were then dried using a paper towel before proceeding to the respective treatment.

Seed germination of F₀ under controlled moisture levels

Germination tests were performed in an incubator (Thermo Scientific, Germany) at the Garmian Agricultural Research Directorate, General Directorate of Agriculture of Garmian, Kurdistan Region, Iraq. Surface-sterilized F₀ seeds were hydroprimed in 1 L purified water (control treatment). For nutrimpriming, 1.5 mg L⁻¹ sodium selenite (Na₂SeO₃) (Thomas Baker, India), hereafter Se, and 5 mg L⁻¹ calcium nitrate [Ca(NO₃)₂·4H₂O] (Casa De Amor, India), hereafter Ca²⁺, were used. Seeds were hydro- or nutrimprimed for 6 h prior to sowing.

A randomized complete design with three replicates was used to assess germination under two moisture levels. Fifteen seeds were placed on double layers of filter paper in a 9 cm Petri dish (Citoglas, China)(Aydinsakir et al., 2013). Six milliliters of purified water were added per dish for the control moisture level, and 3 mL for the limited-moisture treatment. Petri dishes were sealed with Parafilm and incubated under a 16 h photoperiod, 20–22°C, and 65–85% relative humidity(Saadaoui et al., 2023). Germination was recorded daily for 10 days. The following parameters were measured:

- Germination percentage (GP)

GP was calculated daily over 10 days following Irik & Bikmaz (2024), Eq. (1).

$$GP = \frac{\text{Total number of germinaed seeds}}{\text{Total number of sown seeds}} \times 100$$

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Germination energy (GE)

- GE was calculated using Eq. 1, restricted to the first five days of the germination test.

Germination index (GI)

GI (seeds day⁻¹) was calculated following Irik & Bikmaz (2024)

$$GI = \sum \left(\frac{G_t}{T_t} \right)$$

2

- where G_t is the number of seeds germinated on day t, and T_t is the number of days.

Mean germination time (MGT) (day)

MGT was calculated following Irik & Bikmaz (2024), Eq. (3).

$$MGT = \frac{\sum (\text{Germinated seeds} \times \text{day seeds germinated on})}{\sum \text{Germinated seeds}}$$

3

Fresh weight and dry weight (FW and Dry) (mg)

On day 10, five randomly selected seedlings per Petri dish were weighed to obtain fresh weight (FW), then oven-dried (Memmert GmbH, Germany) at 72°C for 48 h and monitored until constant weight to obtain dry weight (DW) (Sheikhnavaz Jahed et al., 2023).

Seedling length (SL) (cm)

Pumpkin exhibits epigeal germination. Hypocotyl length (HL) was measured from the cotyledon to the radicle; radicle length (RL) was measured from the hypocotyl to the root tip. Seedling length (SL) is the sum of HL and RL. Measurements were taken from photographs (Huawei, dual-lens 1:2.2/26 ASPH) and analyzed using Fiji (ImageJ 1.54f) (Fig. 1).

Seedling vigor index (SVI)

SVI was calculated following Irik & Bikmaz (2024), Eq. (4).

$$SVI = (HL + RL) \times GP$$

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Relative chlorophyll content

Total chlorophyll content in the cotyledon of 10-day-old seedlings was determined using a SPAD chlorophyll meter (Opti-Sciences CCM-200, USA)(Saadaoui et al., 2023).

Seed imbibition assay

Surface-sterilized F₁ seeds were used. A randomized complete design with three replicates of 20 seeds each was conducted. Seeds were placed in 250 mL Erlenmeyer flasks (Citoglas, China) containing 150 mL of one of the following test solutions: distilled water (control), 5% PEG, 10% PEG (PEG 8000, MW 7000–9000; Promega, Madison, USA), Se (0.225 mg Na₂SeO₃ dissolved in 150 mL water to yield 1.5 mg L⁻¹), or Ca²⁺ (0.75 mg dissolved in 150 mL water to yield 5 mg L⁻¹).

Prior to immersion (time 0), all seeds were weighed on an analytical balance. Seeds were then submerged in their respective solutions and held at room temperature (15–25°C). At each designated time point, seeds were removed with a 2 mm sieve, gently surface-dried on triple-layered absorbent paper without applied pressure, and immediately reweighed (Pompelli et al., 2023). Measurements were taken at 5, 10, and 30 min, and at 1, 2, 3, 6, 9, 24, 48, 72, 96, 120, 144, 168, and ≥ 192 h, following Duncan et al. (2019):

$$\text{Imbibition rate (\%)} = \left(\frac{wt - w_0}{w_0} \right) \times 100$$

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Where W_t is seed weight at time and W₀ is initial dry seed weight

Seed size was measured at 0, 6, 24, 48, and 72 h of imbibition from captured photographs, analyzed using SmartGrain v1.3 (<http://phenotyping.image.cooacan.jp/>) (Al-Bajalan & Kka, 2025; Tanabata et al., 2012). Parameters measured were area size (AS, mm²), perimeter length (PL, mm), length (L, mm), width (W, mm), length-to-width ratio (LWR), circularity (CS), and the distance between the intersection of length/width and the center of gravity (DS, mm).

Seed germination under PEG-induced drought stress

Newly harvested F₁ seeds were weighed in the dry state, surface-sterilized as described above, and soaked for 6 h in one of the following: distilled water (control), Se (1.5 mg L⁻¹), or Ca²⁺ (5 mg L⁻¹).

Seeds were then transferred to 9 cm Petri dishes lined with double layers of filter paper, moistened with 6 mL of one of the following treatments: distilled water (control, 0 bars), 5% PEG (– 0.50 bars), or 10% PEG (– 1.48 bars). PEG solutions were prepared by dissolving 5.5 g PEG in 110 mL water (5%) or 11 g PEG in 110 mL water (10%) (Kka et al., 2025).

Fifteen seeds were placed in each Petri dish (Aydinsakir et al., 2013). Dishes were sealed with Parafilm and incubated for 10 days at 20–22°C, 65–84% relative humidity, and a 16 h photoperiod. Germination was recorded daily. Parameters measured were GP, GE, GI, MGT, relative chlorophyll content, RL, FW, and DW, calculated as described above (Equations 1–4).

Daily fraction of germinated seeds

The daily fraction of germinated seeds — an indicator of viability used to inform optimal planting density and germination conditions — was calculated (Kka et al., 2025).

$$\text{Fraction of germinated seeds} = \frac{\text{Cumulative number of seeds germinated by day (t)}}{\text{Total number of seeds plated}}$$

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Trichome visualization

Twelve days after planting, fresh seedlings were selected for microscopic examination. A 2 mm section of the adaxial epidermal peel of the cotyledon was excised using fine forceps or a razor blade, stained with 1% safranin O solution (Biochem, Iran), and rinsed with distilled water to remove excess stain(Ekeke & Agogbua, 2018). Samples were mounted in a drop of pure glycerol on clean glass slides (Citoglas, China) and covered with a coverslip. Slides were

examined under a light microscope (Biological Binocular Microscope XSZ-107 TNOVEL, China) at 10× magnification, and trichomes were imaged using a MOCE-5C digital camera (Show Range, China). Total trichome number within the 2 mm imaged area was recorded per slide.

Experimental Design and Statistical Analysis

Each experiment was conducted as a two-factor factorial arranged in a Randomized Complete Block Design (RCBD) with three replications. For the controlled-moisture experiment, the two factors were nutrimpriming treatment (Se, Ca²⁺, control) and moisture level (6 mL, 3 mL). For the PEG-stress experiment, the two factors were nutrimpriming treatment (Se, Ca²⁺, control) and water potential, generated using PEG 8000 (control, 5% PEG, 10% PEG). Data were analyzed using SPSS software (version 25.0, IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was performed for each experiment to evaluate the main effects of each factor and their interaction at the 5% significance level ($P \leq 0.05$). When significant differences were observed, treatment means were separated using Duncan's Multiple Range Test (DMRT). Results are expressed as mean $\pm$ standard error (SE).

Results

Seed germination of F₀ under controlled moisture levels

The two-way interaction between moisture levels (6mL and 3mL) and seed nutrimpriming with (Se, Ca²⁺ and control) demonstrates that seeds primed with Se and exposed to 6 mL moisture level statistically recorded the highest value of GP, GE, GI, reduced MGT, SVI and DW (Table 1). Meanwhile, the statistical analysis showed that of HL, total chlorophyll (SPAD unit) was highest when seeds primed with Ca²⁺ and exposed to the 6 mL moisture level. The 6 mL moisture level per plate increased the value of germination traits compared with limited moisture level (3mL per plate), but statistically there was no significant differences. Subsequently, seeds nutrimpriming with Se and Ca²⁺ significantly increased all related germination traits under limited moisture level compared with control.

Seed imbibition assay

In this experiment, the impact of nutrimpriming seeds with Se 1.5 mg. L⁻¹, Ca²⁺ 5 mg. L⁻¹, PEG 5%, PEG 10%, and a control (hydropriming with purified water only) on imbibition rate and seed size during imbibition.

The result of imbibition rate subsequently increased within the time. Se, Ca²⁺ and the control treatment simultaneously increased until day three (during the first 48–72 h), then the imbibition rate stabilized and decreased. In similar way, when the seeds were exposed to osompriming of 5% and 10% PEG, the imbibition rate increased gradually until day two, and then remained constant. The important finding is that the Se and Ca²⁺ effects did not differ significantly compared with the control, while PEG 5% and 10% restricted the imbibition process significantly.

At the same time, seed size characteristics were measured using smart grain (Table 2). The recorded data show that Ca²⁺ was most effective in the length of the seeds, while Se recorded the highest LWR and DS. The main effect of time was also recorded; the statistical analysis shows that the largest AS (102.24 mm²) and W (8.80 mm) were achieved after 6 h, and PL, L, LWR, and DS after 24 h. The CS was recorded the highest at time 0.

Table 1

The two-way interaction and main effect of nutrimpriming seeds (Se, Ca²⁺ and control), and moisture levels (6mL and 3mL)

Two-way interaction moisture levels and nutrimpriming													
Moisture level	Nutri priming	GP		GE		GI		MGT (day)		HL (cm)		RL (cr	
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	
6mL	Se	82.22 a	3.72	68.89 a	3.72	5.14 a	0.50	3.33 b	0.27	1.50 b	0.08	20.59	
	Ca ²⁺	76.67 a	2.85	62.22 a	4.44	4.50 ab	0.22	3.60 ab	0.22	2.31 a	0.13	19.64	
	Control	58.89 b	3.18	42.22 b	3.30	2.73 cd	0.24	4.22 a	0.19	1.98 ab	0.33	18.38	
3mL	Se	73.33 a	5.16	63.33 a	3.75	3.67 bc	0.34	3.84 ab	0.25	1.53 b	0.09	17.84	
	Ca ²⁺	77.78 a	5.07	62.22 a	5.62	4.29 ab	0.41	3.63 ab	0.11	1.97 ab	0.10	20.96	
	Control	43.33 c	2.28	32.22 b	2.05	2.07 d	0.14	3.95 ab	0.27	1.66 b	0.06	18.65	
Main effect of moisture levels													
6mL		72.59 ns	3.00	57.78 ns	3.45	4.13 ns	0.31	3.72 ns	0.15	1.93 ns	0.14	19.53 ns	0
3mL		64.81	4.41	52.59	4.14	3.35	0.29	3.81	0.13	1.72	0.06	19.15	0
Main effect of nutrimpriming													
Se		77.78 a	3.32	66.11 a	2.65	4.41 a	0.37	3.59 b	0.19	1.52 b	0.06	19.21 a	1
Ca ²⁺		77.22 a	2.78	62.22 a	3.42	4.40 a	0.22	3.62 b	0.12	2.14 a	0.09	20.30 a	0
Control		51.11 b	3.00	37.22 b	2.39	2.40 b	0.17	4.08 a	0.16	1.82 ab	0.17	18.52 a	0
Note: Germination Percentage (GP), Germination Energy (GE), Germination Index (GI), Mean germination time (MGT (day), Hypocotyle length (cm) (HL), Radi weight (g) (FW), and Seedling dry weight (g) (DW). Means in the same column with different letters are significantly different at α = 0.05 according to Duncan													

Table 2

The main effect of priming (hydropriming, nutrimpriming and osmopriming) and time on seed size during imbibition process of pumpkin seeds.

Main effect of nutrimpriming														
Treatment	(AS)[mm ²]		(PL)[mm]		(L)[mm]		(W)[mm]		(LWR)		(CS)		(DS)[mm]	
	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Selenium	95.43 b	2.33	41.77 ab	0.51	15.82 ab	0.22	8.47 b	0.10	1.88 a	0.01	0.68 ab	0.01	1.54 a	0.09
Calcium	97.61 ab	2.15	42.65 a	0.47	16.28 a	0.30	8.57 b	0.10	1.87 a	0.01	0.67 b	0.01	1.53 a	0.08
Control	103.27 a	1.54	42.96 a	0.49	16.02 ab	0.15	8.98 a	0.08	1.79 c	0.01	0.70 a	0.01	1.34 a	0.07
5% PEG	95.13 b	2.06	41.21 b	0.43	15.52 b	0.18	8.45 b	0.10	1.83 b	0.01	0.69 ab	0.01	1.37 a	0.09
10% PEG	97.92 ab	1.92	42.21 ab	0.35	15.81 ab	0.15	8.63 b	0.09	1.83 b	0.01	0.69 ab	0.01	1.34 a	0.06
Main effect of time														
0	99.80 ab	2.18	41.55 a	0.46	15.92 ab	0.18	8.66 ab	0.10	1.84 ab	0.01	0.72 a	0.00	1.07 b	0.05
6h	102.24 a	2.03	42.46 a	0.50	16.09 a	0.18	8.80 a	0.12	1.83 ab	0.01	0.70 b	0.00	1.46 a	0.07
24h	99.66 ab	2.45	42.76 a	0.55	16.12 a	0.20	8.65 ab	0.12	1.87 a	0.01	0.68 c	0.01	1.58 a	0.09
48h	92.61 c	1.50	41.69 a	0.43	15.43 b	0.13	8.42 b	0.08	1.84 ab	0.02	0.67 d	0.01	1.48 a	0.05
72h	95.04 bc	1.36	42.35 a	0.37	15.88 ab	0.31	8.55 ab	0.08	1.81 b	0.01	0.66 d	0.01	1.55 a	0.07

Means in the same column with different letters are significantly different at α = 0.05 according to Duncan test.

Seed germination under PEG-induced drought stress

Furthermore, an experiment was conducted to examine the impact of nutrimpriming seeds of F1 with Se and Ca²⁺ and germinating it under two water potential made using PEG 5% and 10% (Table 3). The results of two-way interaction show that Se and Ca²⁺ under control and osmotic pressure of 5% and 10% improved GP, GE, GI, MGT, RL, SVI, total chlorophyll (SPAD value), FW, and DW (Table 3 and Fig. 3) and the daily fraction of germinated seeds (Fig. 4), in spite of the fact that the highest value of the previously mentioned traits was for seeds treated with Se and Ca²⁺ germinated using purified water (control), as also demonstrated in the main effect data analysis of water potential impact. Moreover, the main effect of priming shows that G%, RL, FW, and DW were the highest in seeds primed with Se. As while Ca²⁺ improved GE, GI, MGT, SVI, and total chlorophyll as the highest values. The daily fraction of germinated seeds increased with time, reached the highest after day 5, and then remained constant. Se and Ca²⁺ significantly improved the cumulative value of germination. The highest germination was recorded after 5 and 6 days of sowing.

Table 3
The two-way interaction and main effect of osmopriming and nutrimpriming on seed germination traits of pumpkin local cultivar F1.

Interaction effect of PEG and nutrimpriming																
PEG	Nutrimpriming	GP		GE		GI		MGT (day)		RL (cm)		SVI		(SPAD value)		FW (g)
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean
Control	Se	92.22 a	2.05	85.56 a	2.68	4.37 ab	0.27	3.75 cd	0.12	27.03 a	0.64	2487.87 a	38.12	26.71 a	0.35	0.72 a
	Ca ²⁺	93.33 a	2.43	85.56 a	4.69	4.80 a	0.31	3.62 d	0.11	26.53 a	0.17	2474.94 a	55.53	27.49 a	0.39	0.70 b
	Control	74.44 bc	4.99	60.00 c	3.44	3.23 de	0.17	4.03 bc	0.11	20.64 b	0.12	1535.94 b	101.55	19.12 b	0.88	0.60 c
PEG 5%	Se	76.67 b	4.13	60.00 c	3.85	3.57 cd	0.28	3.99 abc	0.13	19.76 c	0.30	1518.92 b	97.67	13.97 c	0.59	0.53 d
	Ca ²⁺	81.11 b	6.31	73.33 b	4.22	4.06 bc	0.42	3.63 d	0.08	20.75 b	0.43	1693.70 b	161.97	14.08 c	0.64	0.52 e
	Control	62.22 d	2.22	53.33 cd	2.43	2.70 ef	0.10	4.06 bc	0.17	16.93 d	0.16	1053.72 c	40.83	10.81 d	0.12	0.44 f
PEG 10%	Se	61.11 d	2.05	42.22 e	2.22	2.53 ef	0.17	4.47 a	0.12	15.39 e	0.25	939.67 c	30.31	3.45 e	0.06	0.40 g
	Ca ²⁺	64.44 cd	3.30	46.67 de	2.43	2.80 ef	0.13	4.32 ab	0.11	16.33 d	0.29	1053.94 c	63.98	2.83 ef	0.10	0.40 g
	Control	56.67 d	2.85	42.22 e	2.22	2.39 f	0.18	4.31 ab	0.14	11.37 f	0.06	644.16 d	32.37	1.97 f	0.06	0.33 h
Main effect of PEG																
Control		86.67 a	2.80	77.04 a	3.54	4.13 a	0.21	3.80 b	0.07	24.73 a	0.73	2166.25 a	114.64	24.44 a	1.37	0.67 a
PEG 5%		73.33 b	3.14	62.22 b	2.80	3.44 b	0.21	3.89 b	0.09	19.15 b	0.43	1422.11 b	89.23	12.95 b	0.59	0.50 b
PEG 10%		60.74 c	1.69	43.70 c	1.34	2.57 c	0.10	4.37 a	0.07	14.36 c	0.54	879.26 c	48.45	2.75 c	0.22	0.38 c
Main effect of nutrimpriming																
Se		76.67 a	3.46	62.59 ab	4.61	3.49 a	0.23	4.07 a	0.10	20.73 a	1.19	1648.82 a	158.63	14.71 a	3.37	0.55 a
Ca ²⁺		79.63 a	3.71	68.52 a	4.47	3.89 a	0.26	3.86 a	0.10	21.20 a	1.03	1740.86 a	152.11	14.80 a	3.57	0.54 a
Control		64.44 b	2.64	51.85 b	2.32	2.77 b	0.12	4.13 a	0.08	16.31 b	0.93	1077.94 b	95.35	10.63 a	2.49	0.46 a

Means in the same column with different letters are significantly different at $\alpha = 0.05$ according to Duncan test.

The trichome is known to play an efficient role in reducing water loss through decreasing the rate of transpiration. Trichome intensity and number were counted under the priming and water potential germination conditions. The two-way interaction shows that Se and Ca²⁺ under control, and water potential impact increased the number of trichomes. Although the highest value was recorded with priming seeds with Se (Table 4 and Fig. 5), the lowest number of trichomes was recorded when seeds were hydropriming with water (control) and germinated under high water potential (10% PEG). The main effect of priming showed the highest value when using Se and Ca²⁺, although this result was not statistically significant. Water potential specifically reduced the number of trichomes, which is demonstrated as the main effect of water potential.

Table 4
Two-way interaction and main effect of PEG and nutripripping on trichome number on adaxial of cotyledon leaf of pumpkin local cultivar

Treatment	Two-way Interaction					Main Effect of water potential		
	Selenium		Calcium		Control			
Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	
Control	64.11 a	3.99	61.11 ab	1.94	58.56 ab	3.47	61.26 a	1.81
PEG 5%	57.78 ab	3.78	55.67 ab	1.35	58.89 ab	1.28	57.44 ab	1.31
PEG 10%	57.44 ab	2.63	56.56 ab	2.70	53.44 b	0.80	55.82 b	1.27
Main Effect	59.78 a	2.07	57.78 a	1.33	56.96 a	1.40		

Means in the same column with different letters are significantly different at $\alpha = 0.05$ according to Duncan test.

Discussion

Germination test has been practiced by researcher and farmers to confirm the quality of produced seeds and also the uniformity of growth and development of plant species. Various methods were implemented during this early growth to assay drought resistance and tolerance and seeds mechanism against particular abiotic stress. In this study, limited level of moisture was tested in pumpkin seeds germination as preliminary experiment. Limited evidence is available and resulted similar results compared with using osmopriming (like PEG). In agreement with the result of this study, applying 2.5% PEG influenced on several germination parameters; including germination percentage, MGT, and GRI of pumpkin, however, drought stress had no significant effect on root number or RL (Ashraf et al., 2021). On the other hand, drought stress induced by D-mannitol (-0.24, -0.47, and -0.73 MPa) reduced chlorophyll fluorescence and negatively affected germination and seedling growth traits in squash seedling, including G%, RL, root FW, shoot length, shoot FW, and the shoot-to-root length ratio (Saadaoui et al., 2023). Golob et al. (2020) showed that the G% of pumpkin seeds treated in selenate (SeO_4^{2-}) at a concentration 10 mg. L^{-1} was not significantly affected. On the other hand, pumpkin seedlings were treated with varying concentrations of selenite SeO_3^{2-} (0, 2, 10, 40, 80 μM), the results showed that selenite did not have a beneficial effect on pumpkin development, although it could enhance root activity at doses ranging from 2 to 20 μM . Elevated selenite concentrations (above 40 μM) caused the accumulation of (ROS, $\text{O}_2^{\cdot-}$) in the leaves, inhibited seedling growth, and led to harmful symptoms such as root necrosis and leaf chlorosis (Sun et al., 2024). In additional the application of sodium selenite (Na_2SeO_3) (0, 4, 6, 8, 10 mg. L^{-1}) enhances the G %, promotes shoot and root elongation, and increases both the (FW and DW) of cucumber seedlings under heavy metals stress conditions (Aroiee et al., 2019). Furthermore, the lower concentrations of calcium carbide CaC_2 (10 to 30 mg per plate) significantly promoted HL and RL growth, increased the number of lateral roots, and improved the seedling FW of cucumber seeds when compared with 40 mg and control (Shakar et al., 2016). To conclude, reducing moisture level during the early stage of growth negatively impacts the germination process; however, nutripripping mitigates its effect, particularly using low concentrations of Se 1.5 mg. L^{-1} and Ca^{2+} 5 mg. L^{-1} . In additional soaking melon seeds in sodium selenite (Na_2SeO_3) led to a significant increase in leaf area at 1 mg. L^{-1} during the seedling stage, 30 days after sowing. However, DW increased at 0.1 mg. L^{-1} but not at 1 mg. L^{-1} when compared to the control (de los Santos-Vázquez et al., 2016). The application of 10 mM Ca^{2+} demonstrated in the most favorable outcomes, including a 20.7% increase in the GI, a 15.1% improvement in seedling length and vigor, as well as enhancements in root and shoot FW and DW of tomato seedlings under salt stress conditions (Tanveer et al., 2020).

Priming seed has been practiced to facilitate the process of germination, including imbibition, metabolic activity, cell elongation, and radicle emergence. Various priming techniques including osmopriming, halopriming, hormopriming (Kka et al., 2022, 2025), and nutripripping (Farooq et al., 2019), have been studied and implemented by farmers for their influences on seed germination and later on plant growth performance of vegetable crops.

These findings are supported by (Farooq et al., 2019; Lei et al., 2021; Lutts et al., 2016), who demonstrated that seed priming techniques such as osmopriming with PEG regulate water uptake by creating an osmotic gradient, thus delaying or reducing imbibition. In contrast, priming seeds with Se and Ca^{2+} facilitate water uptake maintains favorable water absorption dynamics by maintaining cell membranes and supporting aquaporin function, allowing normal water uptake during early germination stages. It was reported that osmopriming (i) changes the microstructural features of the seed coat, e.g., leads to the formation of microcracks, (ii) alters the internal structure of the seed by the induction of additional void spaces in the seed, (iii) increases cotyledons cells vacuolization, and (iv) modifies the expression pattern of aquaporin genes (Lechowska et al., 2019). They also were shown that the diameter of primed *Brassica napus* seeds significantly increased after 7 h of plating compared with unprimed seeds. These findings may align with the finding of this study, as in general the seed area size of nutripripping and hydropriming was 2.33% larger than the size of osmopriming on average (Table 3). The most recent articles have demonstrated the impact of nutri-priming with Se and Ca^{2+} on water uptake during imbibition including *Calendula officinalis* L (García-Locascio et al., 2025), *Medicago sativa* (Pan et al., 2025). However, to our knowledge, there is limited published evidence on the influence of Se or Ca^{2+} priming on seed size changes during imbibition, especially within Cucurbits crops. Therefore, the results contribute to understanding the impact of nutripripping Se and Ca^{2+} on seed length, LWR and DS, which represent additional evidence how micronutrient priming modifies morphological hydration dynamics.

In general, it was found that the priming *Vigna radiata* with calcium hydroxide and Se-calcium hydroxide nanocomposites complexed provide better results of shoot length, RL, G%, and dry matter when compared with hydroprimed seeds (Antony et al., 2022). In addition Tamindzic et al. (2025), found that the three studied cucumber cultivars are sensitive to the water stress made using PEG, particularly the germination parameters, including G%, GE, RL, FW, DW, and SVI, were declined at -0.49MPa, (a level considered a moderate drought). In agreement with our data, the recorded values of G%, RL, plumule length, relative water content, protein, carotenoid, and proline were reduced by using PEG6000 (-0.75MP and -1.5MP) compared to hydropriming and non-primed seed of pumpkin

(Jahed et al., 2022). In terms of stress, five different salt concentrations (0.3 dS/m (control); 2.5; 5; 7.5 and 10 dS/m) significantly reduced the SVI, RL, FW, and DW of pumpkin (Irik & Bikmaz, 2024). They also indicate that the germination performance was affected by cultivars and increasing salinity concentrations and reduced Ca^{2+} content. In agreement with the positive impact of priming seeds with Se on germination activities (Table 1) specifically using the form sodium selenite (Na_2SeO_3) 1.5 mg.L^{-1} in this study, Chen & Sung (2001) also demonstrated the form of sodium selenite 1 mg.L^{-1} enhanced the G% and shortened MGT in comparison to the control (hydropriming) and Se 5 and 10 mg.L^{-1} limited the activity of germination under 25°C of two cultivars of bitter gourds (*Momordica charantia* L.). Consequently, Golob et al. (2020) demonstrated no effect of (SeO_4^{2-}) and (SeO_3^{2-}) 10 mg.L^{-1} on the germination of pumpkin seeds when compared with the control. However, he found that the combination between $(\text{SeO}_4^{2-} + \text{I})$ and $(\text{SeO}_4^{2-} + \text{IO}_3^-)$ statistically and significantly enhanced germination in comparison to control. In addition, seeds of zucchini (*Cucurbita pepo* L. var. pepo, cv Courgette d'Italie) primed with CaCl_2 had an improved germination tolerance index, and 15mM was the best concentration when compared with 10, 20 and 25 mM, under controlled conditions. Then, the 15 mM CaCl_2 priming recorded the highest G%, seedling length, and SVI showing no significant difference compared to the control (Tamindzic et al., 2025). The 200 g.L^{-1} CaCl_2 priming recorded the highest G% and the shortest MGT of melon seeds, and the same effect was found when seeds were treated with PEG (Castañares & Bouzo, 2018). On the other hand, the low concentration of Ca^{2+} also influences plant growth and development at different stage (Mosa et al., 2015), he concluded that foliar application of calcium nitrate at 1% concentration positively affected on growth and productivity of "Le Conte" pear trees compared with the control. Therefore, in this study the low concentration of CaCl_2 agent at the concentration of 5 mg.L^{-1} was found to be effective, alongside Se, on germination activity of the local pumpkin cultivar. Therefore, the optimal element concentration and form of Se and Ca^{2+} nutrimpriming are critical to facilitate the imbibition, metabolic activity, cell elongation, and sprouting of seeds.

The defiance chemical and physical mechanism in plants varies according to variety and environmental factors. It was found that trichome density, response to herbivore attack, and mechanical damage was higher in wild populations than in domesticated varieties of squash plants (Ye et al., 2024). As previously mentioned, the trichomes vary in shape, size and function. According to Lin et al. (2023) findings, the trichome observed on the upper surface of cotyledon leaves (Fig. 2) may align and be similar to type I (3mm in length), type II (0.95–1.49 mm in length) and type III (less than 300 μm in length), which is a thick-walled nonglandular trichome. The non-glandular structure was reported to be more effective in impaling predators (Riddick & Simmons, 2014). To our knowledge, due to limited direct publications on the impact of drought and Se and Ca^{2+} on trichome intensity on Cucurbita crops, various studies on different species investigated and presented that drought stimulates the density of trichomes. For example, *Caragana korshinskii* (Ning et al., 2016), *Gossypium Hirsutum* (Ning et al., 2016), and *Arabidopsis thaliana* (Zekri & Lang, 2024). Furthermore, Se and Ca^{2+} in general demonstrate their function in improving drought tolerance in crop plants by regulating water status, reducing oxidative stress, and enhancing growth, respiration, and photosynthesis (Ahmad et al., 2016; Antony et al., 2022; Zhao et al., 2024). A study revealed a significant difference in total trichome counts between glandular and non-glandular trichomes in cucumber and bottle gourd (Kaur & Kariyat, 2023).

In short, priming pumpkin seeds with Se and Ca^{2+} can increase the number of trichomes on adaxial of cotyledon leaf under control and drought induced conditions. While low water potential generally reduces trichome number, possibly diminishing these protective benefits. Further studies are required to clarify how these treatments modulate trichome density and structure at molecular level in pumpkin plants.

Conclusion

Seed priming is widely used to improve early seedling vigor and germination efficiency, particularly under stress conditions, with drought exerting the most pronounced effect on imbibition, metabolism, and cell elongation during early germination. This study evaluated the effects of priming local pumpkin seeds with Se and Ca^{2+} on germination performance under both limited moisture and PEG 8000-induced osmotic stress.

Under controlled moisture, Se-primed F_0 seeds at 6 mL moisture achieved the highest GP, GE, GI, SVI, and DW, while Ca^{2+} -primed seeds showed the highest chlorophyll content and FW. Germination traits did not differ significantly between the 6 mL and 3 mL moisture levels overall, but under the more limited 3 mL level, Se and Ca^{2+} priming produced substantially better germination outcomes than the control.

Maximum imbibition rate and the largest seed size were reached after 6–24 h of imbibition, while 5% and 10% PEG both significantly reduced germination traits in F_1 seeds. Se and Ca^{2+} priming improved germination performance under osmotic stress, and trichome number on the adaxial cotyledon surface increased with priming under both non-stressed and PEG-stressed conditions, though this increase did not fully offset the reduction in trichome number caused by higher PEG concentrations.

Collectively, these results indicate that nutrimpriming with low concentrations of Se and Ca^{2+} improves pumpkin seed germination and early seedling performance under both water-deficit and osmotic stress. This study was limited to a single local cultivar and a single generation cycle (F_0/F_1), and did not examine the molecular basis of the observed trichome and hydration responses; future work extending this approach to additional cultivars and incorporating molecular analyses would help clarify the underlying mechanisms. These findings nonetheless point to Se and Ca^{2+} nutrimpriming as a practical, low-cost strategy for improving crop establishment in water-limited growing environments.

Declarations

Disclosure statement

No potential conflict of interest

Data availability statement

Data will be made available on request.

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Figures

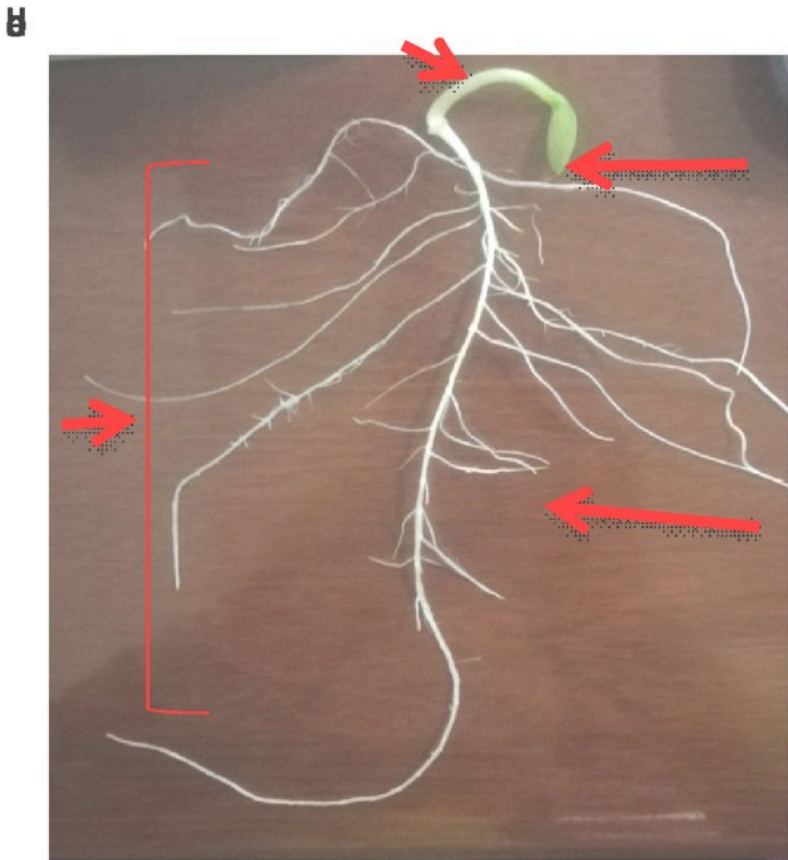


Figure 1

Representative 10-day pumpkin seedling. (a) cotyledon, (b) hypocotyl, (c) radicle, (d) root length.

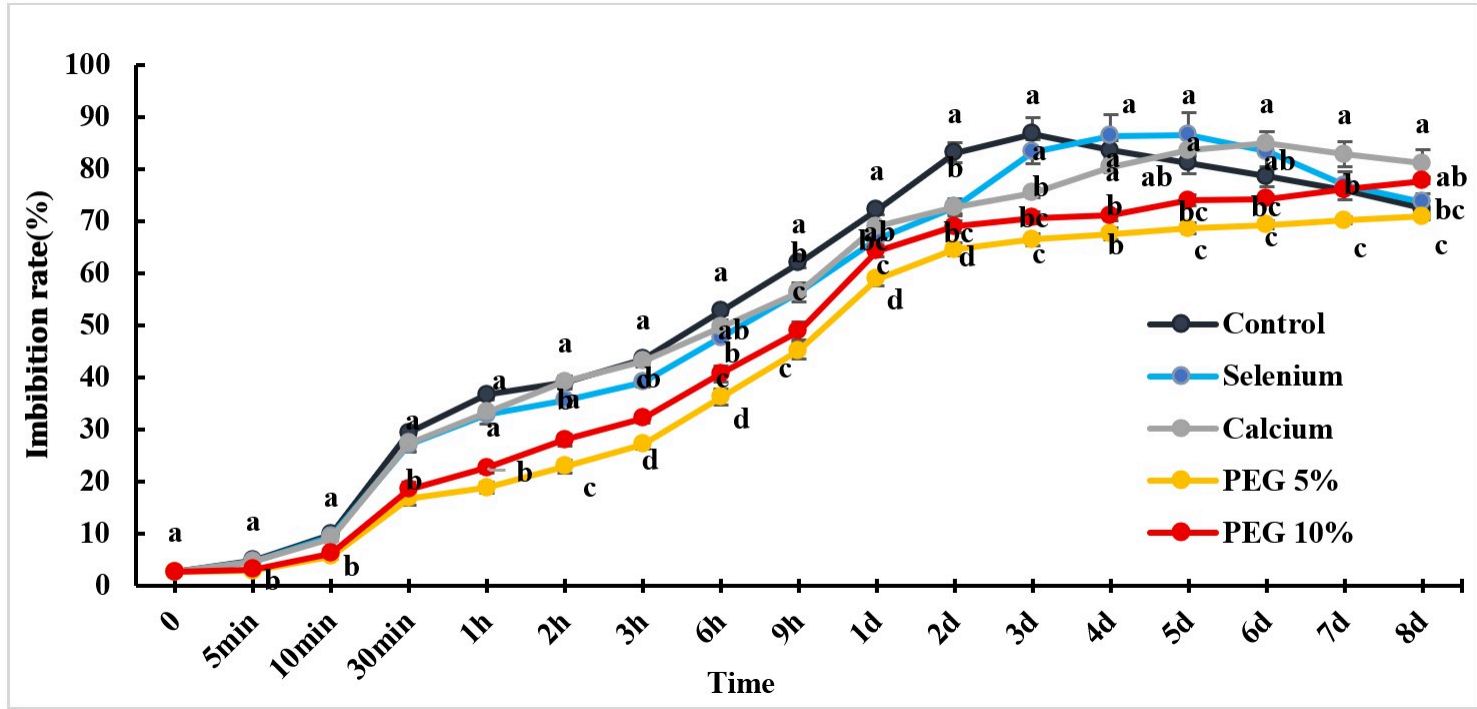


Figure 2

Impact of hydropriming, nutripriming and osmopriming on imbibition rate of pumpkin seeds. Means in the same line with different letters are significantly different at $\alpha = 0.05$ according to Duncan test.

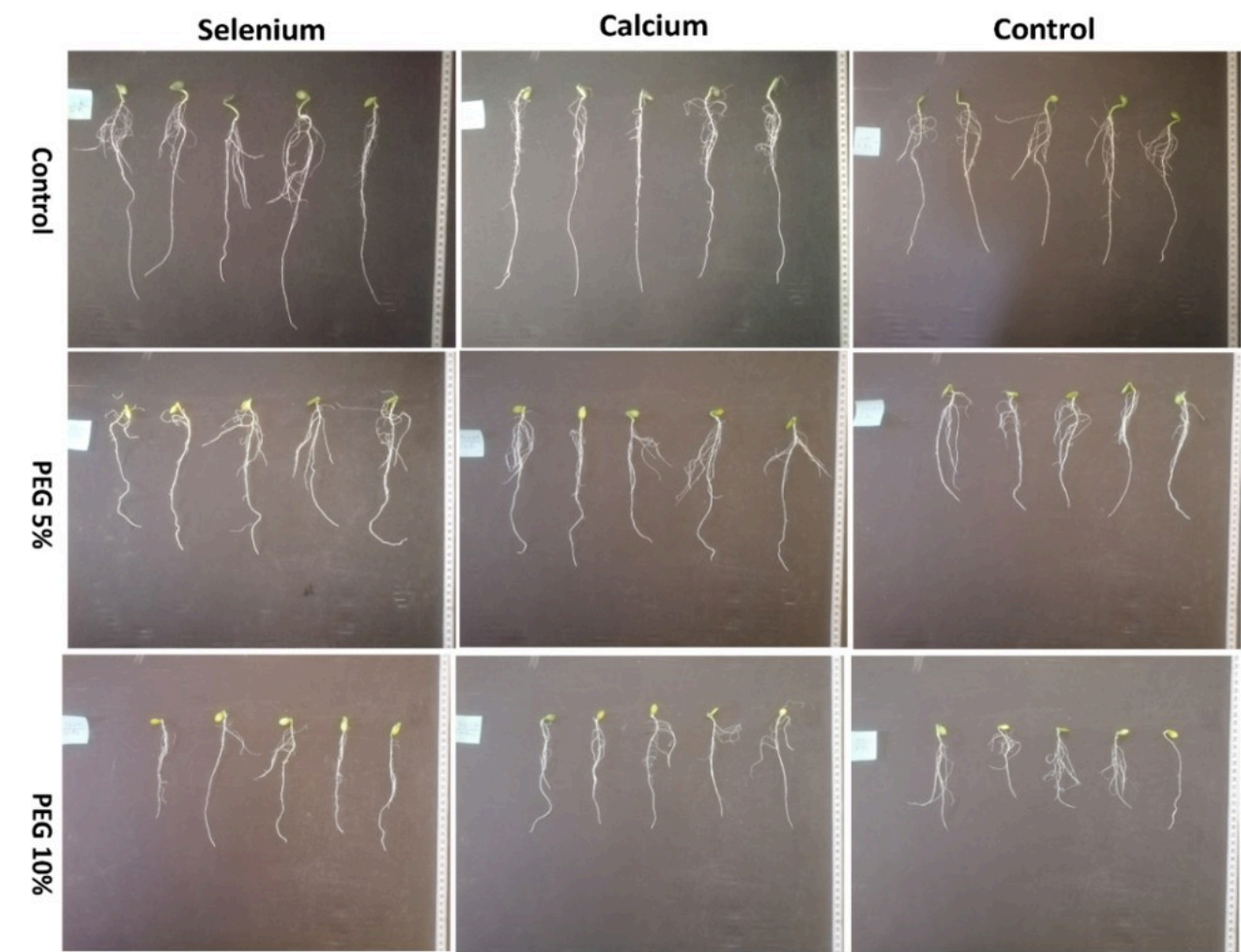


Figure 3
The effect of osmopriming and nutripriming on seedling length of pumpkin local cultivar F1

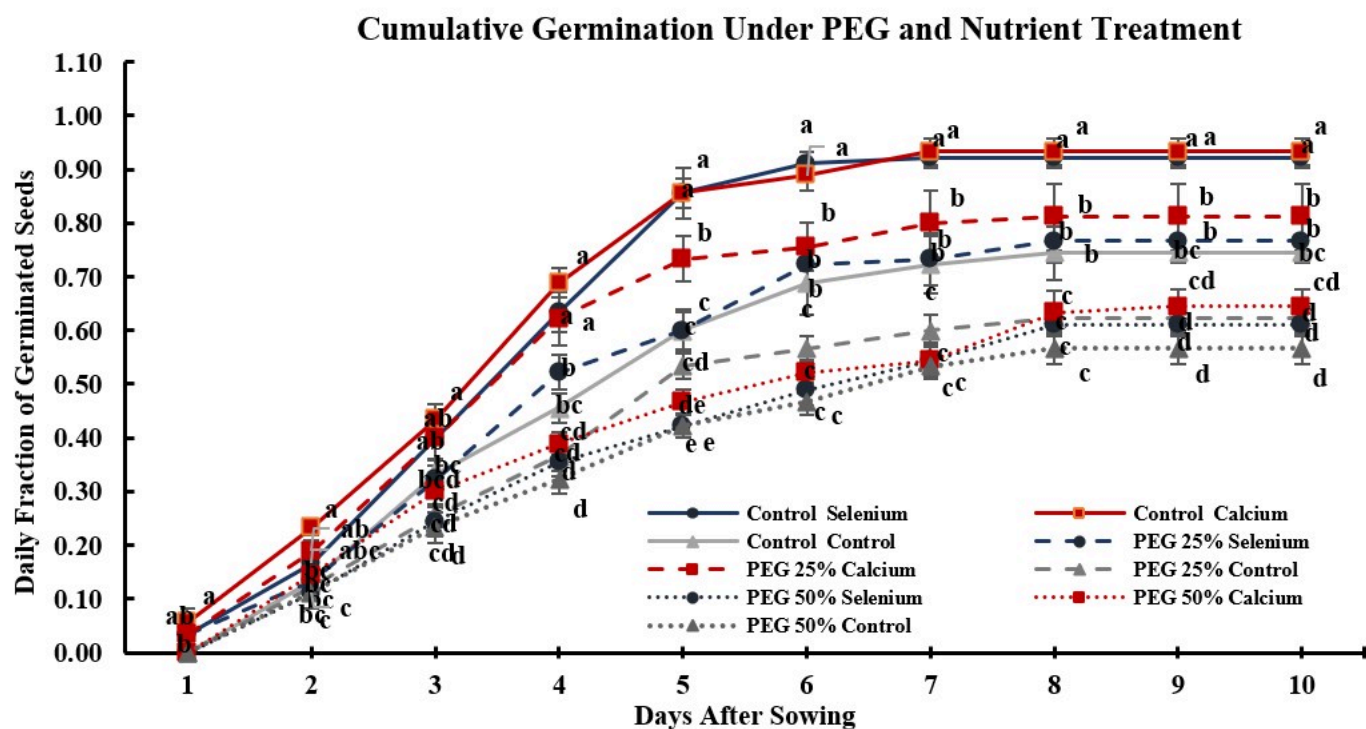


Figure 4

The two-way interaction of osmopriming and nutrimpriming on daily fraction of germination of pumpkin local cultivar F1. Means in the same line with different letters are significantly different at $\alpha = 0.05$ according to Duncan test.

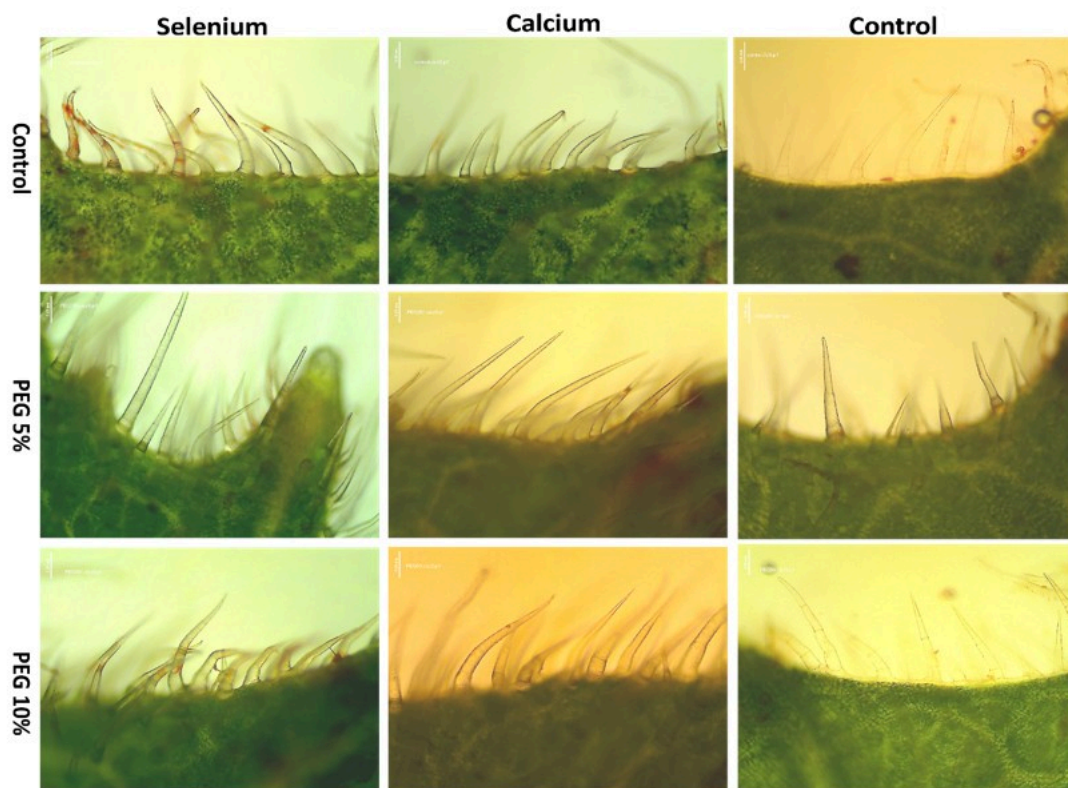


Figure 5

Distributions and structure of trichomes on adaxial of cotyledon leaf of pumpkin local cultivar, as affected by PEG and nutrimpriming.