**Effects of stimulants on the germination and early development of two purple maize (*Zea mays* L.) varieties**

Sandy Vilchez-Navarro1,2†; Arturo Morales-Pizarro2†; Henry Morocho-Romero1,2 Ricardo Peña-Castillo2; Sebastian Casas-Niño1; Gabriela Cárdenas-Huamán1 Nery Tirabante-Terrones1 Esdwin-Oberti Nuñez-Ticliahuanca1,2; Ana Montañez-Artica1; Leslie Diana Velarde-Apaza1; Max Ramirez-Rojas1; Juancarlos Cruz Luis1; Flavio Lozano-Isla1,3#

*1 Dirección de Supervisión y Monitoreo en las Estaciones Experimentales Agrarias, Instituto Nacional de Innovación Agraria. Lima. Perú.*

*2 Universidad Nacional de Piura, Campus Universitario s/n. Urb. Miraflores. Piura, Perú.*

*3 Facultad de Ingeniería y Ciencias Agrarias, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM), Chachapoyas, Peru*

*+ Equal contributing author.*

*#* Corresponding author: [flavio.lozano@untrm.edu.pe](mailto:flavio.lozano@untrm.edu.pe)

Authors’ Information

| Author | ORCID | Email |
| --- | --- | --- |
| Sandy Vilchez-Navarro | 0009-0002-1784-5563 | 0202018140@alumnos.unp.edu.pe |
| Arturo Morales-Pizarro | 0000-0003-3966-6689 | dmoralesp@unp.edu.pe |
| Henry Morocho-Romero | 0000-0002-1520-2372 | 0202018122@alumnos.unp.edu.pe |
| Ricardo Peña-Castillo | 0000-0001-9366-4962 | rpenac@unp.edu.pe |
| Sebastian Casas-Niño | 0000-0002-6576-8761 | 20140231@lamolina.edu.pe |
| Gabriela Cárdenas-Huamán | 0000-0002-8379-5464 | 20200607@lamolina.edu.pe |
| Nery Tirabante-Terrones | 0000-0002-0634-1522 | n229754@dac.unicamp.br |
| Edwin-Oberti Nuñez-Ticliahuanca | 0009-0004-4613-0625 | enunezt@egresados.unp.edu.pe |
| Ana Montañez-Artica | 0000-0003-3580-6621 | 20140725@lamolina.edu.pe |
| Leslie Diana Velarde-Apaza | 0000-0001-6031-6355 | leslie.velarde@upn.pe |
| Max Ramirez-Rojas | 0000-0003-3322-0838 | proyectochira@inia.gob.pe |
| Juancarlos Cruz | 0009-0005-8288-2768 | jcruz@inia.gob.pe |
| Flavio Lozano-Isla | 0000-0002-0714-669X | flavio.lozano@untrm.edu.pe |

**Highlights**

* Stimulants enhance vigorous germination in native and hybrid PMV 581 purple maize.
* Purple maize seeds reach peak imbibition rate in 3 hours, gaining 10%-35% weight.
* Seaweed application boosts plant height and stem thickness in hybrid purple maize.
* Whey and sulfur improve root growth in native purple maize seedlings.

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# Abstract

Maize (*Zea mays* L.) is the world’s third most important cereal crop. Purple maize is an exportable agricultural product due to its high anthocyanin content. This crop is directly sown in the field, where seed germination determines the density of established plants, which directly impacts yield. This research was conducted under controlled laboratory and greenhouse conditions. The genetic material used included seeds of native purple maize and the hybrid PMV 581. The experiment was set up under a completely randomized design with three replications. The effect of three stimulants, milk whey, sulfur dioxide, and seaweed was evaluated on germination and early development. The results demonstrated that the application of stimulants positively affected the germination percentage and seedling development indicators. Compared with the control, the seaweed-based treatments at both doses (1 and 1.5 L/200 L) resulted in greater values for the following variables: number of roots, plant height, stem thickness, leaf width, thickness, and length, and dry weight of the shoots. This finding indicates that its application as a pre-germination treatment promotes root and shoot development in maize seedlings at early growth stages.

**Keywords:** anthocyanin; growth stimulants; purple corn; seaweed; seed germination

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**Author Contributions**

Conceptualization, S.V-N., R.P-C. and A.M-P.; methodology, S.V-N. and G.C-H.; formal analysis, F.L-I. and S.C-N.; investigation, S.V-N.; resources S.V-N.; data curation, F.L-I. and S.C-N.; writing—original draft preparation, S.V-N., H.M-R., G.C-H., N.T-T., E-O.N-T., A.M-A, F.L-I. and S.C-N.; writing—review and editing, F.L-I. and L.V-A.; visualization, F.L-I., and S.C-N.; supervision, J.C. and M.R-R.; funding acquisition, J.C. All the authors have read and agreed to the published version of the manuscript.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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**Data availability**

The original contributions presented in this study are included in the article and supplementary material. The reproducible data analysis and datasets are available in Supplementary File 2 and can be accessed through the GitHub repository at: <https://github.com/Sebass96/prochira_maiz_morado>

# Introduction

Maize (*Zea mays* L.) is part of the Poaceae family and was domesticated in southern Mexico approximately 9,000 years ago [(Panda et al., 2020)](https://www.zotero.org/google-docs/?BjFQmB). Maize is the third most important crop in the world, following wheat and rice [(Kaushal et al., 2023)](https://www.zotero.org/google-docs/?3dryQA). Global maize production has increased in recent decades, and maize is the leading cereal in terms of production volume [(Erenstein et al., 2022)](https://www.zotero.org/google-docs/?T9YK4N). In Peru, four types of maize are produced: yellow maize, starchy maize, green maize, and purple maize. In 2023, purple maize exports reached $2.25 million, with a price of $2.04 per kilogram (AGRODATA PERU, 2024).

Purple maize is the only grain in the world where the kernels, husks, and cobs are purple to black due to the presence of anthocyanins. It has a high anthocyanin content, and its flavonoids are water-soluble, and possess antioxidant and anti-inflammatory properties [(Rabanal-Atalaya & Medina-Hoyos, 2021)](https://www.zotero.org/google-docs/?bHtX0o). Its consumption preferences are primarily based on color, flavor, and nutritional benefits [(Chachar et al., 2024)](https://www.zotero.org/google-docs/?BFA9HY). In addition to its high content of minerals, vitamins such as ascorbic acid and B complex, proteins are present in the endosperm, and it is rich in phytochemicals such as anthocyanins, melatonin, and tryptophan [(Duangpapeng et al., 2019)](https://www.zotero.org/google-docs/?4LMRWP). It is used in the food and pharmaceutical industries as a healthier alternative to synthetic additives [(Rabanal-Atalaya & Medina-Hoyos, 2021)](https://www.zotero.org/google-docs/?5UM357).

Maize is directly sown in the field, and its productivity depends on germination and plant density [(da Silva et al., 2021)](https://www.zotero.org/google-docs/?lLAD3M). Seed germination is a critical stage in crop production that directly affects the yield and quality of maize grains [(Han et al., 2020)](https://www.zotero.org/google-docs/?OA0C6W). Therefore, good initial development of seeds with high sanitary and physiological qualities allows proper adaptability, plant vigor, and high yields [(Huang et al., 2018; Vieira et al., 2019)](https://www.zotero.org/google-docs/?mBBewB). Pre-sowing seed treatments have proven to be effective techniques for achieving early emergence, optimal field density, and a high percentage of germination [(K et al., 2020)](https://www.zotero.org/google-docs/?9McfkN). These treatments, combined with extended imbibition time, increase seed hydration and the initiation of germination [(Morales Pizarro et al., 2023)](https://www.zotero.org/google-docs/?NXgddv). During the germination process, both gibberellic acid (GA) and reactive oxygen species (ROS) act to mobilize reserve substances, break the seed coat, improve imbibition, and promote the emergence of the radicle [(Jhanji et al., 2024)](https://www.zotero.org/google-docs/?UEBZuh). Seaweed extracts of hard yellow maize seeds stimulate root growth, esterase activity, ATP formation, and nutrition [(Ertani et al., 2018)](https://www.zotero.org/google-docs/?sSPmoX). Another input used as a pre-sowing treatment is sulfur in the form of sulfur dioxide (SO₂), which promotes the production of reactive oxygen species (ROS). These ROS contribute to the induction of α-amylase activity, facilitating the release of nutrients from the endosperm and promoting the germination of maize seeds treated with SO₂ [(Guo et al., 2021)](https://www.zotero.org/google-docs/?2DGflE). Recent studies have shown that whey improves maize growth traits, including shoot height, number of leaves, shoot weight, and cob weight [(Abdelraof et al., 2023)](https://www.zotero.org/google-docs/?YTR5bF).

The objectives of this research were: (a) to evaluate the germination parameters of a hybrid variety compared with those of a native strain of purple maize seeds via organic biostimulants, and (b) to identify the best organic biostimulant that positively influences the growth and development of purple maize producing seedlings.

# Materials and methods

## Study location

The study was conducted in the greenhouse of the Department of Plant Morphophysiology at the Faculty of Agronomy, National University of Piura, Peru, located at 5°10’33” S and 80°37’17” W (Figure 1), at an altitude of 30 m above sea level, from December 2022 to February 2023.

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| Figure 1: Geographical location of the laboratory to evaluate the effect of stimulants in the germination and initial biometrics of two varieties of purple maize (*Zea mays* L.) conducted in the Piura Region. |

## Plant material and stimulants

In this study, two purple maize varieties, the native and the hybrid PMV 581, were used. The native variety was obtained from purple maize cobs harvested in August 2022, in the Santa Elena sector of Pueblo Nuevo de Colán, Paita district located at 5°5’21.01” S, 81°6’51.98” W, at an altitude of 3 m above sea level. The best cobs with the most favorable morphological characteristics, including shape, large size, and freedom from diseases and abnormalities were selected. The seeds were subsequently separated, washed, and placed on previously flamed paper towels for drying for 12 hours at room temperature (27±3 °C). The seeds were disinfected with 76% alcohol for 5 seconds and then dried at room temperature.

The hybrid variety is derived from the Morado de Caraz variety [(Medina-Hoyos et al., 2020a)](https://www.zotero.org/google-docs/?1Bt6b0). It was developed by professionals from the Maize Program at the Universidad Nacional Agraria, La Molina. It is adapted to the coastal and lowland regions of Peru and is resistant to certain pests and diseases common in purple maize cultivation. It is characterized by a yield of 6 t/ha with an intermediate vegetative period, and medium-sized cobs ranging from 15 to 20 cm, with a high pigment content.

Two organic products and one inorganic product were used. Seaweed ‘Algaeforte’ (Aris industrial, Peru), dry sulfur powder 930 g/kg (Aris industrial, Peru), and mil whey were obtained from cheese production. For this purpose, a vegetable rennet tablet was added to the milk at a temperature of 37 °C; the liquid suspended at the top was considered whey [(Sousa & Malcata, 1998)](https://www.zotero.org/google-docs/?O9GlRE).

## Seed characterization and presowing treatments

Seed characterization was carried out in the Plant Morphophysiology Laboratory of the Faculty of Agronomy. The dimensions, length, width, thickness (mm), and weight (g) of 150 seeds from each species under study were evaluated. The seed shape was determined based on its length/width ratio: flat seed (<1), round seed (=1), and oval seed (>1), according to the description by Ruesta-López et al. [(Ruesta-López et al., 2024)](https://www.zotero.org/google-docs/?cYbUnP)

For the presowing treatment, the seeds were disinfected by immersion in 70% alcohol for five seconds. They were then placed on previously flamed paper towels for more than three hours for drying. The disinfected seeds were immersed for 12 hours in the six treatments (Table 1). The treated seeds were placed on paper towels, sterilized by flaming, and moistened with distilled water, inside Petri dishes. The treatments used were distilled water (T0), seaweed 1 L/200 L (T1), seaweed 1.5 L/200 L (T2), sulfur 100 g/200 L (T3), sulfur 150 g/200 L (T4), milk whey 10% 10 mL/90 mL (T5), and milk whey 30% 30 mL/70 mL (T6).

**Table 1:** Pre-sowing treatments used on purple maize seeds (*Zea mays* L.) from the native variety and Hybrid PMV 581 to evaluate their influence on germination and early development. Seeds were immersed in the seeds treatment solutions for 12 hours. |

| **Number** | **Description** | **Dose** |
| --- | --- | --- |
| T0 | distilled water | 100% |
| T1 | seaweed | 1 L/200 L |
| T2 | seaweed | 1.5 L/200 L |
| T3 | sulfur | 100 gr/200 L |
| T4 | sulfur | 150 gr/200 L |
| T5 | milk whey | 10% 10 mL/90 mL |
| T6 | milk whey | 30% 30 mL/70 mL |

## Seed germination experiment

The germination experiment was conducted using a completely randomized design (CRD) to evaluate the effect of sowing treatments on the germination of two purple maize varieties. Three replications were established for each treatment. Each replication consists of 25 seeds. Germination was assessed daily over five (5) days at 27±3 °C until the radicle emergence as the criterion for germination [(Lozano-Isla et al., 2019)](https://www.zotero.org/google-docs/?l57MRP).

The traits assessed during the seed germination were carried out under aseptic conditions in the laboratory, according to Morales Pizarro et al., [(Morales Pizarro et al., 2023)](https://www.zotero.org/google-docs/?9NtOdd). The seed imbibition rate (IR) was evaluated during the pre-sowing treatment. Additionally, three germination indices were evaluated, germination percentage (GP), germination velocity (GV), and germination index (GI).

***Imbibition rate*** **(IR, %):** It was expressed according to the formula by Escobar-Álvarez et al., [(Escobar-Álvarez et al., 2021)](https://www.zotero.org/google-docs/?nGSNWt) which relates the percentage increase in seed weight (absorption or hydration of water) to the initial and final weight of the seed [(Ruesta-López et al., 2024)](https://www.zotero.org/google-docs/?4OStxn).

***Germination percentage*** **(GP, %):** GP was calculated using the following formula [(Escobar-Álvarez et al., 2021)](https://www.zotero.org/google-docs/?UxvsxB): .

***Germination speed*** **(GS, days):** GV was determined using the formula proposed by Gutiérrez-Gutiérrez et al., [(Gutiérrez-Gutiérrez et al., 2022)](https://www.zotero.org/google-docs/?cWwtrJ) :

***Germination index*** **(GI):** GI was calculated using the formula proposed by Scott et al. [(Scott et al., 1984)](https://www.zotero.org/google-docs/?2jk6sl):

## Early development stage

In the greenhouse stage, a completely randomized design (CRD) with 3 replications was used, with each replication consisting of 30 seedlings per treatment. The seeds treated with the different stimulants (Table 1) were sown in germination trays measuring 54.5 x 28 cm, with a total of 72 cells, each with a capacity of 5 cc, containing sterile substrate at a 2:1 ratio (humus: river sand). Each cell was sown with 2 seeds and after emergence, only one seedling per cell was maintained.

Fifteen days after emergence (DAE), 15 plants were randomly selected per treatment. Eleven traits were evaluated, root length (RL, cm), root thickness (RT, mm), number of roots (NR), plant height (PH, cm), stem thickness (ST, mm), leaf length (LL, cm), leaf width (LW, cm), number of leaves (NL), dry root weight (DRW, g) and dry stem weight (DSW, g).

## Statistical analysis

Statistical R software version 4.4.1 was used for the data analysis and graphics (R Core Team 4.4.1, Supplementary Material 2, [(R Core Team, 2024)](https://www.zotero.org/google-docs/?H0MnW4)). Analysis of variance was performed to determine if there were differences between the stimulant treatments. The mean comparison test used was Tukey’s test, implemented in the *emmeans* package [(Lenth et al., 2024)](https://www.zotero.org/google-docs/?EO7zMK). The traits under study were subjected to multivariate principal component analysis (PCA) via the *FactoMineR* package. Correlation analysis between the variables was performed via the corrplot package [(Husson et al., 2024)](https://www.zotero.org/google-docs/?Po8Ah4) .

# Results

## Seed characterization and germinability

To standardize the evaluations for determining the imbibition process and germination, both native and hybrid purple maize seeds were characterized (Table 2).

The seed length (L) did not significantly differ between the varieties (p-value = 0.93). The average seed length was 14.85±0.09 mm. The hybrid PMV-581 had a seed width (W) that was significantly greater, measuring 12.67 mm, than the native variety which had a width of 10.6 mm. For the L/W ratio, the obtained values were greater than 1.00, indicating that the varieties had oval shapes. The native variety presented the highest value and the oval shape. For seed thickness, no significant differences were observed quantitatively. In terms of seed weight, the hybrid had a greater weight of 0.64 g, whereas the native seed weighed 0.46 g (Table 2).

**Table 2:** Seed characterization of two varieties of purple maize (*Zea mays* L.). A total of 150 seeds per variety were evaluated, determining the following parameters: length (L, mm), width (W, mm), length/width ratio, thickness (mm), and weight (g). Mean comparison tests using Tukey were employed to compare the means between varieties. Distinct letters in the same row indicate significant differences (p ≤ 0.05) between the varieties for each characteristic. |

| **Variety** | **Seed Length (mm)** | **Seed Width (mm)** | **Length/Width** | **Seed Thickness (mm)** | **Seed Weight (g)** |
| --- | --- | --- | --- | --- | --- |
| Creole | 14.76±0.09 a | 10.69±0.07 b | 1.39±0.01 a | 5.5±0.09 b | 0.46±0.01 b |
| Hybrid | 14.77±0.09 a | 12.6±0.07 a | 1.17±0.01 b | 6.13±0.09 a | 0.64±0.01 a |

**Seed imbibition and germination**

To promote vigorous seed germination and improve the productivity of native and hybrid PMV 581 purple maize. Germination tests were conducted under controlled laboratory conditions. The effects of different stimulants on germination variables were evaluated to determine whether there were significant differences in germination capacity between the two varieties and to identify the most effective stimulants for improving germination parameters.

For the imbibition rate (IR) variable, the seeds of both native and hybrid PMV-581 maize achieved the highest hydration at three (3) hours, increasing in weight to between 10% and 35% (Figure 2a). However, this process was stationary between 3 and 6 hours, and then slightly reactivated between 6 and 12 hours. In the native maize seeds, those in the T1 treatment were 35% greater in weight than those in the other treatments, followed by those in the T0 and T5 treatments, which had an imbibition rate (IR) of 20%. However, treatments T2, T3, T4, and T6 had lower IRs, ranging from 10% to 15%.  In hybrid PMV-581 maize seeds, treatment T0 increased the imbibition rate (IR) to 35%, which was higher than those of treatments T2, T3, T4, T5, and T6, all with an IR of 20%, and higher than that of T1, which had the lowest IR at 16%.

In terms of the germination percentage (GP) variable, native purple maize presented the highest GP in the T0 treatment (86%) and T5 treatment (70%), which were significantly greater than those in the other treatments. For hybrid PMV-581 maize, the treatments did not significantly differ, with GP values ranging from 43% to 86%. However, the highest GP was achieved by treatment T1 ( Figure 2b). On the other hand, for the germination velocity (GV), the native purple maize did not significantly differ between treatments, with values ranging from 1.28 to 3.06 seeds germinated per day. In hybrid PMV-581 maize, treatment T3, with 4.83 seeds germinated per day, was significantly superior to the other treatments (Figure 2c).

In terms of the germination index (GI), native purple maize had the highest GI in the T0 treatment (2.6), followed by the T5 treatment (1.67), with no significant differences between these treatments. In hybrid PMV-581 maize, the treatments did not significantly differ, with GI values ranging from 1.07 to 2.07 (Figure 2d).

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| Figure 2: Seed imbibition and germination indices evaluated under use of stimulants in two purple maize varieties. (a) Imbibition of native purple maize and hybrid PMV 581 seeds (b) Germination percentage (GP), (c) Germination speed (GS), (d) Germination index (GI). Different letters represent significant differences in the Tukey post hoc test (p < 0.05). |

**Effects of biostimulants in the greenhouse**

To evaluate the effects of different stimulants on the growth and initial development of purple maize seedlings, biometric characteristics were assessed 15 days after germination. Treatments with different concentrations of stimulants were compared to a control group (T0, Figure 3).

For the root length variable, significant differences were observed between treatments and varieties (p-value < 0.001). Notably in the native variety, the highest average was achieved with the application of sulfur at 150 g/200 L, at 15.13 cm, followed by the 10% milk whey treatment (10 mL/90 mL), at 15.8 cm. In contrast, the lowest average was recorded in the control treatment at 11 cm. On the other hand, for the hybrid variety, the highest average was recorded in the control treatment at 16 cm, followed by the 30% milk whey treatment (30 mL/70 mL) at 15.06 cm. Conversely, the lowest average was recorded in the treatment with 100 g/200 L sulfur, at 12.46 cm (Figure 3a).

On the other hand, for the root thickness variable, significant differences were found between the treatments and varieties (p-value < 0.001). For the native variety, the highest mean was found with the control treatment at 1.25 mm, followed by the treatment with 10% milk whey 10 mL/90 mL with 1.10 mm. However, the lowest mean was recorded in the treatment with 1 L/200 L seaweed, with a value of 0.74 mm. On the other hand, in the hybrid variety, the highest mean was recorded in the control treatment at 1.08 mm, whereas the lowest mean was observed in the treatment with sulfur 100 g/200 L sulfur at 0.77 mm (Figure 3b).

In terms of the number of roots, significant differences were observed between the treatments and varieties (p-value < 0.001). For creole variety, the greatest mean number of roots was observed in the treatment with 1.5 L/200 L seaweed at 15.06 whereas the lowest mean was recorded in the control treatment at 10.86. For the hybrid variety, the highest mean number of roots was obtained with the T1 treatment at 15.73, whereas the lowest mean was recorded with the 30% milk whey treatment (30 mL/70 mL) at 10.26. These findings suggest that the application of seaweed to both varieties stimulated greater root formation (Figure 3c).

Additionally, for root dry mass, no significant differences were observed between treatments and varieties (p-value = 0.83). However, for the creole variety, the highest average was obtained with the treatment where 1 L/200 L of seaweed was applied, with a value of 1.21 g, whereas the lowest average was recorded in the control treatment with a value of 0.63 g, indicating an increase in root dry mass. For the hybrid variety, the highest average was obtained with the treatment where 100 g/200 L of sulfur was applied, with 1.25 g, whereas the lowest average was recorded in the control treatment with 0.66 g, representing an increase in dry mass of 0.59 g ([Figure 3](#fig-id.bsplcrw9gnh)d).

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| Figure 3: Roots traits of two purple maize varieties, creole and hybrid PMV 581. The analysis is based on fifteen plants per treatment (n = 105). Where (a) root length (cm), (b) root thickness (mm), (c) number of roots, (d) dry root weight (g). Different letters represent significant differences in Tukey’s multiple comparison tests (p < 0.05). |

Statistical analysis via Tukey’s post hoc test (α = 0.05) revealed significant differences in plant height based on the interaction between treatments and varieties (p-value < 0.001). Significant differences were observed between the combinations of each treatment and the varieties, except for the treatments with 100 g/200 L sulfur and 10% milk whey (10 mL/90 mL), where similar effects were noted. When the creole variety was combined with distilled water (30.80 cm) and 150 g/200 L sulfur (40.53 cm), the average plant height was greater than that of the hybrid. In contrast, the hybrid variety, when combined with the 1 L/200 L seaweed, 1.5 L/200 L seaweed, and 30% milk whey (30 mL/70 mL), with heights of 46.33 cm, 44.20 cm, and 28.80 cm respectively, achieved greater average plant heights than did the creole variety. Additionally, the creole variety, with the application of seaweed and sulfur at both doses, presented relatively high average plant heights of 40.20 cm and 40.50 cm, respectively. In contrast, the application of milk whey at both doses resulted in lower values of 24.60 cm, and the control with distilled water had an average height of 30.80 cm. Similarly, for the hybrid variety, the highest average plant heights were achieved with the application of seaweed and sulfur, measuring 46.30 cm and 37.07 cm, respectively. The lowest values were observed with the application of milk whey, at 28.80 cm, and distilled water, at 26.13 cm (Table 3).

For stem thickness, significant differences were also found between the interactions of treatments and varieties (p-value < 0.01). Significant differences were observed between the combinations of the varieties with distilled water, 100 g/200 L sulfur, and 150 g/200 L sulfur, where the creole variety presented higher average values than did the hybrid with stem thicknesses of 4.47 mm, 4.51 mm, and 4.32 mm of stem thickness, respectively. Additionally, the creole variety presented higher averages with the application of seaweed at both doses, reaching 5.03 mm; conversely, the lowest values were observed with the application of milk whey, which had an average of 4.1 mm. For the hybrid variety, the highest averages were also observed with the application of seaweed, with an average of 5.38 mm, in contrast to the application of distilled water and 150 g/200 L sulfur, where the lowest averages were recorded at 3.89 mm and 3.70 mm, respectively. Compared with the hybrid with a 30.2 mm stem thickness, the creole varieties presented, on average, greater values of 31.48 mm (Table 3).

For the leaf length variable, significant differences were found between the interactions of treatments and varieties (p-value < 0.001). Significant differences were observed between the combinations of varieties with distilled water and 150 g/200 L sulfur, where the creole variety presented higher averages of 26.20 cm and 28.27 cm in length, respectively. Seaweed 1 L/200 L and 10% milk whey (10 mL/90 mL), it presented higher averages with values of 28.33 cm and 26.47 cm, respectively. On the other hand, the creole variety presented higher averages with the application of seaweed, with a leaf length of 34.80 cm; conversely, the application of milk whey resulted in the lowest values, with an average of 21.53 cm

Table 3: Early development evaluation of purple maize seedlings in two purple maize varieties. Plant height, stem thickness, number of leaves, leaf length, leaf thickness, leaf width, and shoot dry weight. Calculations were based on fifteen seedlings assessed per treatment in two varieties (n = 105). Different letters indicate significant differences as determined by Tukey’s test at a 95% confidence level. |

| **Variety** | **Treatment** | **Plant height (cm)** | **Stem thickness (mm)** | **Leaves number** | **Leaf length (cm)** | **Leaf thickness (mm)** | **Leaf width (mm)** | **Shoot Dry weight (g)** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Creole | T0 | 30.8 a | 4.47 a | 5.01 a | 26.2 a | 0.97 a | 18.87 a | 0.71 a |
| Hybrid | T0 | 26.13 b | 3.89 b | 4.67 b | 22.67 b | 0.88 a | 16.61 b | 0.43 a |
| Creole | T1 | 40.27 b | 4.85 a | 5.53 a | 28.33 b | 0.63 b | 18.96 a | 1.5 b |
| Hybrid | T1 | 46.33 a | 4.92 a | 5.47 a | 36.27 a | 0.84 a | 20.04 a | 2.03 a |
| Creole | T2 | 39.4 b | 5.03 a | 5.53 a | 34.8 a | 0.7 a | 19.03 b | 1.09 a |
| Hybrid | T2 | 44.2 a | 5.38 a | 5.53 a | 33.87 a | 0.76 a | 21.77 a | 1.21 a |
| Creole | T3 | 38.27 a | 4.51 a | 4.99 a | 28.6 a | 0.63 a | 19.05 a | 1.72 a |
| Hybrid | T3 | 37.07 a | 4.02 b | 5.01 a | 28.93 a | 0.69 a | 17.52 a | 1.41 b |
| Creole | T4 | 40.53 a | 4.32 a | 5.15 a | 28.27 a | 0.69 a | 17.07 a | 1.19 a |
| Hybrid | T4 | 33.07 b | 3.7 b | 5.01 a | 23.33 b | 0.64 a | 16.07 a | 1.1 a |
| Creole | T5 | 27.53 a | 4.2 a | 5 a | 23.4 b | 0.94 a | 18.29 a | 0.65 a |
| Hybrid | T5 | 30.27 a | 4.07 a | 5.01 a | 26.47 a | 0.81 b | 19.52 a | 0.64 a |
| Creole | T6 | 24.67 b | 4.1 a | 5 a | 21.53 a | 0.81 a | 17.55 a | 0.55 a |
| Hybrid | T6 | 28.8 a | 4.22 a | 5 a | 23.07 a | 0.72 a | 17.86 a | 0.46 a |

For the hybrid variety, the highest average length was also observed with the application of seaweed, with an average leaf length of 36.20 cm. In contrast, the lowest averages were observed with the application of distilled water, with a leaf length of 22.60 cm (Table 3).

For leaf thickness, significant differences were also observed between the interactions of treatments and varieties (p-value < 0.01). Significant differences were noted between the varieties in the 1L/200 L seaweed treatment, where the hybrid variety presented greater averages of 0.84 mm; and 10% milk whey treatment (10 mL/90 mL), where the creole variety had higher averages with values of 0.94 mm. Similarly, the creole variety presented higher averages with the application of distilled water, reaching 0.97 mm; in contrast, the application of milk whey resulted in the lowest values, with an average of 0.63 mm. For the hybrid variety, the highest averages were also observed with the application of distilled water, with an average of 0.88 mm, whereas the application of sulfur resulted in the lowest averages, with 0.64 mm of leaf thickness (Table 3).

For leaf width, significant differences were observed between the interactions of treatments and varieties (p-value < 0.01). Significant differences were noted between the combinations of varieties with distilled water treatment, where the creole variety had a greater average leaf width of 18.87 mm; and 1.5 L/200 L algae treatment, where the hybrid variety had higher averages with 21.77 mm of leaf width. Additionally, the creole variety presented very similar averages across the treatments, with notable values for algae and sulfur 100 g/200 L at 19.03 mm and 19.05 mm, respectively. In contrast, the lowest values were observed with 150 g/200 L sulfur, where the average was 17.04 mm. For the hybrid variety, the highest average value was obtained with the application of seaweed, with an average value of 21.77 mm, whereas the lowest average value was observed with distilled water, with an average value of 16.66 mm (Table 3).

Finally, for shoot dry weight, significant differences were also observed between the interaction of treatment and variety (p-value < 0.01). Significant differences were observed between the combinations of varieties subjected to the 1 L/200 L seaweed treatment, where the hybrid variety presented a relatively high average yield of 2.03 g, and the 100 g/200 L, sulfur treatment where the creole variety presented a relatively high average yield of 1.72 g of shoot dry weight. Similarly, these treatments presented the highest averages within each variety (Table 3).

To evaluate the interaction of the variables, a multivariate principal component analysis (PCA) method was used (Figure 4). The first two components explain 52.62% of the variance for dimension 1 and 22.93% for dimension 2, accounting for a cumulative variance of 75.55% (Figure 4a, Supplementary Figure 1a). For dimension 1, the variables shoot dry weight (15.38%), leaf length (15.41%), and plant height (17.35%) contributed the most to this dimension, whereas root length (2.82%) and leaf thickness (5.00%) made the smallest contributions (Figure 4, Supplementary Figure 1b). In dimension 2, leaf width (19.31%), root thickness (21.21%), and leaf thickness (22.60%) had the greatest contributions, whereas root number (0.47%), shoot dry weight (1.22%), and root length (2.34%) had the smallest contributions to this dimension (Figure 4a, Supplementary Figure 1c).

The vectors illustrate the direction of the relationships between variables, showing a positive correlation between root number, root dry weight, plant height, stem thickness, leaf length, leaf width, and shoot dry weight. In contrast, a negative correlation is observed with root length, root thickness, and leaf thickness. Notably, plant height has a very high positive correlation (0.96), whereas root length has a high negative correlation (-0.63) for dimension 1. Additionally, for dimension 2, the variables root thickness and leaf thickness exhibited high positive correlations of 0.70 and 0.72, respectively (Figure 4a, Supplementary Figure 1d).

On the other hand, the combination of T0-creole aligns with the vectors for leaf thickness and root thickness. Therefore, individuals subjected to these treatments presented higher values for these variables. In terms of root length, the combinations T0-hybrid, T6-creole, and T6-hybrid presented the greatest values. Additionally, for the variables leaf width, leaf length, stem thickness, number of roots, and plant height, the combinations T1-hybrid, T2-creole, and T2-hybrid presented the highest values. For the variables shoot dry weight and root dry weight, the combinations of T1-creole, T3-creole, and T3-hybrid presented the highest values (Figure 4b).

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| Figure 4: Principal Component Analysis (PCA) of the biometric traits of purple maize seedlings from the two varieties, creole and hybrid PMV 581. Treatments based on seaweed, sulfur, and milk whey were used for the evaluation of biometric characteristics. Where: Distilled water (T0), seaweed 1 L/200 L (T1), seaweed 1.5 L/200 L (T2), sulfur 100 gr/200 L (T3), sulfur 150 gr/200 L (T4), milk whey 10% (10 mL/90 mL) (T5), milk whey 30% (30 mL/90 mL) (T6). (a) Analysis of variables (b) Analysis of individuals (n = 210). |

# Discussion

Corn is a crop that has excellent nutritional properties and health benefits [7]. However, the effective production of purple corn can be affected by factors related to seed germination. The use of stimulants before planting has emerged as a technique to improve planting outcomes. In the present study, the effects of different stimulants on the germination of purple corn seeds from two varieties were investigated. We evaluated how these stimulants influence the germination and initial development of purple corn seedlings, with the aim of optimizing cultivation conditions and ensuring greater production efficiency.

The germination variables evaluated in purple corn seeds were the IR (%), GP (%), GV (days), and GI. In the present work, the imbibition rates of creole and hybrid-PMV 581 corn seeds varied significantly in response to different treatments (Figure 2a). In the creole variety, water absorption is greater during the first three hours, after which it remains constant. However, for the seeds of the hybrid variety, water absorption was greater at three and nine hours. Different results were reported by Medina-Hoyos et al. [(Medina-Hoyos et al., 2020b)](https://www.zotero.org/google-docs/?3iD2sD) who reported that the highest imbibition rates occurred at 8, 10, and 12 h in corn, black bean, and quinchon seeds, respectively. However, there was no correlation between germination percentage and imbibition rate in the evaluated seeds. For the variables, GP (%), GV (days), and GI ([Figure 2](#fig-id.1pjblht3vonw)b, c, d), seeds of the creole variety, it was observed that in the first three days after germination (DDG), the concentrations used in the treatments did not stimulate germination beyond the control (T0). There were no significant differences between the treatments. On the other hand, in the hybrid variety, in the first three days after germination (DDG), the concentrations used in the treatments (T2, T4, and T6) successfully stimulated germination beyond that of the control. Carrillo-Martínez et al. [(Carrillo-Martínez et al., 2018)](https://www.zotero.org/google-docs/?4fFyZa) reported that seeds require an imbibition process to improve their germination percentage, germination rate, and uniformity of germination. Additionally, they reported that the germination index and energy were high with a shorter average germination time, and a greater germination speed was also observed.

No differences were found in the germination indices with the application of seaweed-based stimulants ([Figure 2](#fig-id.1pjblht3vonw)). However, other studies have reported that seaweed-based biofertilizers offer benefits such as increased plant growth, seed germination, protection against oxidative damage, and improved crop productivity [(Ali et al., 2021; Hernández-Herrera et al., 2023)](https://www.zotero.org/google-docs/?mmP1nc). Similarly, Benítez de la Torre et al. [(Torre et al., 2023)](https://www.zotero.org/google-docs/?JHQ6il) evaluated the phytotoxicity of sweet whey in alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) seeds and seedlings. They reported that sweet whey, at low concentrations (4-6%), has a stimulatory effect on the growth of alfalfa seedlings. However, at a concentration of 40%, germination in seeds of both species decreased by up to 53%. Sweet whey is crucial for the development of 50% inhibition of the seed population. Compared with the other treatments, the T0 and T5 with an additional 10% milk whey show a 20% improvement compared to the other treatments [(Torre et al., 2023)](https://www.zotero.org/google-docs/?aZPARD). Therefore, germination reaches 100% between 0–40% concentrations; higher doses are adversely affected due to the presence of NaCl, which increases osmotic pressure and causes a negative physiological effect. In this regard, T0 and T5 show 86% and 76% germination percentage (GP) in creole corn, respectively. In this sense, it becomes the most toxic during the early stages of germination development [(Zenda et al., 2021)](https://www.zotero.org/google-docs/?tMSwrL). The germination percentage is a crucial physiological process for plant establishment and, therefore, for crop success. In a previous study, the highest GP was observed in the T0 treatments with 86% and in the T5 treatment with 70% [(Shukla & Prithiviraj, 2021)](https://www.zotero.org/google-docs/?lGeNMT)

The germination index (IG) is a parameter that results from the germination time (TI) as it reflects the ability of a seed to germinate and emerge uniformly within a given period. The results obtained in this study indicate that for the creole variety, the T0 treatment had the highest IG of 2.6, followed by the T5 treatment of 1.67, although no significant differences were observed between the two treatments. Other authors, such as Escobar-Álvarez et al. [(Escobar-Álvarez et al., 2021)](https://www.zotero.org/google-docs/?rlsaKC), mention that this can be attributed to the thickness of the seed coat in *Zea spp*. varieties, which varies between 98.9% and 100%, with the IG occurring between 1.43 and 1.53 days.

For early development, the use of seaweed-based stimulants had significant positive effects on both the creole purple corn variety and the improved hybrid variety (Figure 3). The application of seaweed extract as a stimulant is important in the agronomic management of crops and promotes organic agriculture [(Mamede et al., 2024)](https://www.zotero.org/google-docs/?tCrbGM). Compared with the control plants, the seaweed-treated plants presented significant differences in terms of root length, thickness, and number, along with significant increases in stem and leaf biomass (Figure 3 y [Figure 4](#fig-kix.ag5n0ppkuwet)). Similar results were reported by Fatriana et al. [(Fatriana et al., 2020)](https://www.zotero.org/google-docs/?6HSmvU), who evaluated the application of seaweed stimulants at three concentrations (30%, 60%, and 90%) to corn plants (*Zea mays* L). They reported significant differences in stem and leaf biomass, as well as in-ear weight, length, and diameter. In the study by Shukla & Prithiviraj [(Shukla & Prithiviraj, 2021)](https://www.zotero.org/google-docs/?Vdbnp0), seaweed extract was applied as a stimulant in maize cultivation in phosphorus (P) deficient soils. These authors reported a significant difference in shoot and root biomass in the treated plants. Our results revealed that the application of seaweed extract had a significant effect on both the root biomass (length, thickness, and quantity) and the leaves and stems of the plant (Figure 3). Similar results were reported by Ertani et al. [(Ertani et al., 2018)](https://www.zotero.org/google-docs/?dWsAJ1), who evaluated five seaweed extracts (*Ascophyllum nodosum*) from maize plants. In the experiment, they were supplied with a concentration of 0.5 ml/L for two days. We applied two doses of seaweed extract, 1 liter and 1.5 liter per 200 liter. We observed a significant effect in both cases on plant growth and root biomass in both creole corn and improved hybrid corn (Figure 3). Similarly, Gandhi et al. [(Gandhi et al., 2024)](https://www.zotero.org/google-docs/?GD8ROa) evaluated extracts from two cultivable red algae, *Kappaphycus alvarezii* (KAE), *Gracilaria debilis* (GDE), and *Sargassum cinctum* (SCE) in *Zea mays*. The field study lasted for two years, during which they evaluated growth parameters, yield, and grain quality. The study applied doses ranging from 2.5% to 15%. The results revealed significant increases in yields of 19%, 21%, and 20% respectively. Additionally, the study indicated that the most effective dose was 7.5%, as no further improvement was observed beyond this dosage point. The two doses of seaweed applied in our experiment had a significant effect on plant growth and root biomass in creole and hybrid maize.

In the case of inorganic stimulants the use of sulfur in the development of seedlings for both the creole purple maize and hybrid varieties had positive effects at both applied doses (Figure 3, Table 3). This compound plays a crucial role in plant growth and development, as it acts as a structural component of macromolecules and modulates various physiological processes. Additionally, it can function as a key molecule that stimulates root growth and elongation [(Guo et al., 2021; Zenda et al., 2021)](https://www.zotero.org/google-docs/?0bk9Fp). The results obtained in this study revealed that seedlings treated with this stimulant presented increases in root length and dry weight (Figure 3); similarly, the greatest increases in the aerial part were observed in the height and dry weight of the seedlings (Table 3), with those in the control treatment. Pourbabaee et al. [(Pourbabaee et al., 2020)](https://www.zotero.org/google-docs/?pS3sAv) obtained similar results when different doses of sulfur were applied to maize seedlings, which resulted in significant increases in stem diameter, plant height, and shoot weight. On their part. Riffat and Ahmad [(Riffat & Ahmad, 2020)](https://www.zotero.org/google-docs/?9gsQln) reported significant improvements in growth parameters, including root and shoot length and dry weight of maize seedlings, in their experiment with two doses of sulfur. The authors attributed these benefits to the ability of sulfur to increase photosynthesis, photosynthesis attributes, and the concentration of biomolecules that support plant growth and development. In line with the authors, it is presumed that in the present study, the application of sulfur was crucial in stimulating physiological processes such as photosynthesis, enzyme activation, protein and amino acid production, and metabolic reactions necessary for the growth and development of the seedlings [(Narayan et al., 2023)](https://www.zotero.org/google-docs/?dpOYbq)

The use of milk whey as a stimulant in the development of seedlings for both the creole purple corn and hybrid varieties had positive effects at both applied doses (Figure 3, Table 3). The results obtained in this study revealed that seedlings treated with this stimulant presented increases in root length and thickness (Figure 3). Additionally, the greatest increases in the aerial parts were observed for leaf length and thickness (Table 3), to those in the control treatment. Akay and Sert [(Akay & Sert, 2020)](https://www.zotero.org/google-docs/?zKZyn5) obtained results on the effects of different types of powdered whey on soil and maize seedlings at various concentrations. They reported that particularly with undemineralized whey and 50% demineralized whey microbial biomass, soil respiration, and enzymatic activities increased. Additionally, the application of 50% demineralized whey significantly improved plant height, fresh weight, and root weight in maize. This result contrasts with what was observed in the present study, as these effects were not evident in the purple corn seedlings.

Despite these promising findings, this study has several limitations that warrant consideration in future research. First, the sample size for the germination trials was limited because of limited access to plant material from the selected varieties. Although the study provided initial insights, increasing the number of replicates would increase the statistical power, reducing experimental error and providing more robust conclusions. Moreover, the study focused primarily on germination and early seedling development, without assessing the long-term effects of the stimulants on key agronomic variables such as yield, grain quality, and overall crop performance. Future investigations should extend beyond the initial growth stages to encompass the entire crop cycle, allowing for a comprehensive evaluation of the impact of seaweed and sulfur treatments.

Our findings indicate a promising avenue for using seaweed and sulfur as biostimulants in maize cultivation, particularly for enhancing the germination of purple corn seeds. However, further research is essential to confirm the broader applicability of these stimulants across different maize varieties and agroecosystems. In addition, future studies should consider evaluating other seed quality parameters, such as vigor, seedling uniformity, and stress tolerance, to provide a more holistic understanding of the benefits and limitations of these treatments. Addressing these gaps will be crucial for developing effective and sustainable biostimulant strategies in maize agriculture.

C**ONCLUSIONS**

The results obtained in this study revealed that the application of stimulants had a significant influence on the germination and initial growth of two varieties of purple corn. Seed imbibition showed a similar response between both varieties, whereas germination varied significantly, with the creole corn exhibiting greater vigor. The application of stimulants, particularly seaweed and sulfur, promoted more robust root and shoot development, demonstrating a positive effect on seedling establishment. These findings suggest that the use of organic and inorganic biostimulants is a recommended option for purple corn cultivation.

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