

Biostimulant seed priming with humic acid followed by drying: A sustainable technique to enhance early growth of maize

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Abstract: Humic acids (HAs) are increasingly recognized as agricultural inputs due to their biostimulant effects, acting through mechanisms similar to plant growth regulators. This study evaluated the effects of seed priming with humic acid followed by drying on the germination, growth, and early seedling quality of maize (*Zea mays* L.). Five treatments were tested: T1 – control; T2 – imbibition in water for 8 h without drying; T3 – imbibition in humic acid solution for 8 h without drying; T4 – imbibition in water followed by drying to 14% moisture content; and T5 – imbibition in humic acid solution for 8 h followed by drying to 14% moisture content. Germination was assessed at four and seven days after sowing, while at 33 days plant height, stem diameter, root length, shoot and root biomass, total dry biomass, and the Dickson Quality Index were determined. Seeds subjected to Treatment 5 (humic acid, 10 mmol L⁻¹ of carbon, 8 h + drying) showed superior performance compared with all other treatments. They produced more vigorous seedlings, with greater root and shoot biomass accumulation, higher total dry matter, and improved quality indices. These findings indicate that humic acid priming followed by drying is a sustainable and effective technique to improve maize seedling establishment and early crop development.

Keywords: Plant regulator; seed drying; seedling development; *Zea mays* L.

Abbreviations: DMAP_aerial part dry mass; DMRS_root system dry mass; DQI_Dickson quality index; FAM_aerial fresh mass; FMRS_root system fresh mass; LRS_root system length; PH_Average values for plant height; SD_stem diameter; TDM_total dry mass; TFM_total fresh mass.

Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereals globally, serving as food, feed, and industrial raw material (Olugbire et al., 2021). Seed quality strongly influences crop establishment, germination, and seedling vigor, with early root and shoot development being critical for nutrient uptake, stress tolerance, and overall performance (Finch-Savage, 2020; Zinta et al., 2022).

Seed priming with biostimulants has emerged as an effective approach to improve germination and early growth. Humic substances, including humic and fulvic acids, are natural organic compounds derived from the decomposition of plant and microbial residues (Shahrajabian et al., 2024). They enhance nutrient uptake, stimulate hormone-like activity, and increase stress tolerance, largely through modulation of auxin- and cytokinin-mediated pathways (Chen et al., 2022; Rathor et al., 2024).

Humic acids act as sustainable agricultural inputs, improving water and nutrient absorption, regulating metabolism, and enhancing photosynthetic pigments and enzyme activity (Baligah et al., 2022; Li et al., 2024). Their low extraction cost and

Table 1. Analysis of variance for seed germination at 4 (GER4) and 7 (GER7) days after sowing, plant height (PH), stem diameter (SD), root system length (RSL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), total fresh mass (TFM), total dry mass (TDM), and Dickson Quality Index (DQI) of maize seedlings whose seeds were soaked in humic acid (HA) or water according to the following treatments: T1, control; T2, seeds soaked in water for 8 h; T3, seeds soaked in a 10 mmol L⁻¹ C humic acid solution for 8 h; T4, seeds soaked in water for 8 h and dried to 14% moisture content; T5, seeds soaked in a 10 mmol L⁻¹ C humic acid solution for 8 h and dried to 14% moisture content.

Mean Squares													
FV	DF	GER4	GER7	PH	SD	RSL	SFM	RFM	SDM	RDM	TFM	TDM	DQI
Treatment	4	4165.8*	5752.2*	11.08 ^{ns}	0.015 ^{ns}	99.344 ^{ns}	36.084*	18.78*	7.73 ^{ns}	2.35*	97.17*	17.51*	0.0088*
Residue	15	32.2	14.4	11.32	0.010	56.053	9.020	2.31	2.59	0.29	6.15	2.39	0.0008
CV (%)		10.65	5.93	9.76	12.42	16.32	17.33	24.03	52.84	28.16	10.48	31.12	27.03

DF = degrees of freedom; CV = coefficient of variation (%). * Significant by F test at 5% probability; ns = not significant by F test.

potential use from recycled organic residues makes them accessible even to smallholder farmers, reinforcing their role in sustainable agriculture (Ekin, 2019; Lumactud et al., 2022).

Positive effects of humic acids on seed performance have been reported, including increased fresh biomass in maize (Rodrigues et al., 2017), shoot dry mass in common bean (Rosa et al., 2009), improved germination and seedling vigor in lentil under heat stress (Rahim et al., 2023), and enhanced growth and stress tolerance in rice under salinity (Abdel Latef et al., 2023). However, little is known about the feasibility of drying maize seeds after humic acid treatment. Baldotto et al. (2021) demonstrated benefits of seed soaking in humic acid solutions, but prolonged exposure may compromise mechanical sowing and seed integrity. Therefore, this study aimed to evaluate the effects of drying humic acid-soaked maize seeds on germination, early growth, and seedling quality.

Results and Discussion

Germination percentage

For seed germination percentage (Table 1 and Table 2), no significant differences were observed between the treatments in which seeds were soaked and subsequently dried (TGER3 and TGER5) and the control (TGER1), both at four and seven days after treatment application. Notably, soaking in humic acid followed by drying (TGER5) did not negatively affect seed germination, as its values were statistically similar to those of the control. In contrast, these treatments consistently outperformed those in which seeds were soaked without drying (TGER2 and TGER4). Germination was markedly reduced under non-drying conditions, regardless of whether seeds were soaked in water or humic acid solution. This reduction may be associated with stress caused by excess water, leading to decreased or insufficient oxygen availability (Chen et al., 2002). After soaking, seeds were wrapped in Germitest® paper under high-moisture conditions. It is hypothesized that excess water reduced oxygen diffusion, thereby limiting aerobic metabolism during germination (Mortierle et al., 2006). Nevertheless, it should be highlighted that the germination percentages obtained for treatments in which seeds were soaked and subsequently dried were satisfactory. Considering that commercial maize seeds typically present an average germination rate of 85%, these results meet the minimum quality standards established for the crop (Brasil, 2013). Therefore, all treatments involving soaking followed by drying produced germination values above the legally required threshold.

Vegetative development

Analysis of variance (Table 1) revealed significant effects of the treatments on shoot fresh mass (SFM), root fresh mass (RFM), root dry mass (RDM), total fresh mass (TFM), total dry mass (TDM), and the Dickson Quality Index (DQI), while plant height (PH), stem diameter (SD), root system length (RSL), and shoot dry mass (SDM) were not significantly affected. Mean values (Table 2) showed that Treatment 5 (T5), consisting of seed immersion in a 10 mmol L⁻¹ C humic acid solution for 8 h followed by drying to 14% moisture, resulted in the highest values for all biomass accumulation variables. In T5, RFM and RDM doubled compared to the control (T1), and TDM reached 8.48 g, significantly higher than the other treatments. Relative to the control, T5 induced increases of 86.47% in root fresh biomass, 120% in root dry biomass and 114% in total dry biomass. The DQI also increased by 120% in T5 (0.189 versus 0.086 in T1), indicating more vigorous and well-balanced seedlings. Shoot fresh mass in T5 was approximately 30% higher than in the control, whereas T1, T2, T3, and T4 did not differ significantly. These results suggest that humic acid primarily affected root development, which subsequently contributed to increased total biomass.

The DQI, a widely accepted parameter for evaluating seedling quality by integrating plant height, stem diameter, and biomass distribution, was significantly higher in T5, reflecting a better balance between shoot and root growth. This trait is essential for seedling robustness and survival under field conditions (Silva et al., 2013; Binotto et al., 2010). In contrast, T2, T3, and T4 produced lower DQI values, likely due to reduced germination and suboptimal biomass allocation.

The superior performance of T5 can be attributed to the biostimulatory activity of humic acids, which exhibit hormone-like effects. These compounds stimulate the biosynthesis of auxins, cytokinins, and gibberellins, thereby promoting lateral root initiation, nutrient uptake, and balanced plant growth (Trevisan et al., 2010; Silva et al., 2011). In addition, humic acids

Table 2. Mean values for seed germination at 4 (GER4) and 7 (GER7) days after sowing, plant height (PH), stem diameter (SD), root system length (RSL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM), root dry mass (RDM), total fresh mass (TFM), total dry mass (TDM), and Dickson Quality Index (DQI) of maize seedlings whose seeds were soaked in humic acid (HA) or water according to the following treatments: T1, control; T2, seeds soaked in water for 8 h; T3, seeds soaked in a 10 mmol L⁻¹ C humic acid solution for 8 h; T4, seeds soaked in water for 8 h and dried to 14% moisture content; T5, seeds soaked in a 10 mmol L⁻¹ C humic acid solution for 8 h and dried to 14% moisture content.

Treatment	GER4	GER7	PH	SD	RSL	SFM	RFM	SDM	RDM	TFM	TDM	DQI
T1	82.0A	94.5A	36.50A	0.83A	39.63A	16.55B	5.48B	2.45A	1.50B	22.03B	3.95B	0.086B
T2	14.5B	19.5C	35.38A	0.83A	43.38A	15.98B	5.38B	2.08A	1.60B	21.35B	3.68B	0.083B
T3	74.0A	92.7A	35.25A	0.78A	49.75A	13.90B	5.53B	1.83A	1.60B	19.43B	3.43B	0.074B
T4	22.0B	26.0B	35.25A	0.78A	44.65A	18.33B	5.10B	3.70A	1.65B	23.43B	5.35B	0.112B
T5	74.0A	88.0A	39.13A	0.93A	52.00A	21.90A	10.20A	5.18A	3.30A	32.10A	8.48A	0.189A

Means followed by the same letter in the column do not differ by the Scott-Knott grouping test at 5% probability.

enhance plasma membrane H⁺-ATPase activity, improving ion transport and mineral nutrition, while also stimulating Krebs cycle efficiency, chlorophyll biosynthesis, and nucleic acid production, which together increase respiration, photosynthesis, and biomass accumulation (Castro et al., 2019).

These findings are consistent with previous reports in different crops. In common bean, Carvalho et al. (2023) observed greater pod and grain production after humic acid application. In coffee, foliar and soil applications of humic acid improved growth, yield, and bean quality (Kishor, 2021). In maize, Melo et al. (2015) and Baldotto et al. (2016) documented enhanced early growth after seed immersion in humic acid, which was further confirmed under field conditions by Baldotto et al. (2019). Conversely, other studies reported no significant effects of humic or fulvic acids on maize productivity or morphology (Batista et al., 2018; Bernardes; Orioli Júnior, 2018), suggesting that plant responses may depend on factors such as application rate, environmental conditions, and crop management practices. Finally, the post-imbibition drying step likely enhanced the effects observed in T5. Reducing seed moisture may have stimulated enzymatic mechanisms related to germination, maximizing the physiological response to humic acid and resulting in higher seedling vigor and quality. These results highlight the importance of considering not only humic acid concentration and exposure time but also post-treatment handling when evaluating the efficiency of humic substances on seeds.

Materials and Methods

Experimental site

The experiment was carried out at the experimental farm of Univértix College, located in Matipó, Minas Gerais, Brazil (20°16'51" S, 42°20'22" W), at an altitude of approximately 650 m.

Extraction of humic acids

Humic acids were extracted following the protocol recommended by the International Humic Substances Society (IHSS). The procedure consisted of shaking samples of cattle manure (previously stabilized and with an advanced degree of humification) with 0.1 mol L⁻¹ NaOH solution. The resulting supernatant, containing soluble organic matter (humic substances), was acidified to pH ~1.5 to precipitate the humic acid fraction. The precipitate was then neutralized to approximately pH 7.0. Carbon content was determined by the Walkley–Black method, and humic acids were subsequently diluted in distilled water to a final concentration of 10 mmol L⁻¹ of carbon.

Germination test and experimental design

Seed germination was evaluated using the standard germination test, with four replicates of 50 seeds per treatment. The treatments consisted of: untreated seeds (TGER1); seeds soaked in distilled water for 8 h (TGER2); seeds soaked in distilled water for 8 h and dried to 14% moisture content (TGER3); seeds soaked in humic acid solution (10 mmol L⁻¹ C) for 8 h (TGER4); and seeds soaked in humic acid solution (10 mmol L⁻¹ C) for 8 h and dried to 14% moisture content (TGER5). Following immersion, seeds subjected to drying were placed in a forced-air oven until reaching approximately 14% moisture content (dry basis), with the temperature maintained below 40 °C to ensure embryo viability (Borém et al., 2015). Seeds were sown between three sheets of Germitest® paper moistened with distilled water, using a volume equivalent to 2.5 times the dry paper mass. The sheets were rolled so that the seeds were placed between two sheets and covered by the third. The rolls were placed in a germination chamber (B.O.D.) maintained at 25 ± 2 °C with a 12 h photoperiod. The experimental design was completely randomized, with four replicates per treatment.

Seedling evaluation was performed on the fourth day after test initiation, recording the percentage of normal seedlings by counting all emerged seedlings per treatment and converting the values to percentages, according to the Rules for Seed Analysis (Brasil, 2009) and the International Rules for Seed Testing (ISTA, 2023). A second germination count was conducted on the seventh day after sowing, following the same procedure.

Table 3. Composition of treatments applied to maize seeds.

Treatment Description	
T1	Control – seeds without water or humic acid application
T2	Seeds soaked in water for 8 h
T3	Seeds soaked in a 10 mmol L ⁻¹ C humic acid solution for 8 h
T4	Seeds soaked in water for 8 h and dried to 14% moisture content
T5	Seeds soaked in a 10 mmol L ⁻¹ C humic acid solution for 8 h and dried to 14% moisture content

Table 4. Summary of the chemical properties of the soil used as substrate for evaluating maize seedlings under different humic acid and moisture conditions.

pH	MO	P ¹	K	Ca ²	Mg	Al	H+Al	SB	T	V
H ₂ O	dag/kg	----mg/dm ³ ----		-----cmolc/dm ³ -----						%
7.2	2.5	148.4	193	7.7	2.4	0.00	0.00	10.59	10.59	100

¹P and K extracted with Mehlich-1; Ca²⁺, Mg²⁺, and Al³⁺ extracted with 1 mol/L KCl.

Seedling production

To assess morphological traits and seedling quality, seeds were soaked in 100 mL of distilled water with 3 mL of humic acids for different durations, resulting in five treatments (Table 3). Treatments T1 and T3 consisted of soaking for 8 h prior to sowing. Treatments T4 and T5 consisted of soaking for 16 h prior to sowing, followed by drying. After soaking, seeds in T4 and T5 were dried in a forced-air oven until reaching approximately 14% moisture content (dry basis), without exceeding 50 °C to avoid embryo damage.

For seedling production, polyethylene bags (30 × 15 cm) were filled with substrate (Table 4). Basal fertilization was carried out with nitrogen, phosphorus, and potassium at sowing, using 10 g of 04-14-08 NPK fertilizer, according to Ribeiro et al. (1999).

Seedling management and experimental design

Three seeds were sown per container, and thinning was performed after emergence, leaving one seedling per container. The seedlings were subsequently transferred to seedbeds in a greenhouse. Irrigation was carried out manually using watering cans, and water supply was adjusted to maintain optimal conditions for seedling growth, standardized at soil field capacity to ensure homogeneous irrigation across treatments.

The experimental design was completely randomized, with four replicates per treatment, totaling 20 seedlings in the experimental area.

Morphological and seedling quality assessments

At 33 days after sowing, seedlings were evaluated for the following traits: (a) plant height (PH), measured with a graduated ruler (cm); (b) stem diameter (SD), measured with a digital caliper (mm); (c) root system length (RSL), measured with a graduated ruler (cm); (d) shoot fresh mass (SFM); (e) root fresh mass (RFM); (f) total fresh mass (TFM), all determined using a precision balance (g); (g) shoot dry mass (SDM); (h) root dry mass (RDM); (i) total dry mass (TDM); and (j) Dickson Quality Index (DQI), according to Dickson et al. (1960), calculated as:

$$DQI = \frac{SDM + RDM}{\frac{PH}{SD} + \frac{SDM}{RDM}}$$

Dry masses (SDM and RDM) were obtained by drying plant tissues in a forced-air oven at 80 °C until constant weight, and then weighing on a precision balance. TDM was calculated as the sum of SDM and RDM.

Statistical analysis

Data were subjected to analysis of variance using the F-test at a 5% probability level. When significant differences were detected, means were compared using the Scott-Knott clustering test ($p < 0.05$). All statistical analyses and graphical representations were performed using R software (R Core Team, 2025). With the aid of the data packages ExpDes.pt (Ferreira et al., 2018).

Conclusion

Immersing corn seeds in a solution containing 10 mmol L⁻¹ of carbon in humic acid for 8 hours, with humidity reduced to 14%, is the most recommended method, as it provides greater growth, development and quality gains in corn plants.

Author Contributions

DSF and JNR: experiment conduction, data analysis, and manuscript drafting.

ROM and GPO: experimental design and manuscript review.

CSD and SDA: project management and manuscript revision.

Conflict of Interest

The authors declare that there is no conflict of interest.

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