



Research Article

Seed priming boosted waxy corn yield across different water regimes

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ABSTRACT

Plant resistance to drought stress could be improved by seed priming using natural plant growth regulators, such as coconut water. This research aimed examine the physiological characteristics, root development, and yield responses of waxy corn following different seed priming treatments using coconut water and to determine to the optimal coconut water concentration for seed priming under each drought condition. The research was conducted at Banguntapan Sub-district and Laboratory of the Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta - Indonesia, in July-October 2023. The research used a split-plot design with a main plot of watering intervals and a subplot of coconut water concentration with three replications. The main plot consisted of three levels: watering every day, once every three days, and once every six days. The subplot consisted of four levels of coconut water concentrations, i.e., 0, 33, 67, and 100%. The results indicated that seed priming with 100% coconut water increased corn yield. The increased yield could be attributed to the different photosynthetic rates and total seed weight under both well-watered and drought-stress conditions.

Keywords: coconut water; drought stress; root improvement

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INTRODUCTION

Soaking seeds in coconut water is classified as a form of hormonal seed priming (Amir et al., 2024). Hormonal seed priming is a technique involving the immersion of seeds in a solution containing an optimal concentration of phytohormones, which functions to enhance seed metabolism without inducing radicle protrusion through the seed coat. According to Devika et al. (2021), seed priming process consists of three phases. The first phase is imbibition, during which seed rapidly absorb water due to their low water potential. The second phase, known as the activation phase, is characterized by a series of physiological changes. Water uptake is halted at the end of this phase. Rehydration during seed preparation induces various cellular-level changes, such as cell division, nucleic acid and protein synthesis, ATP production, increased cellular energy, a higher ATP/ADP ratio to meet energy demands, accumulation of essential lipid, antioxidant production, and enhanced photosynthetic activity. In the third phase, water uptake resumes, and the emergence of radicle signifies the beginning of cell elongation and growth processes during germination.

Most farmers in East Nusa Tenggara use local corn varieties to meet food demand. The local corn varieties have been cultivated for generations by farmers and they belong to the community and are controlled by the state (Sitorus et al., 2020). The local corn varieties have resistance to biotic and abiotic stress in areas with suboptimal agroecosystem conditions (Putri et al., 2022). Waxy corn (*Zea mays* ceratina Kulesh) was one of the local corn varieties of East Nusa Tenggara. The local corn varieties displayed either a red or white coloration. It was typically harvested at an early stage (60 DAP). It exhibited a soft grain texture.

According to Setiawan et al. (2020), increasing watering intervals resulted in a reduction of soil moisture content. It reduced the plant's relative water content in patchouli (*Pogostemon cablin* Benth). According to Mudhor et al. (2022), plants at 40% field capacity exhibited the lowest stomatal density compared to those at 60%, 80%, and 100% field capacity. According to Murningsih et al. (2015), proline accumulation and sugar content in both plant leaves and roots increased proportionally with the severity of drought stress. Notably, the proline content and sugar accumulation in roots surpassed those in leaves in corn.

East Nusa Tenggara was recognized as a region typified by arid conditions, accompanied by a short-lived rainy season (Seran et al., 2021). The cultivation of corn in rainfed areas was restricted to an annual cycle, resulting in a planting index of 100 (once planting season in a year). Local farmers exhibited a preference for planting short-lived crops and drought-resistant local varieties. It was noted that global warming had the potential to alter rainfall patterns, resulting in prolonged dry periods (Dulbari et al., 2018). The enhancement of the maize plant's drought resistance was achieved through improvements in physiological traits, growth, and maize yield.

The improvement of maize plants' drought resistance was achieved through the utilization of natural growth regulators, such as coconut water. Seed priming in coconut water enhanced physiological attributes, growth, and maize yield (Devika et al., 2021). Optimal coconut water concentrations were considered essential for different levels of drought severity. Seed priming facilitated the activation of internal and external seed resources, thereby optimizing growth and enhancing crop yields. This research aimed to examine the physiological characteristics, root development, and yield responses of waxy corn following different seed priming treatments using coconut water and to determine the optimal coconut water concentration for seed priming under each drought condition

MATERIALS AND METHODS

Research site

The research was carried out from June 1 to September 1, 2023, at the Tridharma Gardens, Faculty of Agriculture, Gadjah Mada University, Banguntapan, Bantul, Yogyakarta, and the Plant Management and Production Laboratory, Plant Science Sub-Laboratory, Department of Agronomy, Faculty of Agriculture, Gadjah Mada University, Yogyakarta.

Research design and procedures

This research used a split-plot design (4x3) with three blocks as replications. The main plot was the watering intervals, consisting of 3 levels: watering once a day, every 3 days, and every 6 days. The subplot was the concentrations of coconut water, i.e., 0, 33, 67, and 100%. The total combination of the two treatments was 12. The number of sample plants per block was 8, so the total number of plants was 8 samples x 12 treatments x 3 replications = 288 plants.

A mixture of planting media in the form of cow manure fertilizer and regosol soil in a ratio of 1:2 (m/m) was prepared and put about 50 kg into a polybag sized 40 cm x 40 cm. Waxy corn seeds were soaked in coconut water for two hours. The coconut water was obtained from young coconut with green exocarp. Two corn seeds were planted per polybag in a planting hole of \pm 2 cm. Then, one plant was selected at 7 days after planting (DAP).

Watering treatment was started at 14 days after planting. Watering was carried out according to the treatment until reaching field capacity indicated by water flowing from the hole at the bottom of the polybag.

The NPK fertilizer applied in this study had a nutrient composition of 16% nitrogen (N), 16% phosphorus (P₂O₅), and 16% potassium (K₂O), reflecting a balanced 1:1:1 ratio. Plant cultivation encompassed manual weeding and the utilization of insecticides for the management of pests and diseases. Harvesting occurred upon reaching physiological

maturity, as indicated by brown or dark red silk, firm and shiny kernels without indentation when pressed with a fingernail, and dry and yellowed husks.

Observed variables

The parameters observed in this study included root length, relative water content, stomatal density, stomatal aperture, photosynthesis rate using LICOR 670 (USA), and total seed weight. Root length observations were undertaken at 24, 48, and 84 DAP. Measurement of relative water content, stomatal density, stomatal aperture, and photosynthetic rate were performed at 48 DAP. Total seed weight was assessed at 84 DAP.

Root length measurements were conducted on all parts of the plant root system, including the primary root, lateral roots, and fibrous roots. Root length was determined using an area meter, which was calibrated prior to measurement. Calibration was performed by placing a 5 cm x 5 cm cardboard piece on the selected area, where a reading of 1000 was obtained. After calibration, the "length" option was selected. A transparent layer was placed on an illuminated table, and the root samples were positioned on the layer for observation. Root length was measured in both vertical and horizontal positions, and the average value of these measurements was used to determine the recorded root length. The recorded length (y) was calculated using the equation $y = 8.2153 + 0.375x$ (in cm), where x represents the actual root length, which was derived from the same equation.

The analysis of relative water content (RWC) was initiated by collecting fresh leaf samples, which were weighed to determine the fresh weight (FW). The samples were then placed in plastic bags and immersed in water for 48 hours to obtain the turgid weight (TW), after which they were reweighed. Subsequently, the leaves were oven-dried until a constant weight was achieved to determine the dry weight (DW). The RWC was calculated using the following formula:

$$RWC = \left[\frac{FW - DW}{TW - DW} \right] \times 100\%$$

Stomatal density and stomatal aperture were evaluated using the imprints method. Stomatal imprints of the abaxial (lower) surface of the leaf were thinly coated with clear nail polish containing acetone and then dried. A piece of transparent adhesive tape was then applied over the coated area. The tape was carefully peeled off, ensuring the abaxial epidermal layer remained attached. The tape containing the epidermal imprint was then affixed to a glass slide. The prepared stomatal imprint was observed under a microscope of 100x and 400x for stomatal density and stomatal aperture, respectively. Measurements were then analyzed using the Image Raster application, with results expressed in micrometers (μm).

Data analysis

Data were analyzed using the analysis of variance (ANOVA) followed by a further Tukey's honestly significant difference (HSD) test and polynomial orthogonal contrast at a significant level of 95%.

RESULTS AND DISCUSSION

Root length

No significant interaction was detected between coconut water concentrations and watering intervals on root length at 24, 48, and 84 DAP (Table 1). However, root length at 24 and 48 DAP was significantly influenced by seed priming in coconut waters, which resulted in respective increases of 98.08% (Figure 1) and 97.44% (Figure 2). The addition of exogenous auxin was found to enhance the plasticity of cell walls (Setyowati et al., 2023). The auxin in coconut water was implicated in cell division and elongation, with increased root length crucial for accessing soil water availability during drought conditions (Rahman et al., 2021).

Table 1. Root length of waxy corn from different priming concentrations and growing water regime.

Treatment	Root length (cm)		
	24 DAP	48 DAP	84 DAP
Coconut water concentrations			
0%	193.87c	1,349.28b	1,667.28a
33%	213.648bc	1,608.54ab	1,839.65a
67%	238.61ab	1,983.80a	1,859.81a
100%	243.06a	2,051.57a	2,102.61a
Watering intervals			
Every day	184.54b	1,949.98a	1,967.77a
Once every three days	212.93ab	1,590.98a	1,909.70a
Once every six days	269.43a	1,703.93a	1,724.54a
Interaction	-	-	-
CV	6%	4%	4%

Note: Numbers in a column followed by the same letter from similar factor were not significantly different based on the 5% HSD test; '-' = no interaction; DAP-days after planting, CV-Coefficient of variations.

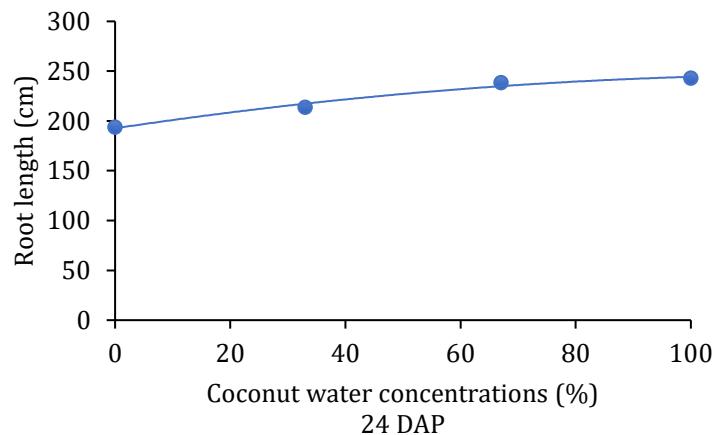


Figure 1. Relationship between coconut water concentrations and root length at 24 days after planting. Description of the regression equation between root length (cm) and coconut water concentrations (%) \rightarrow $y = -0.0035x^2 + 0.8637x + 192.62$ ($R^2 = 0.9808$).

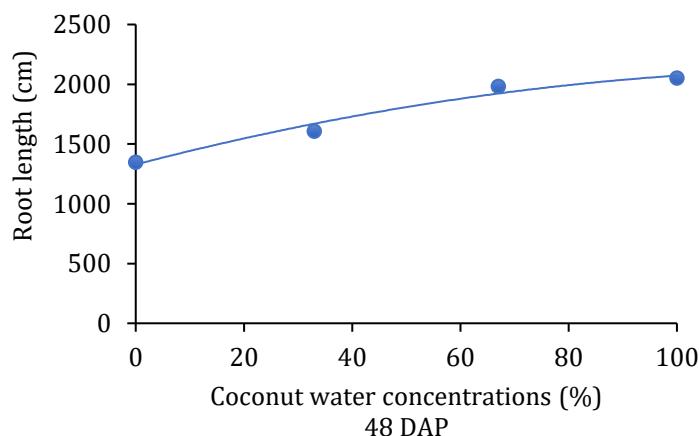


Figure 2. Relationship between coconut water concentrations and root length at 48 days after planting. Description of the regression equation between root length (cm) and coconut water concentrations (%) \rightarrow $y = -0.0433x^2 + 11.769x + 1328.5$ ($R^2 = 0.9744$).

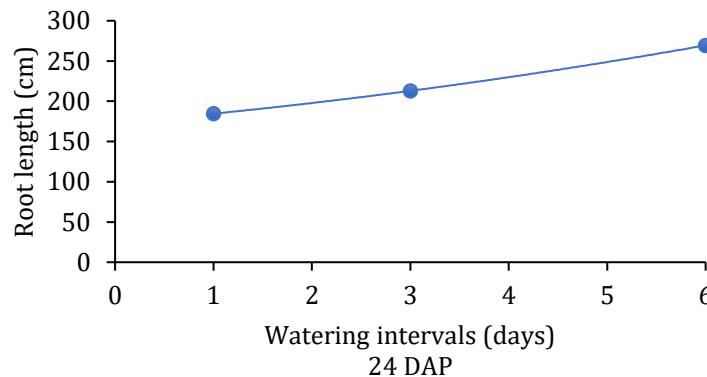


Figure 3. Relationship between root length (cm) and watering intervals at 24 days after planting. Description of the regression equation between root length (cm) and watering intervals (%) → $y = -0.9277x^2 + 10.484x + 173.13$ ($R^2 = 1$).

Seed priming in coconut water at 84 DAP did not result in a significant increase in root length at harvest, which was likely attributed to the onset of the senescence phase as the plants approached the end of their life cycle. The prolongation of watering intervals affected root length at 24 DAP by 100% (Figure 3). The elongation of corn roots is associated with the plant's adaptive response to drought stress aimed at enhancing the acquisition of water to support its growth and development (Nasrudin & Firmansyah, 2020). Extended watering intervals demonstrated no significant effects on root length at 48 DAP and 84 DAP, suggesting that heightened drought stress did not detrimentally affect the root system (Pappula-Reddy et al., 2024).

Table 2. Stomatal density of waxy corn from different priming concentrations and growing water regime at 48 days after planting.

Treatment	RWC (%)	Stomatal density (1/mm ²)	Stomatal aperture (μm)
Coconut water concentrations			
0%	69.76a	529.17b	3.26c
33%	73.32a	575.00ab	4.03b
67%	74.58a	602.78ab	4.51b
100%	75.07a	647.22a	5.26a
Watering intervals			
every day	75.86a	721.88a	5.50a
Once every three days	72.54a	569.79b	4.10b
Once every six days	71.15a	473.96ac	3.18cc
Interaction	-	-	-
CV	14%	3%	14%

Note: Numbers in a column followed by the same letter from similar factor were not significantly different based on the 5% HSD test; '-' = no interaction; CV-Coefficient of variations, RWC = Relative water content.

Relative water content, stomatal density, and stomatal aperture

Seed priming in coconut water did not significantly affect the RWC (Table 2). Seed priming in coconut water did not enhance the RWC of the leaves, as maize inherently possessed sufficient endogenous auxin required to enhance water diffusion (Rahmawati et al., 2023). Auxin enhanced water diffusion into cells by promoting membrane permeability, which facilitated water entry into the cells (Pratiwi & Santika, 2024). According to Zhang et al. (2025), the absence of changes in RWC indicated the plant is capable to preserve the water content within its organs.

No significant interaction was found between coconut water concentrations and watering intervals with respect to stomatal density (Table 2). The elevation of coconut water influenced stomatal density at 48 DAP by 99.07% (Figure 4a). According to Qi &

Torii (2018), cytokinin elicited epidermal cell division. The prolongation of watering intervals affected stomatal density at 48 DAP by 100% (Figure 4b). According to Ginting et al. (2024), the transpiration rate of the plant attenuated under drought-stress conditions.

No significant interaction was observed between coconut water concentrations and watering intervals on stomatal aperture (Table 2). The increase in coconut water concentration had an impact on stomatal aperture width at 48 DAP, reaching 99.15% (Figure 5a). According to (Romero-Muñoz et al., 2022), cytokinin stimulated stomatal aperture. The extension of watering intervals influenced stomatal density at 48 DAP, achieving 100% (Figure 5b). A reduction in stomatal density was a mechanism employed by plant to avoid drought stress (Haghpanah et al., 2024)

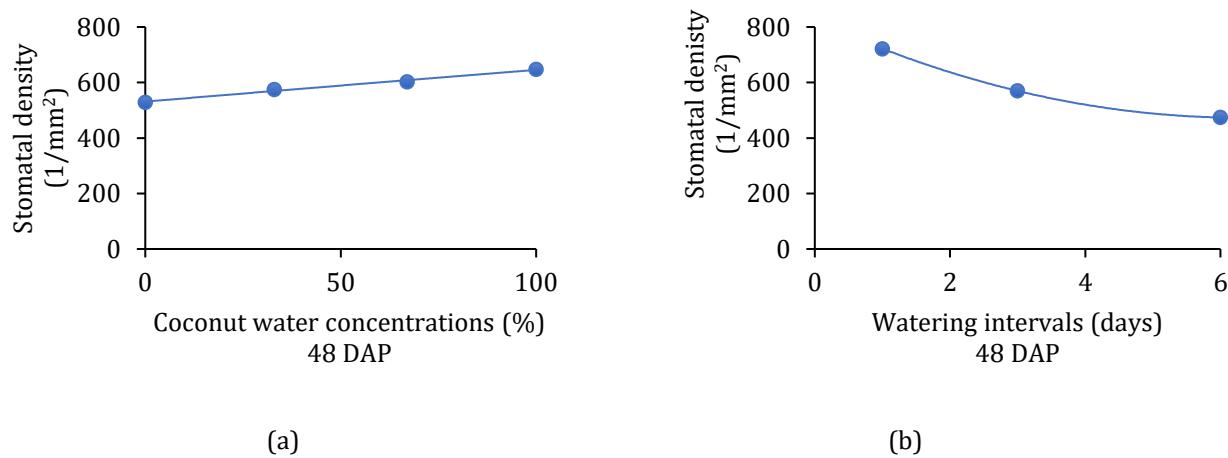


Figure 4. Regression relationship between stomatal density and a. coconut water concentrations b. the watering intervals at 48 days after planting. Description of the regression equation between stomatal density and coconut water concentrations (a) $y = -0.0003x^2 + 1.1743x + 531.05$ ($R^2 = 0.9907$); and the regression equation between stomata density and watering intervals (b) $y = 8.8203x^2 - 111.33x + 824.39$ ($R^2 = 1$).

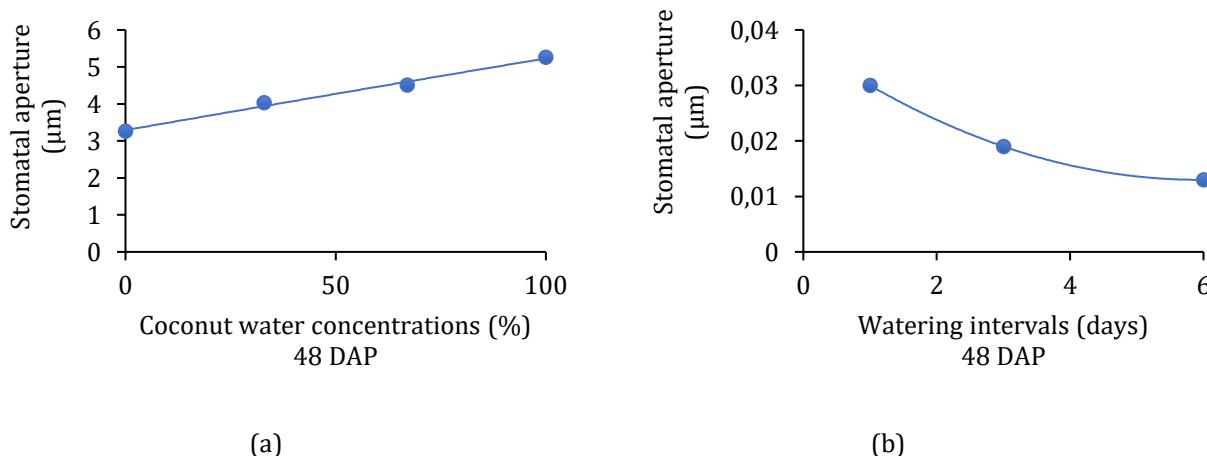


Figure 5. Regression relationship between stomatal aperture (μm) and a. coconut water concentrations b. watering intervals at 48 days after planting. Description of the regression equation between stomatal aperture (μm) and coconut water concentrations (%): (a) $y = -5E-06x^2 + 0.0198x + 3.2905$ ($R^2 = 0.9915$), and (b) regression equation between (μm) and watering intervals (days): (b) $y = 0.0007x^2 - 0.0083x + 0.0376$ ($R^2 = 1$).

Photosynthetic rates

The rate of corn photosynthesis was affected by both seed priming in coconut water and watering intervals. The prolongation of watering interval for seed priming in water and coconut water at concentrations of 33%, 67%, dan 100% affected the photosynthetic rate at 48 DAP, reaching 100% (Figure 6). Cytokinin influenced the regulation of CO_2 diffusion required for carboxylation (Sosnowski et al., 2023) under both well-watered and drought-stress conditions.

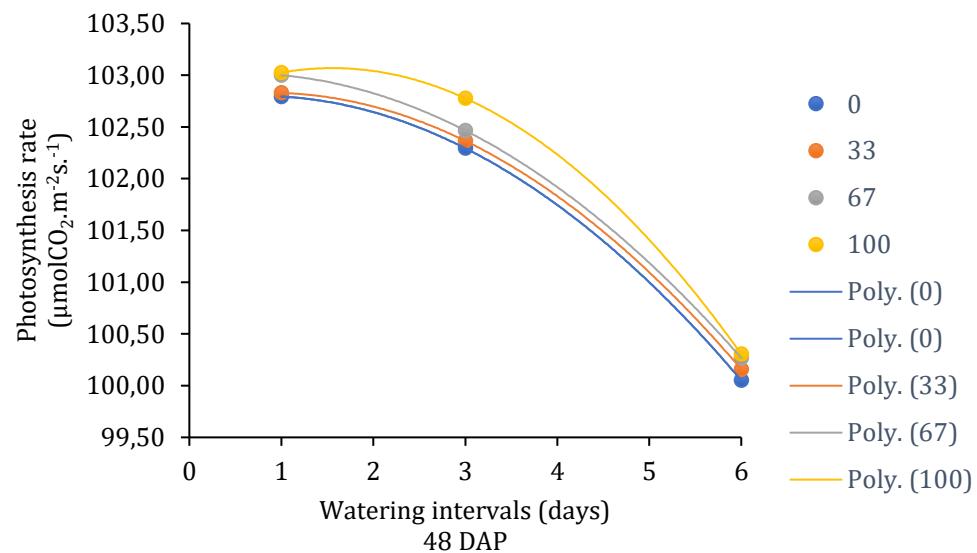


Figure 6. Regression relationship between photosynthesis rate and coconut water concentrations (%) at 48 days after planting. Description of the regression equation: coconut water concentrations: 0% $\rightarrow y = -0.0995x^2 + 0.1479x + 102.75$ ($R^2 = 1$), 33% $\rightarrow y = -0.1007x^2 + 0.1707x + 102.76$ ($R^2 = 1$), 67% $\rightarrow y = -0.093x^2 + 0.1044x + 102.99$ ($R^2 = 1$), and 100% $\rightarrow y = 0.14x^2 + 0.436x + 102.73$ ($R^2 = 1$).

Total seed weight

Coconut water concentrations and watering intervals influenced total seed weight (Table 3). The prolongation of watering intervals for seed priming in water and coconut water at concentration of 33%, 67%, dan 100% affected the total seed weight at 84 DAP, with corresponding values of 100% (Figure 7). The cytokinin enhanced cell size by inducing cell proliferation in corn seeds (Bu et al., 2020) that were subjected to well-watered and drought stress conditions..

Table 3. Total seed weight of waxy corn from different priming concentrations and growing water regime at 84 days after planting (harvest time).

Coconut water concentrations	Total seed weight per plant (g)			Mean
	Every day	Once every 3 days	Once every 6 days	
0%	81.89cd	62.98ef	6.69h	22.55
33%	92.67bc	74.64de	28.86g	33.77
67%	105.87ab	89.57bcd	58.17f	39.60
100%	122.12a	102.74b	62.16ef	40.55
Mean	100.64	36.10	23.90	+
CV		29%		

Note: Numbers in a column followed by the same letter from similar factor were not significantly different based on the 5% HSD test; '+' = present interaction; CV-Coefficient of variantions.

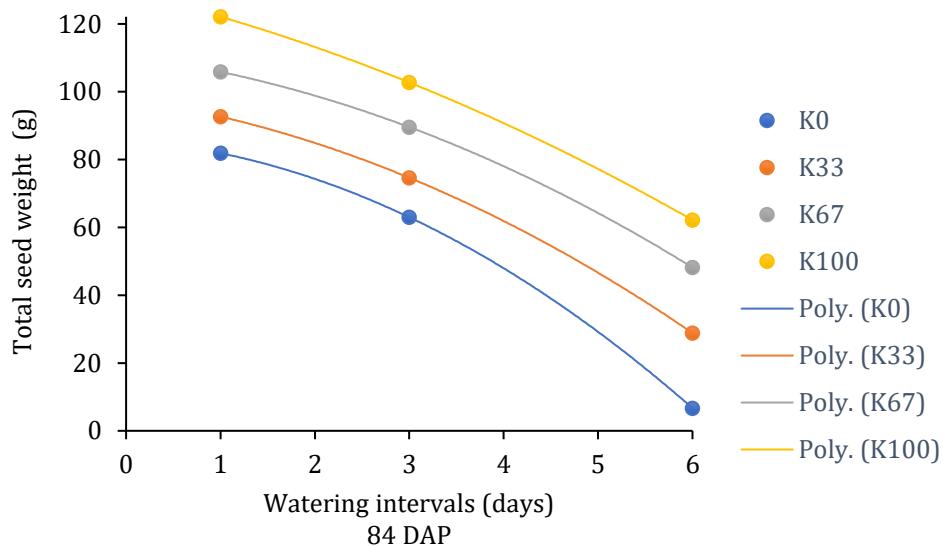


Figure 7. Regression relationship between watering intervals (days) and total seed weight (g) at 84 DAP. Description of the regression equation: coconut water concentrations: 0% $\rightarrow y = -1.8621x^2 - 2.0059x + 85.76$ ($R^2 = 1$), 33% $\rightarrow y = -1.2484x^2 - 4.0239x + 97.942$ ($R^2 = 1$), 67% $\rightarrow y = -1.1304x^2 - 3.6276x + 110.63$ ($R^2 = 1$), and 100% $\rightarrow y = -0.7667x^2 - 6.625x + 129.51$ ($R^2 = 1$).

CONCLUSIONS

Seed priming with 100% coconut water increased corn yield by enhancing photosynthetic rate and total seed weight at all watering intervals in both well-watered and drought-stress conditions.

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