

## Identification of drought stress tolerance of several local Indonesian rice (*Oryza sativa* L.) varieties from Gorontalo at various concentrations of polyethylene glycol (PEG)

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**Abstract:** This investigation aims to evaluate the drought resilience of six indigenous rice varieties from Gorontalo during germination under varying PEG concentrations. The study was conducted in a controlled laboratory setting. An experimental design featuring a two-factorial approach with complete randomization and three replications was utilized. The first factor concerns the rice varieties, specifically: Maraya, Ponda, Pulo Kuku, Pulo Merah, Sonu, and Temo. The second factor involves PEG concentrations, including 0 g L<sup>-1</sup> (control), 8 g L<sup>-1</sup> (low stress), 16 g L<sup>-1</sup> (moderate stress), and 24 g L<sup>-1</sup> (severe stress). Germination performance parameters monitored included germination percentage, germination speed index, T10 germination, T90 germination, mean germination time, mean germination rate, coefficient of variation of germination, and uncertainty, while seedling trait parameters included plumule length, radicle length, fresh weight, and dry weight. Germination traits were calculated based on daily counts of seeds achieving growth greater than 2 mm of radicle. Diverse responses from indigenous rice varieties to drought stress were observed. PEG treatment was found to inhibit both the germination process and seedling development compared to the control. The stress susceptibility index identified Maraya as the only tolerant cultivar concerning plumule length, with a value below 0.5, while the other five cultivars were deemed sensitive across all assessed traits.

**Keywords:** local rice, gorontalo, PEG, stress susceptibility index.

**Abbreviations:** PEG\_polyethylen glycol, SSL\_stress susceptibility index, GP\_germination percentage, GSI\_germination speed index, T10\_time to 10% seed germinated, T90\_time to 90% seed germinated, MGT\_mean germination time, MGR\_mean germination rate, Cvt\_coefficient of variation of germination, and Unc\_uncertainty.

### Introduction

Rice (*Oryza sativa* L.) is essential for fulfilling the dietary requirements of Indonesians. Lowland rice production faces significant obstacles. The loss of arable land to non-agricultural uses is ongoing annually. Concurrently, the population is growing at a rate of 1.25% per year. The Central Bureau of Statistics of Indonesia (2022) indicates that each citizen requires approximately 130 kg of rice annually, leading to a total consumption of about 35,604,367.5 tonnes for the population of 273 million. Consequently, Indonesian rice production is approximately 31.36%, leaving it susceptible to shortages. Kadir et al. (2022) noted that, barring disruptions to land and rice cultivation, future rice requirements could be satisfied through imports. Environmental factors contribute significantly to risks of crop failure and unmet production targets, including droughts during El Nino, flooding during La Nina, and threats from pests and diseases, along with various unavoidable biotic and abiotic challenges.

The principal determinant in preserving the sustainability of optimal food crop cultivation, particularly rice, is the accessibility of water requisite for the support of plant growth and development. Water plays a pivotal role in the growth of plants (Wang et al., 2012). Persistent irrigation is not a prerequisite for the cultivation of local rice with elevated production levels (Wu et al., 2017). Insufficient water availability per unit area of plant growth is counterproductive, as water is subject to losses through mechanisms such as evaporation, seepage, and percolation (Alberto et al., 2011). A

**Table 1.** Cultivation location.

No	Variety	Coordinate	Region
1	Maraya	0°52'35.2"N 123°07'23.4"E	Atinggola, Gorontalo, Indonesia
2	Ponda	0°51'47.4"N 123°07'12.6"E	Atinggola, Gorontalo, Indonesia
3	Pulo Kuku	0°51'47.1"N 123°07'10.4"E	Atinggola, Gorontalo, Indonesia
4	Pulo Merah	0°51'47.1"N 123°07'10.4"E	Atinggola, Gorontalo, Indonesia
5	Sonu	0°51'44.1"N 123°07'08.8"E	Atinggola, Gorontalo, Indonesia
6	Temo	0°51'44.5"N 123°07'09.0"E	Atinggola, Gorontalo, Indonesia

deficiency in water results in the closure of stomata, which diminishes cellular CO<sub>2</sub> levels, while dehydration within leaf mesophyll cells inflicts damage upon photosynthetic structures (Sopandie, 2014). Photosynthesis represents one of the fundamental metabolic processes that governs plant growth and productivity and is significantly influenced by water scarcity or drought stress. A lack of water constitutes an abiotic stressor capable of disrupting all metabolic processes within plants, culminating in impediments to plant growth, development, and a reduction in crop yields (Tripathy, 2000; Manickavelu et al., 2006; Hosain et al., 2020).

Germination represents the initial pivotal stage of plant development. The assessment of plant characteristics in response to drought stress may be conducted during the germination, seedling, or vegetative phases of plants through field experimentation (Chutia and Borah, 2012). The application of polyethylene glycol (PEG) solution serves as an alternative methodology frequently employed to identify plant resistance to drought stress. Polyethylene glycol is an inert macromolecule characterized by an extensive polymeric structure, which has found extensive application in scientific research (Steuter, 1981). PEG is recognized as a pertinent compound for screening purposes, given its capability to regulate seed imbibition and hydration processes. Additionally, PEG is utilized in evaluating seed resilience against drought by considering the drought index (Nemoto et al., 1995; Bouslama and Schapaugh, 1984; McDonald et al., 1988; and Casbuslay et al., 1999). PEG functions as an *in vitro* agent for inducing drought stress due to its ability to diminish the osmotic potential of water without eliciting toxicity, thus effectively simulating drought conditions in plants. PEG has been employed for an extensive period to facilitate the selection of drought-tolerant plant varieties within laboratory settings during the germination phase (Chutia and Borah, 2012). The utilization of a 20% concentration of PEG 6000 can delineate rice varieties that exhibit resistance to drought stress based on the metrics of plumule length, root length, and drought tolerance index, informed by both plumule and root lengths (Maisura et al., 2016). As the concentration of PEG increases, the binding of water by ethylene subunits intensifies, thereby impeding the seed's capacity for water absorption, which culminates in plants experiencing drought stress (Verslues et al., 2006). PEG precipitates germination inhibition as it is intimately linked to the phenomenon of osmotic stress (Sidari et al., 2008).

This investigation seeks to ascertain the drought resilience of six indigenous rice varieties from Gorontalo through the application of various polyethylene glycol (PEG) concentrations. It is anticipated that the findings of this study will yield valuable insights regarding local rice varieties exhibiting drought tolerance, which may subsequently be employed in rice breeding initiatives aimed at producing superior cultivars that are well-adapted to arid environments or regions frequently encountering water scarcity. Through the identification of varieties possessing robust drought resistance, it is envisioned that food security in Gorontalo and other regions confronting analogous challenges will be enhanced. Moreover, this research is instrumental in bolstering the conservation of local varieties with potential for further enhancement, aligning with efforts to diversify food sources and optimize the utilization of indigenous genetic resources.

## Results

Based on the results of the analysis of variance, it shows that the interaction between variety and PEG concentration had a significant influence on the parameters of germination percentage, time to 10% seed germination, and mean germination time. Then, each variety factor and PEG concentration separately had a significant influence on the parameters of germination speed index, time to 90% seed germination, mean germination rate, coefficient of variation of germination, and uncertainty.

In Table 2, it is explained that there is an interaction effect between variety and PEG concentration on germination percentage. In general, the germination percentage ranges from 85.3% to 100%. Especially for the Pulo Kuku variety, increasing the PEG concentration causes a decrease in seed growth capacity. PEG treatment at a concentration of 24 g L<sup>-1</sup> decreased seed growth capacity by 14.7% compared to the control. Then, in other varieties, there were no statistical differences in germination percentage values at different PEG concentrations.

The parameter time to 10% germinated seeds presented in Table 3 shows that there is also an interaction between variety and PEG concentration. In general, all treatments succeeded in growing seeds by 10% starting on day 2. However, the interaction between the Sonu variety with a PEG concentration of 8 g L<sup>-1</sup> recorded the shortest duration to achieve 10% test seed germination, 2.31 days. Then, the interaction between the Temo variety with a PEG concentration of 24 g L<sup>-1</sup> recorded the most extended duration, namely 3.40 days. The same thing was also found in the mean germination time parameter (Table 4), where the interaction between the Sonu variety and a PEG concentration of 8 g L<sup>-1</sup> recorded the fastest mean germination, 3.71 days. Then, the interaction between the Temo variety with a PEG concentration of 24 g L<sup>-1</sup> recorded the longest mean germination, 4.92 days.

The variety factor alone significantly influences the parameters of germination speed index, time to 90% seed germination, mean germination rate, coefficient of variation of germination time, and uncertainty. The PEG concentration factor alone

**Table 2.** Germination Percentage (%).

Variety	PEG Concentration (g L <sup>-1</sup> )			
	0	8	16	24
Maraya	100.0aA	98.7aA	96.0aA	97.3aA
Ponda	98.7aA	98.7aA	97.3aA	100.0aA
Pulo Kuku	100.0aA	98.7aA	94.7aA	85.3bB
Pulo Merah	100.0aA	97.3aA	97.3aA	96.0aA
Sonu	97.3aA	100.0aA	98.7aA	100.0aA
Temo	100.0aA	100.0aA	100.0aA	97.3aA

Notes: Averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test ( $p < 0.05$ ).

**Table 3.** Time to 10% seed germinated (d).

Variety	PEG Concentration (g L <sup>-1</sup> )			
	0	8	16	24
Maraya	2.54aB	2.40bB	3.07aA	2.72bAB
Ponda	2.57aB	2.72abAB	2.65abB	3.22abA
Pulo Kuku	2.66aB	2.86abAB	3.02aAB	3.37aA
Pulo Merah	2.54aA	2.64abA	2.98abA	2.85abA
Sonu	2.34aA	2.31bA	2.42bA	2.76bA
Temo	2.39aB	3.09aA	2.93abA	3.40aA

Notes: Averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test ( $p < 0.05$ ).

**Table 4.** Mean germination time (d).

Variety	PEG Concentration (g L <sup>-1</sup> )			
	0	8	16	24
Maraya	3.91abB	3.92bB	4.24bcdAB	4.29bA
Ponda	4.00abB	4.05abB	4.00cdB	4.76aA
Pulo Kuku	4.17aB	4.32aB	4.80aA	4.87aA
Pulo Merah	4.19aB	4.37aAB	4.51abAB	4.61abA
Sonu	3.72bB	3.71bB	3.89dB	4.25bA
Temo	4.09abB	4.35aB	4.31bcB	4.92aA

Notes: Averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test ( $p < 0.05$ ).

**Table 5.** Germination speed index, time to 90% seed germinated (d), mean germination rate (day<sup>-1</sup>), coefficient of variation of germination (%), and uncertainty (bit).

Variety	Germination Traits				
	GSI	T90 (d)	MGR (day <sup>-1</sup> )	CVt (%)	Unc (bit)
Maraya	6.11b	4.49b	0.24b	14.17c	1.33bc
Ponda	6.04b	4.59b	0.23b	14.97bc	1.27c
Pulo Kuku	5.44c	4.99a	0.22c	18.74ab	1.53ab
Pulo Merah	5.74bc	4.91a	0.22c	19.21a	1.59a
Sonu	6.54a	4.22c	0.25a	15.94abc	1.28c
Temo	5.80bc	4.90a	0.22c	15.49abc	1.35bc
PEG Concentration (g L <sup>-1</sup> )	GSI	T90 (d)	MGR (day <sup>-1</sup> )	CVt (%)	Unc (bit)
0	6.39a	4.46c	0.24a	17.54	1.39
8	6.19a	4.49bc	0.24a	16.27	1.34
16	5.86b	4.68b	0.23b	16.23	1.36
24	5.34c	5.09a	0.21c	15.64	1.48
CV (%)	5.22	4.59	4.00	21.16	13.62

Notes: Averages followed by the same lowercase letter in the column do not differ by the Tukey test ( $p < 0.05$ ).

significantly influenced the germination speed index, time to 90% seed germination, and mean germination rate (Table 5). The Sonu variety recorded the highest germination speed index value, namely 6.54, the highest duration of time to 90% seed germination, namely 4.22 days, and the highest mean germination rate, 0.25. So, each of the Maraya and Ponda varieties recorded the lowest coefficient of variation of germination time value, 14.17, and the lowest uncertainty value, 1.27 bits. Then, it was noted that the treatment without PEG recorded the highest germination speed index value, namely 6.39, the shortest time for time to 90% seed germination, 4.46 days, and the highest mean germination rate, 0.24.

When observing seedlings' morphology, variety, and PEG concentration, there was a single and separate significant influence on plumule length, radicle length, fresh weight, and dry weight (Table 6). The Maraya variety recorded the longest

plumules and heaviest fresh and dry weights, with 8.19 cm, 2.14 g, and 0.50 g. The Sonu variety recorded the longest radicle, namely 12.43 cm, while Pulo Merah recorded a dry weight, the same as the Maraya variety, 0.50 g. Then, the PEG concentration factor consistently showed that the treatment without PEG recorded the longest plumules and radicles and the heaviest fresh and dry weights, with values of 7.16 cm, 11.88 cm, 1.94 g, and 0.53 g, respectively.

Several varieties were obtained in the SSI calculation, which could be categorized as tolerant or moderate to drought stress due to PEG application (Table 7). Four parameters are used as references: plumule length, radicle length, fresh weight, and dry weight. The plumule length parameter noted that the Maraya variety was included in the tolerant category because the SSI value was  $\leq 0.5$ , while the Sonu and Temo varieties were classified as moderate with an SSI value of  $\leq 1.0$ . Ponda, Pulo Kuku, and Pulo Merah were classified as sensitive with an SSI value  $> 1$ . For the radicle length parameter, it was recorded that the Ponda, Pulo Merah, and Temo varieties were included in the moderate category because the SSI value was  $\leq 1.0$ , while the Maraya, Pulo Kuku, and Sonu varieties were classified as sensitive with SSI values  $> 1$ . In the fresh weight parameter, it was recorded that the Pulo Merah variety and Sonu are included in the moderate category because the SSI value is  $\leq 1.0$ , while the Maraya, Ponda, Pulo Kuku, and Temo varieties are classified as sensitive with SSI values  $> 1$ . Then, in the dry weight parameter, it is noted that the Pulo Kuku and Pulo Merah varieties are included in the moderate category because the SSI value is  $\leq 1.0$ , while the Maraya, Ponda, Sonu, and Temo varieties are classified as sensitive with SSI values  $> 1$ .

## Discussion

In general, all varieties responded differently to increasing the PEG concentration given. In the germination percentage parameter, the Pulo Kuku variety experienced a linear decrease in growth capacity as the PEG concentration increased, but the other varieties did not experience this. Of course, this is an initial indication that the Pulo Kuku variety will experience growth problems in certain drought conditions. This differs from the other tested varieties, which did not respond linearly to increasing PEG concentrations. It was recorded that the Ponda and Sonu varieties had a maximum growth capacity of 100% in the PEG treatment with the highest concentration. The same thing was also found by Siddique et al. (2023), who showed that increasing PEG concentration indicated a decrease in the percentage of germinating seeds in rice. Apart from that, research conducted by Purbajati et al. (2019) also found that increasing PEG concentrations in a drought stress study on several rice varieties could significantly reduce the ability of seed germination.

Then, a similar trend was also recorded in the parameter time to 10% germinated seeds, where the Pulo Kuku variety experienced a slowdown in the germination process as the PEG concentration increased, even with a difference of 1 day when compared to the control treatment with the highest PEG concentration. The mean germination time parameter noted that the Maraya, Pulo Kuku, and Pulo Merah varieties were sensitive to increasing PEG concentrations. The higher the PEG concentration, the longer the mean germination time, reaching four days. Research conducted by Evamoni et al. (2023) showed a decrease in germination potential in several rice genotypes that were given drought treatment using PEG.

Other germination traits such as germination speed index, time to 90% seed germination, and mean germination rate show that the Sonu variety performs better than others. Then, the Maraya variety has the lowest Cvt. The coefficient of variation of germination time can be used to evaluate the uniformity of germination or to determine the variability of germination related to the meantime of germination (Ranal and Santana, 2006). The Ponda variety has the best germination synchronization with the lowest uncertainty value. Uncertainty shows the value of seed growth synchronization. The lower the value, the higher the growth synchronization, and vice versa (Genze et al., 2020). A lack of water during periods of high osmotic stress first diminishes the seed's ability to absorb water and reduces the activity of hydrolytic enzymes, which hampers nutrient delivery to the embryo. This combination results in decreased germination, cell division, and elongation (Fahad et al., 2017). Furthermore, drought conditions interfere with water movement through the xylem and lead to reduced turgor pressure (Hussain et al., 2018).

Administering PEG at a specific concentration inhibits the germination of rice seeds, which is evident through a reduced germination speed index, slower seed germination, and a lower average germination rate. The sensitivity of each rice cultivar to drought stress can vary based on its resilience traits (Cabello et al., 2013). Plants respond differently to water scarcity; some demonstrate tolerance, while others employ avoidance strategies (Nazar et al., 2015). Consequently, variations among rice genotypes reveal distinct reactions to drought stress. The differing responses of various rice genotypes to drought are attributed to variations in their morpho-physiological and biochemical traits (Bhandari et al., 2023). The capacity of plants to withstand and recover from drought stress is influenced by differences in genotype responses, the severity of the stress, and the growth stages of the plants (Abid et al., 2018).

The application of PEG at specific concentrations has not only affected germination traits but has also been shown to successfully inhibit the growth of rice seedlings in terms of sprout morphology. Compared to the control group, the radicles, plumules, and fresh and dry biomass accumulation were shorter and lower in the PEG-treated samples. According to a study by Zheng et al. (2016), drought stress significantly impacted early seedling growth in rice, leading to notable reductions in both shoot and root lengths and fresh and dry weights. The characteristics of seed germination and seedling growth are crucial determinants of yield (Rauf et al., 2007). Swain et al. (2014) reported that seed germination and early seedling development are among the most critical stages severely affected by water stress. Therefore, selecting drought-resistant genotypes early in the growing season appears essential and effective (Xie et al., 2013).

Using the SSI value derived from four reference parameters, plumule length, radicle length, fresh weight, and dry weight local Gorontalo rice varieties were identified as either tolerant or moderately tolerant. The Maraya variety was classified as tolerant, while the Sonu and Temo varieties were deemed moderate concerning plumule length. The Ponda, Pulo Merah, and Temo varieties were categorized as moderate for root length characteristics. Additionally, the Pulo Merah and Sonu

**Table 6.** Plumule length (cm), radicle length (cm), fresh weight (g), and dry weight (g).

Variety	Seedling Traits			
	PL (cm)	RL (cm)	FW (g)	DW (g)
Maraya	8.19a	9.99bc	2.14a	0.50a
Ponda	5.52e	10.85abc	1.66b	0.43b
Pulo Kuku	7.94a	9.67c	1.58b	0.48a
Pulo Merah	7.07b	11.54ab	1.74b	0.50a
Sonu	6.09d	12.43a	1.68b	0.49a
Temo	6.66c	10.76abc	1.48b	0.37c
PEG Concentration (%)	PL (cm)	RL (cm)	FW (g)	DW (g)
0	7.16a	11.88a	1.94a	0.53a
8	6.92ab	11.76a	1.67b	0.45bc
16	6.90ab	11.50a	1.83ab	0.45b
24	6.67b	8.35b	1.41c	0.42c
CV (%)	4.38	13.14	12.75	7.56

Notes: Averages followed by the same lowercase letter in the column do not differ by the Tukey test ( $p < 0.05$ )

**Table 7.** Drought sensitivity index based on plumule length, radicle length, fresh weight, and dry weight.

Variety	PL	RL	FW	DW
Maraya	-0.57	1.17	1.06	1.04
Ponda	1.45	0.92	1.22	1.02
Pulo Kuku	1.72	1.06	1.05	0.78
Pulo Merah	1.03	0.81	0.62	0.82
Sonu	0.80	1.00	0.97	1.30
Temo	0.58	0.63	1.36	1.72

Notes: If the value is  $\leq 0.5$  (tolerant),  $0.5 < \text{value} \leq 1.0$  (moderate), and  $> 1.0$  (sensitive).

varieties showed moderate fresh weight characteristics, whereas the Pulo Kuku and Pulo Merah varieties were moderate in dry weight characteristics. The genetic traits of each variety primarily influence this variation in sensitivity. A similar finding was reported by Salsinha et al. (2020), who identified several local rice cultivars from East Nusa Tenggara, Indonesia, that exhibit drought tolerance.

## Materials and Methods

### Plant materials and experimental design

This research was conducted in laboratory conditions, precisely at the Seed Science and Technology Laboratory, Faculty of Agriculture, Hasanuddin University. This research was conducted as an experiment using a two-factorial with a completely randomized design (CRD). The first factor is rice variety, which consists of 6 levels: V1 = Maraya, V2 = Ponda, V3 = Pulo Kuku, V4 = Pulo Merah, V5 = Sonu, and V6 = Temo. The second factor is the PEG concentration which consists of 4 levels, namely: P0 = 0 g L<sup>-1</sup> (control), P1 = 8 g L<sup>-1</sup> (low-stress simulation), P2 = 16 g L<sup>-1</sup> (moderate-stress simulation), and P3 = 24 g L<sup>-1</sup> (severe stress simulation). Each treatment was repeated 3 times to obtain a total of 72 experimental units. These local Gorontalo rice seeds were obtained from farmers in Sigaso Village, Atinggola District, North Gorontalo Regency. Seeds are selected to receive a uniform size and not experience physical damage. Lokasi pertanaman enam varietas lokal tersebut dapat dilihat pada Tabel 1.

### Germination assay

Germination assay was done with petri dishes with a layer of filter paper (Šerá, 2023). Petri dishes measuring 90 mm were used and lined with filter paper. The filter paper was moistened until saturated with distilled water for control and PEG 6000 for drought testing, about 10 mL each; then, seeds were placed evenly on it. Each petri dish contained 25 seeds, then replicated three times. The petri dishes were then placed in a room-temperature environment, and the humidity of the filter paper was checked daily. Observations were made for 14 days.

### Observation and data analysis

The observed parameters of germination performance include germination percentage (%) (GP), germination speed index (GSI), T10 germination (d) (T10), T90 germination (d) (T90), mean germination time (d) (MGT), mean germination rate (MGR), coefficient of variation of germination (Cvt), and uncertainty (bit) (Unc). All germination traits were calculated based on the number of seed successes to growth ( $> 2$  mm of radicle) daily. The parameters of seedling traits include plumule length (cm), radicle length (cm), fresh weight (g), and dry weight (g). Seedling traits were calculated by measuring the seedlings sample at the end of observation. Dry weight measurements were carried out after the seedling samples were oven-treated for 4 hours at 90°C. The stress susceptibility index (SSI) calculation is carried out based on the formula

proposed by Fischer and Maurer (1978) is  $SSI = \frac{1 - \left(\frac{Y_s}{Y_p}\right)}{1 - \left(\frac{Y_s}{Y_p}\right)}$  where  $Y_s$  is the value of the treatment in non-stress conditions,  $Y_p$  is

the value of the treatment in stressful conditions,  $\bar{Y}_s$  is the average value of all treatments in non-stress conditions, and  $\bar{Y}_p$  is the average value of all treatments in stress conditions. The highest concentration, namely 24 g L<sup>-1</sup>, is the reference data in SSI calculations. If the SSI value is  $\leq 0.5$  (tolerant),  $0.5 < \text{SSI value} \leq 1.0$  (moderate), and  $> 1.0$  (sensitive) (Widyastuti et al., 2016). Data were analyzed with R Studio software (R Core Team, 2024), with a SeedCalc package for germination traits (Silva et al., 2019). To calculate the analysis of variance (ANOVA) and Tukey test to detect differences among treatments, both individual factors or interactions, we used the AgroR package (Shimizu et al., 2024). Suppose there is an interaction between variety and PEG concentration. In that case, the test is carried out in two directions, namely looking at differences in varieties at the same PEG concentration or differences in PEG concentration in the same variety.

## Authors Contribution

Ria Megasari: Investigation, formal analysis, Muhammad Darmawan: Writing original draft, validation, supervision, methodology, Asmuliani Rasyid: Validation, methodology, Muhammad Faried: Data analysis, data visualization.

## Conclusion

Based on the research found, it was found that there were different responses of local rice varieties from Gorontalo to drought conditions. Compared to the control, PEG treatment had a slowing effect on the germination process and sprout growth. Through the SSI value, one variety was obtained, namely Maraya, which was classified as tolerant of the plumule length character with an SSI value below 0.5.

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