

ISSN : 2321-9602



Indo-American Journal of Agricultural and Veterinary Sciences



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Tests of Rice Seedlings' Resistance to Drought Stress (*Oryza sativa* L.)

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Article Info

Received: 30-03-2025

Revised: 14-04-2025

Accepted: 29-04-2025

ABSTRACT

The need to enhance yield by creating drought-resistant rice varieties is driven by the ever-increasing demand for rice. There is a great deal of genotype-specific variability in drought tolerance, a complicated polygenic characteristic that is heavily dependent on plant developmental phases. At the glasshouse of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, researchers characterized the seedling stage drought tolerance and revealed genotype variability for morphological and biochemical traits using drought tolerant (Binadhan-19, BRRI dhan83) and drought susceptible (BRRI dhan26, BRRI dhan48) rice genotypes. In the experiment, a fully randomized design (CRD) was used with three replications. The treatments included a control group and one group that was subjected to drought stress caused by 20% PEG. With the use of the right breeding equipment, we collected and evaluated data on length of shoots and roots, weight of fresh roots and shoots, weight of dried roots and shoots, chlorophyll content (SPAD value), MDA, and H₂O₂. Shoot length, root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll, H₂O₂, and MDA were among the variables identified as showing significant variation ($p < 0.01$) in relation to genotype and treatment. Root and shoot growth are both drastically reduced by drought stress, while levels of hydrogen peroxide and malondialdehyde are dramatically elevated. Genotypes BRRI dhan28 and BRRI dhan48, which are sensitive, showed a steeper decline in root and shoot development than genotypes BRRI dhan71 and Binadhan-19, which are resistant. When comparing susceptible and tolerant genotypes, it was shown that tolerant genotypes showed a much smaller rise in H₂O₂ and MDA. H₂O₂ exhibited a strong positive association with root and shoot traits in the control condition, but a strong negative association with chlorophyll content in the drought condition. Under circumstances of adequate watering, MDA had a strong negative association with the majority of the characteristics that were examined. After analyzing all of the seedling-stage traits, the genotype Binadhan-19 was chosen for additional field studies and genetic improvement against drought stress because it is tolerant of both well-watered and drought conditions.

Keywords: Drought stress, Rice, Morphological characterization, Biochemical marker, and PEG.

INTRODUCTION:

Many people across the globe, particularly in Asia, rely on rice (*Oryza sativa* L.) as their primary source of nutrition (Samal et al., 2018). When looking at output and consumption, Asia ranks first. eating rice. The Food and Agriculture Organization of the United Nations estimates the average annual rice harvest to be 499.1 million metric tons, and by 2030, the demand is projected to reach 2,000 million metric tons, driven by population growth. The phenomenon known as climate change a serious risk to farming, especially in developing countries. Turrall et al. (2011) found that plants experience a variety of

abiotic pressures due to climate change, which affects the frequency and magnitude of hydrological variations. In rainfed habitats, rice production is severely constrained by drought, the most important abiotic factor (Pandey & Shukla, 2015; Alam et al., 2022).

Rice is the most significant crop in Bangladesh and the world's second-most-important grain for human consumption. About Bangladesh produces 34.7 million tons of rice annually and devotes 75% of its arable area to growing rice (BBS, 2016). The rice industry accounts for half of the agricultural GDP and one-sixth of the national revenue. The



sustainability and production of Bangladesh's rice crop is perpetually jeopardized by abiotic factors such as heat, cold, and drought. Climate change is making things worse by increasing the likelihood and intensity of these abiotic pressures. Extreme heat and subsequent drought is a major factor reducing harvest success. In moderate drought, rice yields may drop by 10–30%; in extreme drought, the loss can reach 70–90% (Rahman, 2011). It is crucial to note that the ever-increasing population requires an increase in rice productivity, yet according to climate change models, Bangladesh's rice yield would decline by

33% in 2100 (Karim et al., 2012). Therefore, the nation's food security would be severely compromised if rice output were to drop as a result of climate change. Therefore, in order to guarantee food security and sustainable agricultural output, it is critical to create enhanced rice varieties or management practices that are more resistant to drought stress. Given these background details, the current study set out to accomplish two main goals: first, to catalog rice genotypes according to their drought stress tolerance; and second, to identify physiological and biochemical indicators associated with drought stress tolerance.

MATERIALS AND METHODS:

Experimental materials

Plant materials included four different varieties of rice: two drought resistant (BRRI fhan71 and Binadhan-19) and two drought susceptible (BRRI dhan28 and BRRI dhan48). The seeds came from two places in Bangladesh: the Bangladesh Institute of Nuclear Agriculture (BINA) and the Bangladesh Rice Research Institute (BRRI).

Experimental setup and procedures

The control group (C) and the group subjected to 20% PEG-induced drought stress were the subjects of the experiment, which followed a fully randomized design (CRD) with three replicates.

Date and location of the experiment In the glasshouse of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, the experiment was carried out from January to March 2022. To begin the process of growing seedlings in a hydroponic system, we sterilised the rice seeds, submerged them in distilled water for 24 hours, and then placed them on moist filter paper inside of petri dishes. Under glasshouse conditions, seeds

were planted in a row on Styrofoam sheets that floated in trays with regular water. We used two sets of floats—one for stress treatment and the other for control—to arrange the seedlings from each line. Here, the seedlings spent three days in water maintained at 28 °C. Under open glasshouse circumstances, the seedlings were moved to Peter's nutritional medium solution on the fourth day and kept there until they reached a pH of 5.2, with occasional adjustments made with NaOH and/or HCl.

Hydroponic water shortage alleviation

Seedlings that were 14 days old were grown under hydroponic conditions and tested for drought stress for 5 days at (28±2) °C. The seedlings were mostly cultivated in Peter's nutrient solution, which included 20% PEG-6000 and had an osmotic potential (OP) ranging from -4.05 to -4.95 MPa. On the other hand, plants that were not subjected to stress were grown using a regular concentration of Peter's nutrient solution in hydroponic systems.

Information on biochemical and physical characteristics in seedlings

There were five seedlings of each genotype used for each replication to determine the following parameters: root length in centimeters, fresh shoot weight in milligrams, and shoot length in centimeters. After the samples were dried in an oven at 60 °C for three days, we were able to measure the dry root weight (mg) and dry shoot weight (mg). Following a week of drought treatment, biochemical markers such as chlorophyll content (SPAD value), malondialdehyde (MDA), and hydrogen peroxide (H₂O₂) were assessed in the tissues of flag leaves.

Hydrogen Peroxide (H₂O₂) Quantification In order to determine the amount of hydrogen peroxide (H₂O₂) in the flag leaf tissue, the following procedure was used: (Velikova et al., 2000). The extinction coefficient is expressed as nmolg⁻¹FW and has a value of 0.28 μM⁻¹cm⁻¹.



Analysis of Malondialdehyde (MDA)

The MDA concentration was calculated from leaf tissue using the 155 mM⁻¹ cm⁻¹ extinction coefficient and expressed as nmolg⁻¹FW, according to the standard technique outlined by Heath and packer (1968).

Ascertainment of Chlorophyll Concentration (SPAD valuation)

Using a SPAD meter (Model: SPAD 502), the chlorophyll content of the flag leaf, first, second, and third leaves was measured at the conclusion of the drought stress.

Evaluation using statistical methods

A statistical analysis was conducted using the Minitab 17 statistical software program (Minitab Inc. State College, Pennsylvania) and software R, version 3.3.2. The data collected for various parameters were compiled and tabulated in the correct way. We assessed the varietal performance of the genotypes we looked at using a battery of statistical tests, including a two-factor ANOVA, mean performance, percent decrease, correlation coefficient, combined effects of genotype treatment, and more.

Calculation of percent yield reduction

For comparing stressed plants with control plants, the percent reduction was calculated by the following formula:

$$(\%) \text{ Reduction} = \frac{(\text{value of stressed plants}) - (\text{value of control plants})}{\text{value of control plants}} \times 100$$

RESULTS:

Effect of drought stress on rice genotypes at the seedling stage

Morphological traits

The result of analysis of variance (ANOVA) for all the characters (viz., shoot length, root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll, H₂O₂, MDA) showed highly significant ($P \leq 0.001$) variation among the genotypes studied. The present study found a considerable variation in shoot length. On average, shoot length was the highest in BRRi dhan48 (31.84 cm) and the lowest in BRRi dhan71 (19.65 cm) under control condition (**Table 1**). Drought stress resulted in a remarkable decrease in shoot length and the highest decrease was observed in BRRi dhan48 (36.99 %) followed by BRRi dhan28, Binadhan-19 and BRRi dhan71 (25.08, 21.06, and 15.12%, respectively) compared to control (**Fig. 1**). Drought stress lead to a significant decrease in root length among all the genotypes studied. The highest reduction was observed in drought susceptible BRRi dhan28 (25.58 %) whereas the lowest reduction was found in BRRi dhan71 (9.53

%) (**Fig. 1**). The result found that the highest fresh shoot weight was noted in BRRi dhan48 (1066.78 mg) and the lowest was recorded in BRRi dhan71 (343.00 mg) (**Table 1**). Drought stress resulted in significant reduction in fresh shoot weight among all of the genotypes; the highest reduction was found in BRRi dhan48 (77.18 %) whereas the lowest reduction was found in BRRi dhan71 (44.73 %) as compared to control (**Fig. 1**). For shoot dry weight, the highest reduction was observed in drought susceptible BRRi dhan48 (66.55 %) whereas the lowest reduction was found in BRRi dhan71 (17.22 %) compare to their control (**Fig. 1**). Drought stress resulted in a sharp decrease in root fresh weight among all genotypes; the highest reduction was found in BRRi dhan48 (72.21 %) and the lowest reduction was found in BRRi dhan71 (53.30 %) related to their control (**Fig. 1**). Under drought stress condition, maximum decrease was



recorded in BRR1 dhan48 (64.26 %) and the lowest in BRR1 dhan71 (22.69 %) as compared to control (**Fig. 1**).

Biochemical traits

In the study, the treatment effects showed a significant variation in MDA content among the genotypes. MDA was noted maximum in BRR1 dhan48 (25.98 nmolg⁻¹) and minimum in Binadhan-19 (22.17 nmolg⁻¹) under control condition (**Table 1**). Drought stress lead to a significant increase in MDA content in all of the genotypes; the highest induction was found in BRR1 dhan28 (185.76 %) and the lowest increase in Binadhan-19 (104.23 %) as compared to control (**Fig. 1**). Drought stress resulted in a sharp increase in H₂O₂ level, the highest increase was recorded in susceptible variety BRR1 dhan28 (63.77 %) & the lowest induction in tolerant variety Binadhan-19 (34.43 %) as compared to control (**Fig. 1**). Imposition of drought stress resulted in a significant decrease in chlorophyll content (SPAD) among all of the genotypes compared to control. Due

to drought stress, the highest decrease in SPAD was found for BRRI dhan28 (56.49 %) and the

lowest in Binadhan-19 (39.51%) (Fig. 1).

Table 1: Mean performances of 4 rice genotypes based on different morphological and biochemical traits grown under control and drought stress conditions at the seedling stage.

Genotypes	Trt	Shoot length (cm)	Root length (cm)	Shoot fresh wt. (mg)	Shoot dry wt. (mg)	Root fresh wt. (mg)	Root dry wt. (mg)	MDA (nmolg ⁻¹ FW)	H ₂ O ₂ (nmolg ⁻¹ FW)	Chlorophyll (SPAD)
Bina dhan-19	C	26.01 c	15.61 b	612.44 c	110.96 c	327.78 c	44.40 b	22.17 g	26.11 f	75.69 a
	D	20.53 d	13.17 d	279.50 e	75.50 d	188.17 e	28.03 c	45.27 d	35.33 d	48.73 d
BRRI dhan71	C	19.65 d	14.37 c	343.00 d	55.65 f	250.83 d	29.82 c	22.52 g	25.21 g	76.36 a
	D	16.57 e	12.38 e	181.83 g	45.90 h	125.50 g	20.48 d	47.31 c	37.49 c	50.90 c
BRRI dhan28	C	27.49 b	14.69 c	723.33 b	120.63 b	466.89 b	43.61 b	24.19 f	25.86 f	73.13 b
	D	20.08 d	10.83 f	274.67 e	62.23 e	173.00 f	21.67 d	69.12 b	43.78 b	39.10 e
BRRI dhan48	C	31.84 a	18.53 a	1066.78 a	151.12 a	617.89 a	60.51 a	25.98 e	27.10 e	75.50 a
	D	20.12 d	14.13 c	243.33 f	49.30 g	175.00 f	21.58 d	20.48 a	46.60 a	38.77 e
Max.		31.84	18.53	1066.78	151.12	617.89	60.51	69.12	46.60	76.36
Min.		16.57	10.83	181.83	45.90	125.50	20.48	22.17	25.21	38.77
CV		2.23	3.06	2.09	2.16	1.95	3.83	0.77	0.79	1.06

Here, Trt= Treatments, C= Control condition, D= Drought condition.

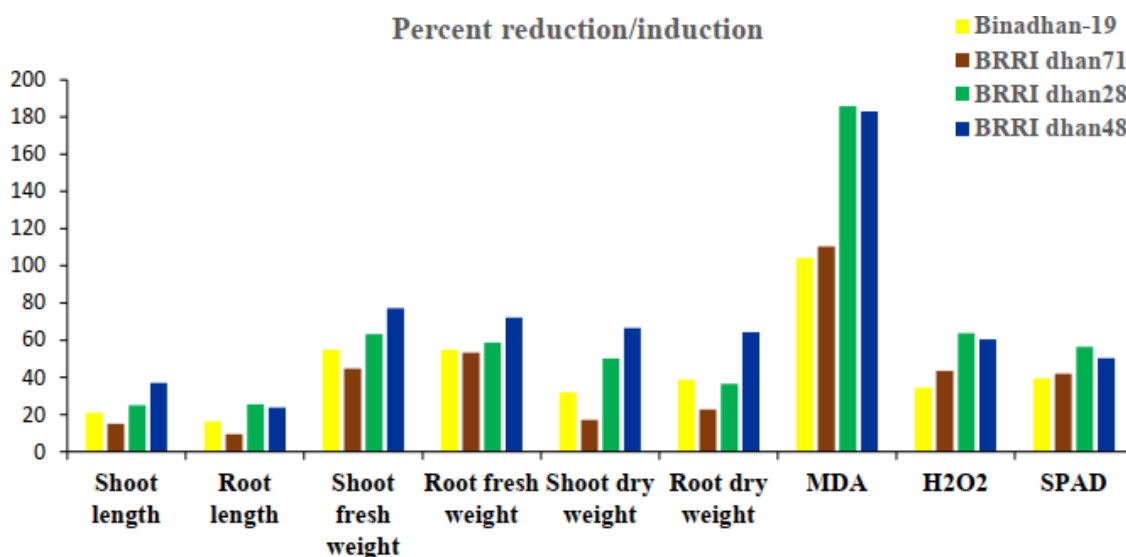


Fig. 1: Percent reduction/induction due to drought stress in morphological and biochemical traits among 4 rice genotypes at the seedling stage.



Estimation of phenotypic correlation co-efficient among nine characters of rice genotypes under control and drought stress conditions at the seed-ling stage

Correlation co-efficient is a measure of intensity or degree of linear relationship between two variables. In the present study, relationship among morpho- logical and biochemical traits were studied through analysis of correlation between them. Phenotypic correlation co-efficient among 9 traits of 4 rice genotypes are presented in **Table 2**. In the present study out of 72 associations, 27 associations were signifi- cant at phenotypic level in control condition and 12 associations were significant at phenotypic level in drought stress condition (**Table 2**). The positive and non-significant associations referred information of inherent relation among the pairs of combination. The negative and non-significant association referred a complex linear linkage among the pair of com- binations.

Table 2: Phenotypic correlation coefficient among nine characters of rice genotypes under control and drought stress condition at the seedling stage.

Traits		Shoot length	Root length	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.	MDA	H ₂ O ₂
Root length	C	0.790							
	D	0.123							
Shoot fresh wt.	C	0.978***	0.859***						
	D	0.929***	-0.090						
Shoot dry wt.	C	0.994***	0.760**	0.966***					
	D	0.652**	-0.145	0.822***					
Root fresh wt.	C	0.936***	0.792**	0.974***	0.915***				
	D	0.955***	0.204	0.942***	0.736**				
Root dry wt.	C	0.968***	0.907***	0.976***	0.954***	0.915***			
	D	0.565	0.249	0.631**	0.881***	0.657***			
MDA	C	0.799**	0.753**	0.883***	0.763**	0.954***	0.797**		
	D	0.419	-0.008	0.273	-0.309	0.307	-0.484		
H ₂ O ₂	C	0.917***	0.922***	0.932***	0.902***	0.858***	0.961***	0.738**	
	D	0.317	0.049	0.139	-0.438	0.200	-0.567	0.988***	
SPAD	C	-0.385	0.182	-0.284	-0.383	-0.369	-0.188	-0.273	-0.071
	D	-0.578	0.068	-0.472	0.091	-0.484	0.313	-0.973***	-0.931***

Here, C= Control condition and D= Drought condition.



DISCUSSION:

The ultimate goal of plant breeding is to develop superior crop varieties with potential to give maximum yield under optimum growth condition as well as able to produce a satisfactory yield under any stressful conditions. The negative effects of drought stress are well studied but the variability of drought stress response at various plant developmental stages by using tolerant and sensitive genotypes has not been well documented. In the present study, an attempt was made to investigate the impact of drought stress on morphological and biochemical traits by using drought sensitive and drought tolerant rice genotypes. A detail discussion of the results found is presented below.

Root length was severely affected by the induction of drought. As drought decreases the growth of plants, the decrease in root length might be due to the negative consequence of drought stress. The reduction in root length due to drought was also found by other researchers (Lanna *et al.*, 2021; Sourour *et al.*, 2017; Omisun *et al.*, 2018; Susanto *et al.*, 2018). Shoot length was also significantly reduced by the imposition of drought stress. The highest reduction of shoot length was found in drought susceptible genotypes compared to their tolerant genotypes. Similar to our results, the reduction of shoot length due to drought was also reported by others (Lanna *et al.*, 2021; Ahmed *et al.*, 2019; Omisun *et al.*, 2018; Susanto *et al.*, 2018).

Root fresh weight was also significantly affected by the imposition of drought stress. The highest reduction of root fresh weight was observed in drought susceptible genotypes compared to tolerant genotypes. The reduction of root fresh weight due to drought was found by Lanna *et al.* (2021), Sourour *et al.* (2017), and Ahmed *et al.* (2019). Shoot fresh weight was severely affected by drought. The reduction of shoot fresh weight was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of shoot fresh weight due to drought was found

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Chlorophyll concentration was also significantly affected by the imposition of drought stress. The reduction of chlorophyll concentration was highest in drought susceptible genotypes compared to tolerant genotypes. The reduction of chlorophyll content due to drought was found by other researchers (Beena *et al.*, 2012; Sourour *et al.*, 2017; Susanto *et al.*, 2018; Ray *et al.*, 2022).

H₂O₂ level was significantly increased due to drought. The reduction of H₂O₂ was highest in drought susceptible genotypes compared to tolerant genotypes. Similar results were also indicated by other researchers (Nahar *et al.*, 2018; Beena *et al.*, 2012; Susanto *et al.*, 2018, Sourour *et al.*, 2017; Omisun *et al.*, 2018). MDA level was significantly increased due to drought. The reduction of MDA was highest in drought susceptible genotypes compared to tolerant genotypes. Similar results were also indicated by others (Nahar *et al.*, 2018; Beena *et al.*, 2012, Sourour *et al.*, 2017; Omisun *et al.*, 2018; Susanto *et al.*, 2018).

In a nutshell, the decrease in growth and development of drought stressed seedlings might be due to the negative effects of high osmotic potential of the nutrient solution that lowered



absorption of water and nutrient (Shrivastava *et al.*, 2015). The morphological parameters of all genotypes usually decreased under drought stress though some drought tolerant genotypes, namely BRRI dhan71 and Bina-dhan-19 maintained higher growth by the adjustment of drought stress (Sourour *et al.*, 2017). Reduction/induction level could be used as an important physiological assortment criterion for drought tolerance in many plant species, such as tomato (Juan *et al.*, 2005; Nahar *et al.*, 2018), wheat (Oyiga *et al.*, 2016) and barley (Chen *et al.*, 2005).

The results of the study also reflected that drought treatment led to the enhancement of H₂O₂ and MDA contents in all genotypes but the accumulations of H₂O₂ and MDA were lower in drought tolerant genotypes compared to drought susceptible genotypes (Table 2). The lower accumulations of MDA and H₂O₂ in drought tolerant genotypes imply protection against oxidative damage by better regulating mechanism to control the formations of more MDA and H₂O₂, and therefore, these genotypes displayed more drought tolerance (Akram *et al.*, 2017; Sourour *et al.*, 2017). In contrast, the higher accumulations of H₂O₂ and MDA contents in drought susceptible genotypes probably due to higher rate of ROS production as well as higher inactivation of antioxidant enzymes (Sourour *et al.*, 2017) leading to oxidative stress and membrane permeability by assaulting membrane lipids (Sourour *et al.*, 2017; Saini *et al.*, 2018; Karim *et al.*, 2021).

Association among nine characters of rice genotypes under control and drought stress condition at the seedling stage

Phenotypic correlation provides the information about the relationship between the two or more than two independent variables. In plant breeding correlation analysis can be used to estimate the value of different traits (Ahmad *et al.*, 2012).

Phenotypic correlations of morphological and biochemical traits at the seedling stage

In the present experiment, the relationship

among morphological and biochemical traits were studied through phenotypic correlation (Table 2). Results reflect a significant positive correlation between shoot length with most of the morphological traits under both control and saline condition. This finding may help to conclude that shoot length and morphological traits might be the better descriptors of drought tolerance of genotypes. However, in saline condition, root length, H₂O₂ content and chlorophyll have not found such significant association. It may be suggested that insignificant relation of root length, H₂O₂ content and chlorophyll under drought stress may not be a good descriptor of drought tolerance. MDA with morphological traits showed significantly negative association indicate that higher the MDA negatively affects the root and shoot growth characteristics. Similar result was also found in H₂O₂ level with chlorophyll content. Thus for screening the tolerant genotypes at seedling stage, its shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, MDA may be considered as selection criteria.

CONCLUSION:

One big problem that hinders the development of rice is drought in the agricultural sector. and efficiency, which causes food insecurity and poor yield potential. Drought tolerance is a complicated polygenic feature that is particular to a plant's genotype and is heavily influenced by the stage of development at which the plant is in. By analyzing morphological and biochemical characteristics of seedling rice, this research aimed to discover possible determinants of drought tolerance. A CRD design with three replications and two treatments (control and drought) was used to carry out the experiment and achieve the suggested goals. Following is a synopsis of the experiment's findings:

For 72 hours, one set of seedlings was dried out with 20% PGE. Data on nine morphological and biochemical parameters were collected and evaluated using suitable breeding techniques after drought stress treatment was finished. Analysis of



variance revealed that genotype, treatment, and genotype all contributed significantly to the observed variation. \times interaction between treatment and majority of the qualities that were investigated. Responses to drought stress included substantial increases in H₂O₂ and MDA levels and marked decreases in root and shoot growth traits. The tolerant genotypes (BRRI dhan71, Bina-dhan-19) showed less of a decline in root and shoot traits than the sensitive genotypes (BRRI dhan28, BRRI dhan48). The tolerant genotype, on the other hand, showed just a little rise in H₂O₂ and MDA levels compared to the tolerant genotype. When tested under controlled circumstances, H₂O₂ correlated positively with root and shoot traits but negatively with chlorophyll concentration. H₂O₂ exhibited a strong inverse relationship with chlorophyll concentration when drought stress was present. Despite showing a strong negative association with the majority of the qualities examined, MDA had no statistically significant link with the majority of these attributes when tested in a controlled environment. Taking into account all of the characteristics seen in seedlings, the genotype Binadhan-19 was determined to be drought tolerant in both controlled and uncontrolled environments.

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