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Arbuscular Mycorrhizal Fungi as Seed Coat of Soybean Grown on Ultisol Applied with Various Doses of Lime

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ABSTRACT

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Indonesia has a wide area of acid-dry land with potential food crops, especially soybeans. The study was conducted to determine the effect of arbuscular mycorrhizal fungi (AMF) as seed coat of soybean on ultisol, applied with various doses of lime. The study was conducted in Ultisols in Kuranji Village, Kuranji District, Padang City, West Sumatra, Indonesia. The experiment was arranged in a split-plot design. The main plot was the dose of CaCO_3 lime with two levels, namely 0 (without lime) and lime equivalent to 2 x Al-dd. Subplots were the application of seed coat in five ratios of AMF inoculant: former soybean planting soil, consisting of 0:4, 1:4, 2:4, 3:4, and 4:4. Each treatment was replicated three times. Data were analyzed for variance and the least significant difference (LSD) test with a significance level of 5%. The results showed that applying lime could increase P uptake in soybean plants by 25.58%. The highest soybean yield was 2.045 tons ha^{-1} , yielded from seed coat treatment with a ratio of 2:4 without lime and the lime treatment equivalent to 2xAl-dd without seed coat.

Keywords: *Acaulospora tuberculata*, lime material, seed coat, soybean cv. Mutiara-1, Ultisol

ABSTRAK

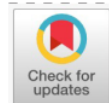
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Indonesia memiliki lahan kering masam yang cukup luas dan potensial untuk tanaman pangan khususnya kedelai. Tujuan penelitian adalah mengetahui peranan kapur dan seed coat inokulan fungi mikoriza arbuskula (AMF) dengan tanah bekas kedelai meningkatkan serapan hara P dan hasil kedelai. Penelitian telah dilaksanakan pada Ultisol di Kelurahan Kuranji, Kecamatan Kuranji, Kota Padang, Sumatera Barat, Indonesia. Percobaan disusun dalam rancangan petak terbagi. Petak utama adalah pemberian kapur CaCO_3 dengan 2 taraf yaitu 0 x Al-dd kapur dan setara 2 x Al-dd. Anak petak adalah aplikasi seed coat sebanyak 5 taraf perbandingan (inokulan AMF : tanah bekas kedelai) yang terdiri atas (0:4); (1:4); (2:4); (3:4) dan (4:4). Percobaan diulang sebanyak 3 kali. Data dianalisis sidik ragam dan uji beda nyata terkecil dengan taraf nyata 5%. Kesimpulan penelitian ini adalah pengapuran mampu meningkatkan 25,58% serapan P tanaman kedelai saat fase pengisian biji dibandingkan dengan yang tidak dikapur. Hasil kedelai tertinggi mencapai 2.045 ton ha^{-1} dari perlakuan seed coat (2:4) yang tidak diberi kapur dan sama hasilnya dengan yang diberi kapur setara 2 x Al-dd tanpa seed coat.

Kata Kunci: *Acaulospora tuberculata*, kapur, kedelai Mutiara-1, seed coat, Ultisol

2 INTRODUCTION

Soybean (*Glycine max* L.) is one of the highest protein-producing plants from the legume group. Indonesian soybean production is generally low. According to BPS data (2018), in 2017, the average Indonesian soybean productivity only reached 1.569 tons ha^{-1} , while West Sumatra only reached 1,193 tons ha^{-1} . Indonesia has only met 30% of the 2.2 million ton⁻¹ demand for soybeans. As a result, Indonesia had to import a 70% of the soybeans demand.

One of the problems with low soybean yields is soil fertility, caused by the development of soybean plants on ultisols reacting acidic with high Al-dd. Indonesia has a wide area of acid-dry land, which has the potential to be developed for food crops, especially soybeans (Bulgarelli et al., 2017). High levels of aluminum and iron are the main problems in these soils. Ultisols have kaolinite clay minerals type 1:1 and sesquioxide clay minerals that have an acidic reaction with low soil cation exchange



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capacity and high phosphate binding capacity by colloidal clay, which are not even available to plants (Faozi et al., 2019). The acidity of ultisols significantly inhibits the life and activities of microorganisms such as Rhizobium bacteria that live on soybean roots (Bulgarelli et al., 2017). Since soybean plants require a neutral soil pH (6-7.5), liming is necessary to raise the pH. The dose of lime must be calculated so to avoid excess lime. The calculation of lime requirements is based on Al-dd equivalence. This approach is significantly better for tropical soils (Krisnawati & Bowo, 2019).

Soybean cv. Mutiara-1, released by the National Atomic Energy Agency (BATAN) in 2010, is a superior variety with super large seed sizes and exceeds the size of soybean seeds produced in America. The weight of 100 soybean seeds cv. Mutiara-1 reaches 23 g, while the weight of 100 g of soybeans from America is only 18 g (BATAN, 2018). This variety has the potential to be developed in West Sumatra.

Arbuscular mycorrhizal fungi (AMF) is commonly suggested in farming communities to be proven through research results by various researchers (Sukmawan et al., 2020). The recommended dose of 10-100 g of AMF inoculant in each planting hole made the AMF inoculant product infeasible for farmers (Muis et al., 2013; Diastama & Wirawan, 2015; Nakmee & Techapinyawat, 2016). If a farmer wants to plant 1 hectare of soybean plants with a spacing of 15 x 20 cm with 10 g of inoculant per planting hole, then the inoculant needed can reach 20.8 tons. This is very ineffective and inefficient. Many AMF inoculants is difficult to provide. Therefore, other alternatives must be available to apply AMF.

The technique of coating this material on each soybean seed (seed coat) can be an alternative. Seed coat on soybean seeds is done by mixing the former soybean planting soil and inoculant in a certain ratio to wrap each soybean seed. A study

was conducted to test the liming and the ratio of soybean seed coat materials (AMF inoculant and used soybean soil) in ultisol soil.

MATERIALS AND METHOD

This research was carried out in Ultisols with a high Al-dd reaction. The experimental location is Kuranji Village, Kuranji District, Padang City, West Sumatra Province, with an altitude of 20 m above sea level.

The research was arranged in a split-plot design, with the main plot of CaCO₃ lime given in 2 levels, namely K0 (without lime) and K1 (equivalent to 2xAl-dd). The subplots consisted of seed coat application at five ratios of AMF inoculant: soil former soybeans, consisting of 0:4, 1:4, 2:4, 3:4, and 4:4. The combination of 10 treatments consisted of 30 replications, resulting in 30 experimental units.

Soil acidity (pH) was measured using a pH electrode with a soil and water ratio of 1:2.5. Soil samples for each treatment plot were taken as much as 10 g, dried, and sifted through a 2 mm sieve. The sample was put into a plastic bottle, mixed with distilled water, and shaken using a shaker for 60 minutes. Next, the sample was allowed to stand for 5 minutes, and the pH was measured (Bindraban et al., 2015).

Analysis of plants was carried out to determine plant P level (%). Plant nutrient content was determined by destroying the plants from the sample plants in each plot. Plant P analysis was carried out using the wet ashing method regarding (Eviati & Sulaeman, 2009). A spectrophotometer was used to measure P levels in plant tissues. Plant P absorption was determined by % P plant x dry weight of plant topsoil, including all parts of the plant from the neckline of the roots to the shoots.

Agronomical observations included plant height, number of branches, number of root nodules, root crown ratio, number of pods per plant,

percentage of empty pods, the weight of 100 seeds, seed weight per plant, and dry seed weight per hectare at 14% moisture content. The number of root nodules was calculated by counting all effective root nodules on the sample plant roots. The characteristic of an effective root nodule is that if the nodule is broken, it will produce a pink liquid. The pink color is a leg-hemoglobin material that is active in fixing N from the air.

The experiment started with soil tillage in the experimental field. The field was divided into 30 plots, measuring 2 x 2 meters and spaced 30 centimeters. In each plot, two seeds were placed into each planting hole, which had a 15 x 20 cm spacing. AMF inoculant (*Acaulospora tuberculata*) was propagated in compost media (62.12 propagules per g inoculant) using the wet sieving and decanting method (Bundrett et al. (1996). According to the treatments, the AMF inoculant was mixed thoroughly with the former soybean planting soil in air-dry conditions that had passed through a 2-mm-diameter sieve. The seeds were coated by soaking them in water for four hours. The seeds were then

mixed with the coating material until it wrapped each seed, with the composition of the seed coat and soybean seeds of 1: 1 (v/v). The water used was sterile distilled water. Next, the coated seeds were planted in each planting hole.

The data obtained from the experimental results were analyzed using the F test at a 5% significance level and then tested using the Least Significant Difference (LSD) test at 5% significance level (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Statistical analysis showed that the liming and seed coat treatments on soybean seeds did not affect soil pH. However, liming and seed coat treatments affected the number of roots, root length, and nodules on soybean roots (Table 1).

The insignificant effect of liming treatment on changes in soil pH is thought to be due to the long-term effect of lime, in which the changes in the reaction are expected to occur after entering the second growing season. Besides, soil pH in the rhizosphere is influenced by root exudates, which

Table 1. Effects of liming and seed coat treatments on the soil pH, number of branches, root length, the number of soybean nodules cv. Mutiara-1 during the generative phase

| Treatments | Soil pH at the rhizosphere | Number of roots | Root length (cm) | The number of root nodules |
|------------------------------|----------------------------|---------------------|---------------------|----------------------------|
| Without lime | | | | 34.20 |
| Mo | 5.30 | 16.33 ^{Ab} | 21.33 ^{Aa} | 40.00 ^{Aa} |
| M1 | 5.53 | 24.00 ^{Aa} | 13.00 ^{Bb} | 16.33 ^{Bb} |
| M2 | 5.07 | 12.67 ^{Ab} | 21.33 ^{Aa} | 41.33 ^{Aa} |
| M3 | 5.23 | 23.67 ^{Aa} | 24.00 ^{Aa} | 36.67 ^{Aa} |
| M4 | 5.25 | 17.67 ^{Ab} | 16.33 ^{Bb} | 36.67 ^{Aa} |
| Lime equivalent to 2 x Al-dd | | | | 35.46 |
| Mo | 5.10 | 15.67 ^{Aa} | 19.00 ^{Ab} | 44.00 ^{Aa} |
| M1 | 5.12 | 19.00 ^{Aa} | 20.33 ^{Ab} | 45.67 ^{Aa} |
| M2 | 5.33 | 10.33 ^{Ab} | 23.00 ^{Ab} | 28.33 ^{Bb} |
| M3 | 5.33 | 11.67 ^{Bb} | 19.83 ^{Ab} | 18.33 ^{Bb} |
| M4 | 5.47 | 20.67 ^{Aa} | 39.33 ^{Aa} | 41.00 ^{Aa} |
| CV Liming (%) | 6.16 | 11.05 | 18.09 | 16.35 |
| CV Seed coat | 6.60 | 19.70 | 13.75 | 15.00 |
| LSD at 5% | - | 5.85 | 5.17 | 9.04 |

Remarks: Means followed by the same uppercase letters are not significantly different between liming treatments, while means followed by the same lowercase letters are not significantly different between seed coat treatments based on LSD test at 5%. M= AMF: former soybean planting soil ratio of: M0=0:4, M1=1:4, M2=2:4, M3=3:4, and M4=4:4.

Table 2. Effects of liming and seed coat treatments on the plant height, number of branches, shoot and root ratio, and P level and uptake of soybean cv. Mutiara-1 during the seed filling stage

| Treatments | Plant height (cm) | Number of branches | P level (%) | P uptake (mg/plant) | Shoot and root ratio |
|------------------------------|----------------------|----------------------|-------------|----------------------|----------------------|
| Without lime | | | 0.58 | 174.70 ^B | 9.63 ^B |
| Mo | 37.33 ^{Aa} | 11.67 ^{Aab} | 0.57 | 164.49 ^{Aa} | 10.71 ^{ab} |
| M1 | 42.00 ^{Aa} | 10.33 ^{Ab} | 0.74 | 190.17 ^{Aa} | 8.88 ^{ab} |
| M2 | 44.00 ^{Aa} | 10.33 ^{Ab} | 0.53 | 157.28 ^{Aa} | 9.24 ^{bc} |
| M3 | 44.33 ^{Aa} | 14.67 ^{Aa} | 0.56 | 182.97 ^{Aa} | 8.48 ^c |
| M4 | 40.67 ^{Aa} | 10.33 ^{Bb} | 0.52 | 178.60 ^{Ba} | 10.87 ^a |
| Lime equivalent to 2 x Al-dd | | | 0.62 | 219.40 ^A | 10.85 ^A |
| Mo | 42.33 ^{Aa} | 7.00 ^{Bc} | 0.57 | 147.19 ^{Ab} | 9.89 ^{ab} |
| M1 | 28.00 ^{Bb} | 6.67 ^{Bc} | 0.64 | 231.28 ^{Ab} | 13.57 ^{ab} |
| M2 | 37.67 ^{Aab} | 11.67 ^{Aab} | 0.58 | 216.66 ^{Ab} | 9.08 ^{bc} |
| M3 | 35.83 ^{Aab} | 8.33 ^{Bbc} | 0.60 | 149.19 ^{Ab} | 8.32 ^c |
| M4 | 47.67 ^{Aa} | 13.67 ^{Aa} | 0.70 | 352.66 ^{Aa} | 13.38 ^a |
| CV Liming (%) | 12.66 | 9.06 | 1.79 | 27.08 | 22.05 |
| CV Seed coat (%) | 11.79 | 15.50 | 10.07 | 18.87 | 13.56 |
| LSD at 5% | 8.16 | 2.81 | | 64.36 | 2.40 |

Remarks: Means followed by the same uppercase letters are not significantly different between liming treatments, while means followed by the same lowercase letters are not significantly different between seed coat treatments based on LSD test at 5%. M= AMF: former soybean planting soil ratio of: M0=0:4, M1=1:4, M2=2:4, M3=3:4, and M4=4:4.

neutralize the alkaline effect caused by the lime. (Krisnawati & Bowo, 2019) explained that giving lime equivalent to 1-2 x Al-dd reduced Al saturation below the tolerance limit for soybean plants. Even giving lime can reduce the soybean need for P.

The number and length of roots are strongly influenced by liming and seed coat of a mixture of AMF inoculants and former soybean planting soil. Table 1 shows that plants with more roots also have longer roots than those with fewer roots. Plants that were not given lime but were given seed coats with a ratio of 1:4 had the highest number of roots compared to other treatments. On this characteristic, the single effect of lime is not significant. However, root length was more influenced by liming with the most AMF in the composition of the seed coat formula. Therefore, AMF and lime are simultaneously able to increase root elongation through the addition of root cells to grow longer. According to Gao et al. (2017), AMF can produce glomalin to increase plant resistance to the environment. Glomalin can also be produced from other organic materials. Glomalin is an adhesive produced by

AMF and protects hyphae from drying out and damage by microbes. (Jamilah & Novia, 2010) have also proven the role of AMF in spurring the growth of maize plants on Ultisol marginal land.

Longer roots will enhance the range of nutrient uptake. Nutrients and water will be more easily obtained by longer roots if soil conditions provide a limited source of nutrients for plants. Faizi et al. (2019) and Bulgarelli et al. (2017) have explained that inorganic P, taken by plants, comes from areas accessible to roots. Inorganic P is generally one of the less available nutrients. Its availability varies with soil redox potential, pH, mechanisms for immobilizing and mineralizing organic phosphorus, temperature, and climate.

There is no direct relationship between soil pH and root nodule formation. Root nodules are influenced more by soybean root exudate, which can invite Rhizobium bacteria to come and carry out symbiosis with plant roots. Soybean root nodules develop more at the root base than at the tip of the root. The developing root nodules always form a cluster as if small grains experience modification

Table 3. Effects of liming and seed coat treatments on the number of pods, filled pods, weight of 100 seeds, and seed dry weight/ha

| Treatments | Number of pods/plant | Filled pods (%) | Weight of 100 seeds (g) | Seed dry weight kg/ha |
|-------------------------------------|----------------------|-----------------|-------------------------|------------------------|
| Without lime | | | | |
| Mo | 42.00 ^{Abc} | 21.26 | 21.08 ^a | 604.375 ^{Bc} |
| M1 | 32.33 ^{Bcd} | 24.78 | 23.59 ^a | 1240.408 ^{Bb} |
| M2 | 50.00 ^{Ab} | 33.70 | 23.45 ^a | 2046.192 ^{Aa} |
| M3 | 23.00 ^{Ad} | 13.72 | 21.10 ^b | 729.225 ^{Ac} |
| M4 | 67.33 ^{Aa} | 26.79 | 22.19 ^{ab} | 1122.975 ^{Ab} |
| Lime equivalent to 2 x Al-dd | | | | |
| Mo | 52.33 ^{Aa} | 42.55 | 23.05 ^a | 2044.717 ^{Aa} |
| M1 | 47.33 ^{Aab} | 28.34 | 23.76 ^a | 1553.167 ^{Ab} |
| M2 | 37.00 ^{Bbc} | 22.61 | 23.18 ^a | 1004.958 ^{Bc} |
| M3 | 29.00 ^{Ac} | 10.96 | 17.42 ^b | 384.2583 ^{Bd} |
| M4 | 53.67 ^{Ba} | 10.79 | 20.08 ^{ab} | 457.3083 ^{Bd} |
| CV (%) | | | | |

LSD at 5%

Remarks: Means followed by the same uppercase letters are not significantly different between liming treatments, while means followed by the same lowercase letters are not significantly different between seed coat treatments based on LSD test at 5%. M= AMF: former soybean planting soil ratio of: M0=0:4, M1=1:4, M2=2:4, M3=3:4, and M4=4:4.

into one large grain. However, this study needs to be followed up on whether this assumption is correct because there is no information regarding nodule modification. The higher the ratio of former soybean planting soil compared to the AMF inoculant in the composition of the seed coat, the higher the number of nodules, both on limed and unlimed soils. The former soybean planting soil contains *Rhizobium* from the growth of previous soybeans, which easily infects the roots and forms nodulations on the soybean roots. Putra et al. (2017) and (Sucahyo & Wijayanto, 2018) explained that a higher dose of *Rhizobium* inoculant could increase soybean root nodules.

Soybean plant height ranged from 28-47 cm, with the number of branches ranging from 6-14 (Table 2). According to the variety description (BATAN, 2018), the height of soybean cv. Mutiara-1 is around 46.8 cm; the weight of 100 seeds is 23.2 g; and the average yield is 2.4 tons.ha⁻¹. In general, plant height in this study was below the variety description. Nutrients may still not be optimally obtained by soybean plants during their growth. When compared to other varieties, the ap-

pearance of Mutiara-1 cultivar is still shorter than the Wilis cultivar (88-92 cm), Tanggamus cultivar (77-91 cm), and Burangrang cultivar (133.9 cm) planted on Ultisols in Lampung Province (Fauzi & Puspitawati, 2018). It is proven that this plant absorbs low P, ranging from 147-352.66 mg plant⁻¹. According to (Ismail et al., 2018), soybeans cv. Detam, Anjasmoro, and Tanggamus have P levels ranging from 0.7% and P absorption reaching 3-8 g, while soybeans cv. Mutiara-1 have P level ranging from 0.5% -0.7%. However, it is different from the results of (Bulgarelli et al., 2017), reporting that the P level of soybean plants at the flowering and seed filling stages ranges from 0.4-1.3%.

The highest P uptake was found in soybean treated with lime and AMF with ratio of 4:4. This shows that AMF inoculation can increase P uptake, producing the highest shoot and root ratio. Similar thing was also reported by (Wardahani et al., 2019), that plant P uptake increased as AMF inoculant increased. In the limed treatment, the increase in the composition of AMF in the seed coat media resulted in an increase in P uptake in soybean plants. (Nakmee & Techapinyawat, 2016)

also reported that the application and type of AMF given to *Sorghum bicolor* plants affected plant nutrient uptake. The highest plant P uptake was found in the plants treated with *Glomus* type AMF compared to other types, such as *G. Agregatum*, *G. occultum*, *A. spinosa* dan *Scutellospora*

Giving lime equivalent to 2 x Al-dd was able to increase 25.58% P uptake of soybean plants compared to plants without lime treatment. There is an effect of calcification on P uptake of soybean plants during the seed filling stage. Although liming did not increase the pH significantly, it was able to eliminate the interference effect of Al on the uptake of P nutrients carried out by plants. (Ismail et al., 2018) and (Krisnawati & Bowo, 2019) explain that monomeric Al forms are considered toxic to plants. Giving lime can reduce the level of monomeric Al poisoning. It is recommended that giving lime should be calculated based on Al-dd levels.

In general, the role of AMF is sufficient to help plant growth in the filling stage. However, this still does not determine the yield of seeds. The application of lime and soybean seed coat resulted in 23-67 pods, 10-42% filled pods, 17 to 23.76 g of 100 seeds, and seed dry weight of 384-2046 kg ha⁻¹. The highest number of pods was obtained from seed coat treatment with a ratio of 4:4 without liming. However, the seed filling rate was very low. Very few photosynthates could fill all the pods, characterized by the ability to form seeds or pods with only 13-33% (Table 3).

Mutiara-1 cultivar is a new soybean variety in West Sumatra, especially Padang, which has large seeds. However, in this study, its ability to fill seeds was very low. The weight of 100 seeds of Mutiara-1 cultivar is up to three times of that of Wilis, Tanggamus, Sibayak, and Kaba varieties (Krisnawati & Bowo, 2019); (Faazi et al., 2019). Large seed need adequate fertilizer, especially P and K elements, because N elements can be fulfilled from the activity

of the nitrogenase enzyme in fixing N₂ from the air carried out by *Rhizobium* bacteria. This can be seen in Table 1, that Mutiara-1 cultivar has quite numerous nodules and great potential in helping the absorption of N nutrients from the air.

The effect of liming and seed coat on the number of pods, number of filled pods, weight of 100 seeds, and seed weight per hectare is presented in Table 3. The number of pods meets the requirements, indicating that the plant has reached optimal pod growth. The highest number of pods was achieved in the soybean treated with seed coat at a ratio of 4:4 without liming. This result shows that Mycorrhiza and *Rhizobium* have the same role in producing pods. Susanti et al. (2018) proved that the application of AMF inoculants could increase the the number of pods in soybeans. It can be compared with the Wilis variety of 22-100 pods (Lukiwati & Kristanto, 2018); (Erlinda et al., 2019) and 21-45 pods of various soybean varieties. The number of soybean pods of the Mutiara 1 cultivar ranged from 23-67 pods, higher than the Wilis, Kaba, and Tanggamus varieties, which had 21-45 pods. According to (Susanti et al., 2018), the average weight of 100 seeds in Mutiara-1 cultivar reached 23.2 g, which is greater than the weight of imported soybean seeds from the USA, which is around 18 g.

Regardless of whether AMF was applied as a seed coat, the pod filling was generally poor in soybean with and without lime treatments, showing only 42% of filling capacity. Seed coat has been reported successful (Erlinda et al., 2019). The poor filling capacity caused low yield per hectare, with an average production of less than two tons per hectare. Despite the fact that Mutiara-1 cultivar could produce 4 tons ha⁻¹ in its location of origin, its productivity reached only 50% in West Sumatra, particularly in Padang City, and even some treatments couldn't yield 1 ton ha⁻¹. Despite normal

performance of the plants, there are signs of P deficiency, which is quite significant. This can be seen from P level at seed filling stage reached, which was only 0.58% and 0.62% in plants with and without liming, respectively. According to (Faoziet al., 2019), the P level of soybean plants is > 0.7%. It is necessary to test the addition of intensive P fertilizer to increase plant metabolism to increase soybean yields. In this experiment, the addition of the P element has not been tried, which is considered to have exceeded the dose already used.

According to Table 3, if the plants are given lime, the seed coat treatment is not necessary. However, if the plants are not given lime, the soybean seeds need to be coated with a ratio of 2:4. Besides requiring P, plants also need other elements, which are strongly influenced by the formation of root nodules. In the same treatment, the number of root nodules was also the highest, so there was a relationship with the adequacy of N nutrients carried out by N fixation by Rhizobium bacteria.

CONCLUSION

Liming was able to increase the P uptake of soybean plants by 25.58% during the seed-filling stage. The highest soybean yield was 2.046 tons ha⁻¹, resulted by the seed coat treatment (2:4) without liming, and the result was the same as those given lime equal to 2 x Al-dd without seed coat.

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