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Direct Organogenesis of Different Explants of Aceh Patchouli (*Pogostemon cablin* Benth.) with Several BAP Concentrations

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Abstract. The patchouli plant (*Pogostemon cablin* Benth.) is a tropical herbaceous plant that produces essential oil. One of the problems is that the production is not yet optimal. Fulfillment of superior seedlings can help increase patchouli productivity. Conventional patchouli propagation through stem cuttings is ineffective and takes longer. Patchouli propagation can be done using a tissue culture approach via direct organogenesis to produce seedlings quickly and efficiently. Effective patchouli propagation methods and successful acclimatization are very important to research to support the propagation and breeding of patchouli plants. The aim of this research was to determine the best of BAP concentration in direct organogenesis of leaf and stem explants. The research design used a completely randomized series of hormone BAP, it has 5 levels, namely 0 mg/L (as control), 0.25 mg/L, 0.50 mg/L, 0.75 mg/L, and 1.0 mg/L. The explants used were the leaves and stems of Aceh patchouli. Plantlets are acclimatized in compost media and covering treatment. Based on the results of observations, the best BAP concentration is 0.25 mg/L with the initial observation parameters of the early emergence of shoots, number of shoots, and length of shoots on leaf explants were 10 daps, 35.33 shoots, and 2.83 cm respectively. The use of leaf explants showed a better response compared to stem explants. Patchouli plantlets were successfully acclimatized and can adapt to the ex vitro environment using the covering method. Successful patchouli propagation and high acclimatization can help produce effective patchouli seeds.

Key words: Explants; BAP; Direct Organogenesis; Patchouli.

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INTRODUCTION

The patchouli plant (*Pogostemon cablin* Benth.) is a tropical herbaceous plant that produces essential oils (Rahayu and Heriani, 2021). It has the highest essential oil content compared with the other patchouli varieties, reaching 2.5-5% (Sahwalita and Herdiana, 2016). Patchouli essential oil is needed in industrial fields such as perfume, soap, cosmetics and medicine. Pogostemon has been widely explored to obtain key chemical compounds to produce new drugs (Swamy and Sinniah, 2015). Patchouli plants contain bioactive compounds including terpenoids, flavonoids, organic acids, and patchouli alcohol (Junren *et al.*, 2021). One of the problems of patchouli plant is the oil production in Indonesia still limited and the production is not optimal yet (Wahyudi *et al.*, 2022). The availability of superior seedling can increase plant patchouli productivity. Patchouli plant can be propagated by conventional method such as stem cuttings, but its not efficient planting material and needed a long time to produce seeds. Based on research (Rikatari *et al.,* 2016), the successfull of stem patchouli cuttings requires soaking on a high concentration hormone, namely NAA 200 mg/L.

The propagation of patchouli by tissue culture approach can produce healthy and efficient seedling. The propagation of patchouli can be done through either direct or indirect organogenesis (Yusniwati *et al.*, 2021). The propagation through tissue culture can produce plants quickly and potentially free from viruses and diseases (Florenika *et al,* 2022). Direct organogenesis can produce plant organs such as shoots, roots, and leaves which can arise directly from meristems or undifferentiated cells such as

callus (Oseni *et al.*, 2018). The explants be used can influence on the direct organogenesis induction. However, each explants shows different response. According to Yusniwati *et al.* (2021) the node explant can induce shoots faster than the leaf explant. However, it produces a smaller number of shoots compared with leaf explants.

Direct organogenesis can form organs or shoots multiplication that produce seedlings quickly and effectively (Maulia and Zuyasna, 2021). The success of patchouli in producing shoot multiplication is largely influenced by optimal hormone concentrations. It is important to use the right type of hormone and concentration to effectively induce direct organogenesis of Aceh patchouli. According to Deepa and Thomas (2022), the type and concentration of cytokinin hormones influence shoot formation, the BAP hormone alone shows the best results. BAP can increase the shoot multiplication (Manurung *et al.,* 2021). Based on previous research, the addition of BAP at low concentrations showed a better response to patchouli shoot multiplication. According to Lalthafamkimi *et al.* (2021), the use of leaf explants can produce the highest number of shoots with an addition BAP hormone concentration is 1.0 mg/L, with a number of shoots is 29.2/explant compared to a higher concentration, namely BAP 1.5 mg/L which produced 21.9/explant and BAP 2.0 mg/L with a number of shoots is 19.3/explant. This is in line with research by Kumara Swamy *et al.* (2010) stated that the addition of 0.5 mg/L BAP produced higher shoots compared with BAP concentrations of 1 mg/L and 2 mg/L. According to research by Mayura *et al.*, (2020), the BA hormone 0.01 mg/L is the best treatment for organogenesis of patchouli plant accession Rimba Binuang with the parameters are percentage of live explants, number of shoots per explant, and total number of leaves. The aim of this research is to determine the best BAP concentration in direct organogenesis of leaf and stem explants. Effective patchouli propagation and successful acclimatization are very necessary to produce healthy patchouli seedlings. Healthy patchouli seedlings can be used as parents for conventional patchouli propagation. Based on research by Swamy and Sinniah (2016), the success of acclimatization of patchouli reaches 80-90%. The success of acclimatization is influenced by light conditions, temperature, humidity and nutrition of the planting media (Anis and Ahmad, 2016).

METHODS

The research was conducted at the Ecophysiology and Plant Tissue Culture Laboratory of the Program Study of Agronomy, Faculty of Agriculture, University of Jember, starting from January 2023 to August 2023.

Research Design

The research design used a completely randomized series of hormone benzylaminopurine (BAP). BAP has 4 levels, namely 0.25 mg/L, 0.50 mg/L, 0.75 mg/L, 1.0 mg/L, and control. The medium used was murashige skoog (MS) medium with the addition of BAP at different concentrations. The medium was added with 30 g/L sugar and 8 g/L gel. The tissue culture medium was sterilized using an autoclave at 121˚C and 17.5 psi pressure for 60 minutes.

Planting Explants

Propagation of patchouli plants can be divided into third stages namely establishment of direct organogenesis, proliferation, and acclimatization. The explants used in this research are leaves and stems of the Aceh patchouli plant. The explants sterilized by washing gently with sodium hypochlorite and the explants were cut around 1 cm. Then, planting explants in the culture media. The results of the planting were stored in an incubation room with a temperature of 22˚C and in bright conditions or illuminated with an LED lamp (3000 lux). Proliferation of shoot was carried out on the same medium, namely MS medium with the addition of the hormone benzylaminopurine (BAP). The propagation of patchouli plants through organogenesis is cultivated to produce a root system.

Acclimatization of Seedlings

Plantlets resulting from the propagation of patchouli plants through organogenesis are acclimatized in compost media. Compost media is sterilized by autoclaving at a temperature of 121˚ C. Acclimatization is carried out by removing the plantlets and cleaning them from the culture medium with sterile water. Next, the plantlets are immediately planted in compost media in a glass with covering treatment. The plantlets were kept in an incubation room at a temperature of 25^{o} C and illuminated with LED lights for 24 hours. During acclimatization care, the plantlets are watered every 3 days with MS nutrient solution in the field capacity or approximately 10 ml. Acclimatization was carried out for one month and

the patchouli seedlings were transferred to the greenhouse. Patchouli seedlings were cultivated in a greenhouse and fertilized with 5 g/L foliar fertilizer.

Observation Variable

Observed variables in research included early emergence of shoots, number of shoots, shoot length, and histology of early shoot emergence. The early emergence of shoots was observed from the start of explant planting until the start of the response to shoot emergence. The number of shoots and shoot length were observed in 4th week.

Histology

The best of early emergence of shoot observed by histology. Histological observation by making preparations with stages including sample fixation using formaldehyde for 12-24 hours at a temperature of 25-30˚C. Then the samples were dehydrated using acetone with graded concentrations (70%, 80%, and 90%) for a day. Next, clearing using xylol for 15 minutes twice. Then, embed it by placing the sample in liquid paraffin. Next, blocking was carried out using paraffin. The sample cut uses a microtome knife. The samples were selected and stained using heamatoxylin-eosin. The sample is put down on the glass slide and label. Next, observations were using a binocular microscope at 100x magnification (Karabiyik and Sen., 2023).

Data Analysis

The observation parameters used in this study include the early emergence of shoots, the number of shoots, and Shoot Length. Data obtained from observations were analyzed using analyze of variance (ANOVA), if the results obtained showed significantly different results, they would be analyzed using the Duncan multiple range test (DMRT) at a 95% confidence level.

RESULTS AND DISCUSSION

Histology of early shoot emergence

Histological observations help analyze morphogenetic events and determine tissue development that occurs during plant regeneration in vitro conditions (Santana *et al.*, 2023). The propagation of patchouli can be done by direct organogenesis or organ formation without going through the callus formation phase. Direct organogenesis in leaf and stem explants is successful in showing shoot formation with a success percentage of 100% (F2). Based on observations, leaf explants showed better growth compared to stem explants. This is because leaf explants are parts of plants that actively divide and are maristemic in nature. Patchouli leaf explants are capable of producing a large multiplication of shoots reaching around 4.5-16.3 shoots (Yusniwati *et al.*, 2021). The results of initial histological observations of the early emergence of shoots on patchouli leaf explants (F1).

Based on the results of histological observations at the beginning of the emergence of shoots on patchouli leaf explants, the surface of the leaf explants gave rise to meristematic spots on the explant cut scars (ms=meristematic spots) (F1a). Meristematic spots show optimal cell division and develop to form shoot protrusions and produce shoot multiplication (F1b). In early observations of the early stages of multiplication, it was seen that there were many shoots that would develop into many shoots of organogenesis or multiplication. According to Deepa and Thomas (2022), Histology of regenerated leaf explants forming the initiation of shoot apical meristem (SAM), leaf primordia (LP), and vascular connections (VC) from the explant to the shoot apex. Histology shoot organogenesis showing multiple shoot buds originating from explant. The initial buds will develop into shoots and form leaf organs.

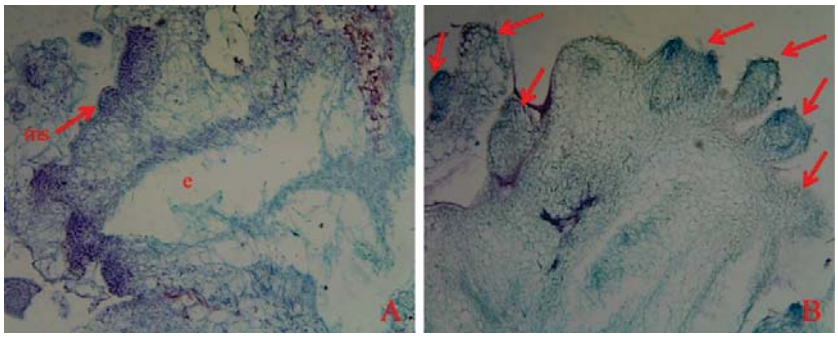

Figure 1. Histology of direct organogenesis in patchouli leaf explants. (A) early onset of shoot emergence, ms=meristematic spots, e=explant; (B) beginning of shoot multiplication.

Direct organogenesis of stem and leaf explant

Stem and leaf explants succeeded in inducing shoots on MS media with the addition of the BAP hormone. Shoots formed from explants show various sizes. Each explant produces shoots continuously so that the growth in each explant is not uniform in size. The shoots will continue to develop and produce multiplication as in Figure 2.

Based on the results, show that BAP hormone treatment has a significant effect on the multiplication of leaf and stem explants. Providing the BAP hormone can produce good shoot proliferation (Lalthafamkimi *et al.*, 2021). The concentration of BAP affects cell division. Cell division that occurs will affect the number of daughter cells formed. Each daughter cell will differentiate into a shoot primordia (Rahayu and Banowati, 2022). Leaf explants grown on MS media with the addition of 0.25 mg/L BAP showed the best multiplication growth with the highest number of shoots, the highest shoots, and the fastest initial shoot emergence (Table 1).

The propagation of patchouli plants by organogenesis can directly produce rapid shoot multiplication. Based on observations, treatment with 0.25 mg/L BAP on leaf explants was able to show early emergence of shoots within 10 days after planting. The application of 0.25 mg/L BAP to stem explants showed early emergence 12 days after planting. Providing the BAP hormone with a smaller concentration shows a rapid initial response to the emergence of shoots. The rapid emergence of shoots indicates that the explants responded well to the administration of the BAP hormone. Leaf explant induces shoots faster than stem explant. According to Yusniwati *et al.* (2021), node explant induces shoots faster than leaf explant. In addition, a low BA concentration of 0.3 mg/L was able to induce rapid shoots, namely around 9.3 days after planting compared to higher BA concentrations of 0.4 mg/L and 0.5 mg/L (Yusniwati *et al.*, 2021).

The best number of shoots was obtained from leaf explants treated with a BAP concentration of 0.25 mg/L with an average of 35.33 shoots. Using stem explants with an addition of 0.25 mg/L BAP showed the best number of shoots with an average of 11.67 shoots. The BAP 0.25 mg/L showed the best results compared to the control and other higher BAP concentrations. The best shoot height was obtained from leaf explants treated with 0.25 mg/L BAP with an average of 2.83 cm at the age of a month. Leaf explants showed better shoot height compared to stem explants. Treatment of 0.25 mg/L BAP on stem explants only produced shoot height with an average of 1.63 cm. According to Yusniwati *et al.* (2021), a low concentration of BA 0.3 mg/L in leaf explants was able to induce more shoots and the highest shoot, namely around 16.3 shoots and 2,1 cm, compared to higher BA concentrations, namely 0.4 mg/L and 0.5 mg/L (Yusniwati *et al.*, 2021). The concentration of BA that is too high can cause callus growth more dominant and can inhibit the growth of shoots and leaves (Dewi *et al.,* 2022).

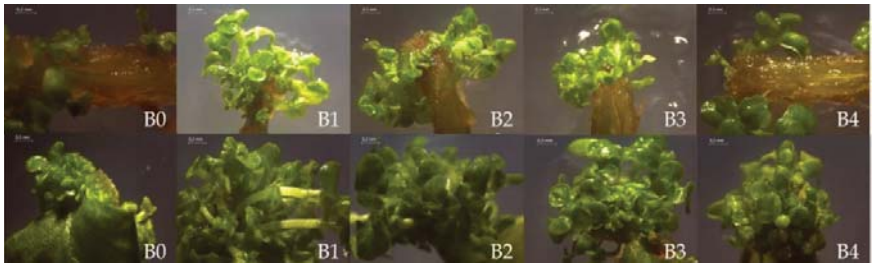

Figure 2. Direct organogenesis in stem and leaf explants of Aceh patchouli at one month of age. (B0) control, (B1) BAP 0.25 mg/L, (B2) 0.50 mg/L, (B3) 0.75 mg/L, (B4) 1.0 mg/L.

Table 1. The parameters of early shoot emergence (DAP), number of shoots, and shoot length in direct organogenesis of Aceh patchouli

Treatment	Early shoot emergence (DAP)		Number of Shoots		Shoot Length (cm)	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Control	16 ± 0.58^{d}	18 ± 0.58^{d}	8.33 ± 1.53^{e}	5.33 ± 0.58^{d}	0.43 ± 0.06^{d}	0.27 ± 0.06^{c}
BAP 0.25 mg/L	10 ± 0.00^{a}	12 ± 0.00^{a}	35.33 ± 3.06^{a}	11.67 ± 2.52^{a}	2.83 ± 0.15^{a}	1.63 ± 0.15^{a}
BAP 0.50 mg/L	10 ± 0.58^{ab}	14 ± 0.58^{b}	30.33 ± 2.52^{b}	10.67 ± 1.53^{ab}	1.47 ± 0.21^{b}	1.00 ± 0.20^{b}
BAP 0.75 mg/L	11 ± 0.00^{b}	15 ± 1.00^{c}	22.67 ± 2.08^{c}	8.33 ± 0.58^{bc}	1.33 ± 0.15^{b}	0.87 ± 0.12^{b}
BAP 1.00 mg/L	12 ± 0.58^{c}	17 ± 1.00^{d}	18.33 ± 1.53^{d}	6.67 ± 0.58^{cd}	0.83 ± 0.06^{c}	0.43 ± 0.06^{c}

The addition of BAP hormone at higher concentrations shows increasingly stunted growth. Shoot multiplication of patchouli at low concentrations showed the best results on leaf and stem explants. The control treatment showed the worst growth compared to the highest concentration, namely BAP 1 mg/L. This is in accordance with research by Swamy *et al.* (2014), which showed that the addition of BAP showed better results compared to the control. However, based on research by Swamy *et al.* (2014), the best BA concentration is 0.5 mg/L which effectively induces shoots and increases shoot multiplication with an average of 46.1 shoots/explant. According to research by Lalthafamkimi *et al. (*2021), the use of patchouli leaf explants was able to produce the highest number of shoots at a BAP hormone concentration of 1.0 mg/L compared to higher concentrations, namely BAP 1.5 mg/L and BAP 2.0 mg/L.

Proliferasi of shoot

The multiplication results were carried out by proliferation on MS media with the addition of 0.25 mg/L BAP. The results of shoot proliferation showed shoot growth and elongation at the age of 2 months (F3b). Patchouli plantlets were observed at the age of 3 months with good shoot and root growth (F3c).

The propagating of patchouli plants by organogenesis can directly produce seedlings quickly and effectively. Based on observations, treatment with a BAP concentration of 0.25 mg/L on leaf explants was able to produce early shoot emergence for 10 days after planting. Patchouli plantlets can be produced at the age of 3 months after sub-culturing. Propagating patchouli through tissue culture can produce lots of patchouli seedlings quickly and more efficiently. Propagation of patchouli plants through multiplication has been reported to produce seedlings quickly and effectively (Maulia and Zuyasna, 2021).

Acclimatization of Seedlings

The propagating of patchouli from in-vitro propagation cannot be planted directly and needs to be acclimatization gradually adapted to field conditions and optimal humidity. Acclimatization is the transition process of plants from in-vitro conditions to ex-vitro environments. Acclimatized plants will be subjected to abiotic stress due to differences in environmental conditions and higher transpiration rates (Khandel *et al.*, 2022). One of the success factors during acclimatization is maintaining humidity to reduce the rate of transpiration. According to Khandel *et al.* (2022), the duration of exposure and light intensity are the main factors for success during the acclimatization. Acclimatization of patchouli seedlings from in-vitro propagation was carried out using the covering method and using compost media. Acclimatization was carried out in stages starting from storing in incubation room conditions for 2 weeks. Then transplanted in polybags and moved to the greenhouse at the age of a month.

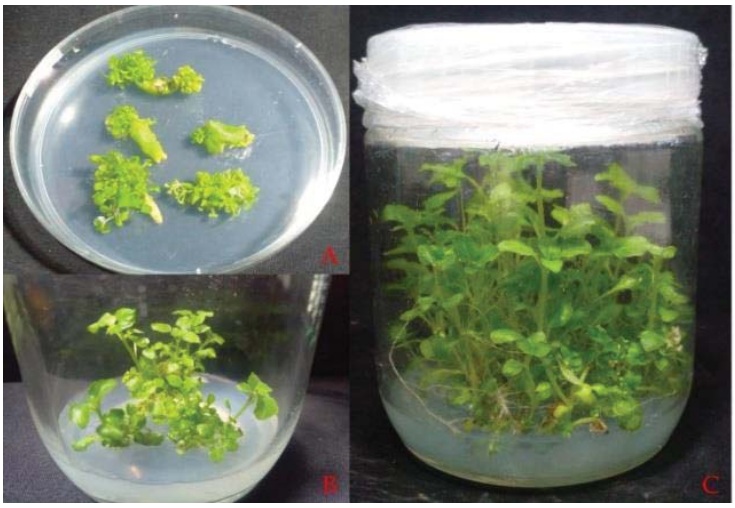

Figure 3. Direct organogenesis in leaf and stem explants of Aceh patchouli. (A) shoot multiplication (1 month), (B) shoot proliferation (2 months), (C) plantlet (3 months).

Figure 4. Acclimatization of patchouli plantlets using the covering method. (A) initial acclimatization under cover conditions, (B) condition of plantlets at the beginning of opening the cover, (C) condition of plantlets at the age of 2 weeks after opening the cover, (D) condition of plantlets seen from above, (E) growth of patchouli after transplanting into polybags.

The initial acclimatization conditions of the patchouli that were covered showed good and adaptive growth (F4a). The lid was opened in the 2nd week and the small plantlets died. Small plantlets show nonadaptive growth and experience drought. Defense ability in small plantlets during adaptation is low. Small plantlets lose water due to the high transpiration rate after the cover treatment is opened. During the acclimatization stage, plantlets can be treated with anti-transpiration to increase the percentage of survival (Anis and Ahmad, 2016). According to Hidangmayum *et al.* (2019), chitosan can effectively reduce transpiration by closing stomata. Chitosan treatment can induce ABA as an antitranspiration which affects stomata closure (Hidangmayum and Dwivedi, 2022). Jasmonic acid treatment can minimize water loss by regulating stomata opening and closing in Arabidopsis thaliana (Wang *et al.*, 2020).

Plantlets that can survive and are able to adapt are shown in figure (F4c). Plantlets that are a month old and have adapted to the external environment are planted in large polybags and stored in a shaded greenhouse. Figure 4e shows patchouli that has been transferred to polybags and is able to adapt to greenhouse environmental conditions. The successful acclimatization of patchouli seedlings influences the level of success in adapting to the field. According to Deepa and Thomas (2022), pogostemon quadrifolius seedlings that had been acclimatized for 3 months showed an adaptation level reaching 94% in the field environment. Acclimatization using the cover method can increase humidity and reduce the transpiration rate which affects the successful adaptation of plantlets. Acclimatization of elatior plantlets using the cover method for 45 days produces plants with higher fresh and dry mass, higher photosynthetic pigments (Pinheiro *et al.*, 2021).

The acclimatization stage must be carried out gradually at lower humidity, higher light, and temperature conditions (Anis and Ahmad, 2016). The process of adaptation to environmental changes is very necessary for success and optimal growth. Failure at the acclimatization stage is an important problem and needs to be controlled. Acclimatized plants will be subjected to abiotic stress due to differences in environmental conditions and higher transpiration rates (Khandel *et al.*, 2022). Based on previous research, acclimatization of algarbiensis plants in the greenhouse showed a decrease in H_2O_2 content indicating recovery due to oxidative stress (Gonçalves *et al.,* 2017).

Patchouli plant breeding to increase essential oils and produce superior patchouli is very necessary. In several previous studies, efforts to increase essential oils in patchouli were carried out by triggering mutations in patchouli leaf explants using cholchicin which was added to the culture medium to produce tetraploid patchouli with greater morphological characteristics and the potential to become a superior variety (Widoretno, 2016). According to Sugimura *et al.* (2005), improving the characteristics of patchouli plants was carried out through genetic transformation to produce virus-free plants by infecting A. tumefaciens strain EHA101/pIG121-Hm which carries the b-glucuronidase (GUS) and hygromycin phosphotransferase (HPT) genes. Procedures and methods for in-vitro propagation of patchouli that are effective and have high acclimatization success are very helpful for patchouli plant breeders. Based on the research results, in vitro propagation of patchouli is effectively carried out through direct

organogenesis in the leaves with the addition of the BAP hormone 0.25 mg/L in MS media. High acclimatization success with the application of cover during the acclimatization stage. The in vitro patchouli propagation method along with acclimatization is very useful for producing healthy and disease-free patchouli to produce patchouli seeds.

CONCLUSION

Leaf explants showed a better response compared to stem explants in direct organogenesis. A BAP concentration of 0.25 mg/L showed the best response in terms of early emergence of shoots, number of shoots, and shoot height. Propagating patchouli by tissue culture can produce many seedlings within 3 months during in-vitro growth and a month during adaptation in an ex-vitro environment. Further research needs to be carried out regarding the success factors for patchouli acclimatization as well as changes in the biochemical content of patchouli during abiotic stress at the acclimatization stage.

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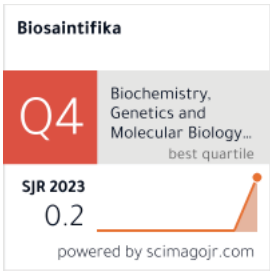

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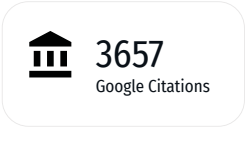

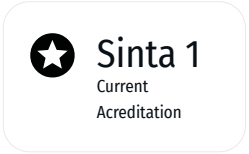

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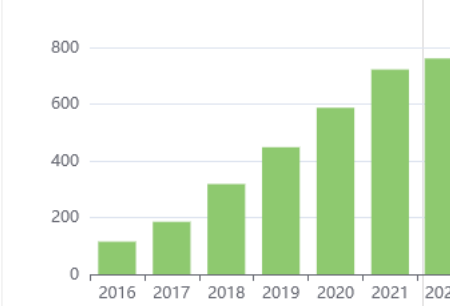

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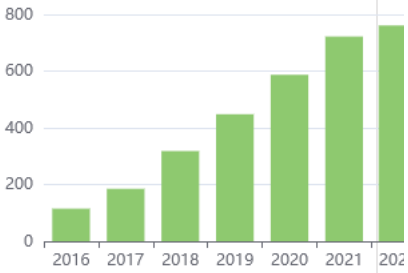

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