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Growth and phytochemical properties in differences weight of porang bulbil (*Amorphophallus muelleri* B.) var. Madiun 1

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Abstract

The increase of Indonesian's *porang* export has led into a new opportunity for better prosperity of local farmers. Thus, the demand for *porang's* bulbil/ *katak* has risen due to its function for vegetative propagation. This study aimed to observe the growing and phytochemicals properties of *porang* tubers from bulbil with 10 bulbil weight categories samples, which were 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, and 5 g. The parameters observed were statistically analyzed in PAST 4.03. This study was conducted for three months and the results showed that samples from bulbil weighing 5 g had significant higher value than the others for the plant height, crown diameter, and stem diameter, but bulbil samples with other weights did not show any significant difference in *porang* tuber growth. Phytochemical's analysis for dissolved protein resulting in 0.5 g weight sample was the best of all but random results for carbohydrate, antioxidants, polyphenol, and saponin.

INTRODUCTION

As one of the agricultural countries in the world, Indonesia continues to carry out sustainable development in order to achieve food security, one of which is through the optimization of agricultural products. In the 2020 State Revenue and Expenditure Budget (APBN), it was reported that from January to June only the agricultural sector had a positive growth of 9.6% (Dahiri and Fitri, 2020). Based on data from the Agricultural Quarantine Agency-Ministry of Agriculture of the Republic of Indonesia (2019), there was an increase in exports of *porang* commodities from 11,000 tons in 2018 (equivalent to 220 billion rupiah) to 11,300 tons (equivalent to 226.4 billion rupiah) in 2019. *Porang*, in the form of chips or intermediate products for food or industrial raw materials, was exported to China, Hong Kong, Vietnam, Thailand and Pakistan (Administrator, 2019). *Porang* is a promising agricultural commodity which opens new opportunities to improve farmers' welfare and state income.

Porang (*Amorphophallus muelleri*) or known as *iles-iles* is a native plant in Indonesia growing in the wild forest, under a bamboo grove, or even in farmland. It comes from family Araceae and spreads worldwide in South Asia and Southeast Asia, from India, Myanmar, Thailand, and Indonesia. This perennial plant can easily grow in a tropical region, even up to 1.5 meters in height (Supriati, 2016). *Porang* grows better in a shading environment especially under trees, so it can be added as an intercropping plant in plantation crops (Lontoh et al., 2019; Srzednicki and Borompichaichartkul, 2020).

Indonesia has several types of *porang*, and one of which has been officially released by the Ministry

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of Agriculture, namely Madiun 1 variety. Based on the official website of the Indonesian Agency for Agricultural Research and Development, the Madiun 1 variety was released in February 2020 as proof of the collaboration between the Madiun Government and the Indonesian Agency for Agricultural Research and Development.

Morphologically, *porang* has the characteristics of mostly *Amorphophallus* plant. Its stem grows vertically, *teres* (tube-like stem), the upper side looks like a bowl and it has a brown color with nodules, but stem branches and petioles have green color and white spots on the bark. The solitary leaf has flat lamina form, *integer partitus*, and *penninervis nervatio*. The flower will grow when the leaf enters degeneration phase; it has a short stylus and an ovule per locus. The lower part is female flower part, the middle one is male, and the upper part is steril (Srzednicki and Borompichaichartkul, 2020). The appearance of the flower signifies the maturity of *porang*.

Porang propagation can be done in vegetative and generative way. Vegetatively, it uses bulbil/*katak* as a propagation organ which is similar to a tuber to grow a new stem and every other normal part of a plant. *Porang* propagation generatively is used the seeds. Planting bulbil in conventional method will grow and undergo some morphology changes to be *porang*, and then it can develop to be a perfect plant entering generative phase. This process needs a long time. *Porang* flowers and harvests in 3 or 4 years after planting and at the same time it becomes optimal in size and weight, approximately 2 kilograms (Lontoh et al., 2019). That is why farmers prefer bulbil better than seeds. Bulbil grows at axillary bud stem branch in between leaves, it has a round shape, and it has yellow-brownish color with several brown spots on the surface. In a branch stem, there can be several bulbils in different sizes. However, commonly, bulbil will grow bigger along with the age of the plant.

Porang has also known for its benefit in the food industry, cosmetics, and medical ingredients. This *porang* is usually exported as dry chips or thin slices. *Porang* contains carbohydrates, fat, protein, and fibers (Laignier et al., 2021). The mature *porang* may contain glucomannan, often known as KGM (Konjac Glucomannan) up to 73.70% in water solvent and 63.20% in ethanol solvent (Aryanti and Abidin, 2015). Enzymatic hydrolysis can lead to pure glucomannan up to 93%, 84.5°C in temperature, with pH 3.6, and for 3.6 h (Wardhani et al., 2019). It also contains phytochemicals, such as antioxidants (Firman et al., 2016) to prevent free radical compounds that damage body cells and polyphenol and (Zhong et al. 2017) to prevent cancer, heart disease, and diabetes.

Porang farmers or bulbil sellers never pay attention to its quality especially the size or weight in line to prevent free radical compounds that damage body cells and polyphenol. The market system is having no previous standard to begin with for quality. Raw bulbils were mixed without regard to quality, but just to have them soldin a bigger heterogeneous size group, for example, in 1 or 3 kilograms. This research aims to know growth and phytochemical properties in *porang* bulbil with different weights.

MATERIALS AND METHODS

Bulbils of *Amorphophallus muelleri* B. var. Madiun 1 was planted in the greenhouse of Agronomy Department, Faculty of Agriculture, Jember University. This experiment was conducted from May until July 2021. The greenhouse location is 8° 10' 26.076'' S and 113° 41' 42.576'' E, latitude -8.173910 and longitude 113.695160, has an elevation of 89 masl, warm temperature in dry season, and some rain. The bulbils were divided into 10 categories based on weight; 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, and 5 g. Each category was made into three replications. All bulbils were planted in polybags using soil media. Every month, the growth observations were recorded such as plant height, canopy diameter, and stem diameter. Observations were carried out until the third month. At harvest (three months after planting), the weight of *porang* tuber, its diameter, and tuber height was recorded too. One way-ANOVA and tukey tests were used to analyse growth data using PAST 4.03 software.

Phytochemical analysis was conducted in Plant Analysis Laboratory and Ecophysiology and Plant Tissue Culture Laboratory of Agronomy Department, Faculty of Agriculture, Jember University. The analysis conducted were carbohydrate, dissolved protein, antioxidant, phenolic, and saponin. Carbohydrate values for each sample were measured with some steps of preparation and analysis. The sample preparation started with measuring flour as much as 0.5 g, and inserted it in a screw bottle and added with HCl 0.5 N, at ratio of 1 : 3 = 0.5 g : 1.5 mL HCl (0.5 N). Then, it was placed at oven with temperature 50°C for 17 hours, followed by 80°C for 3 hours. Added NaOH

0.5 N 3 mL was placed at oven temperature 80°C for 4 hours. It cooled and centrifuged at 5000 rpm for 10 minutes and the supernatant was measured. This method was applied spectrophotometric with absorbent of visible light and UV (Quero-Jiménez et al., 2019). Supernatant amount of 50 µL was added with 450 µL buffer phosphate of 0.1 N, and pH 7,2. It was incubated in temperature of 37°C – 40°C for 30 minutes, then subsequently it was added with DNS 500 µL and incubated again in boiling water for 5 minutes. It cooled at a room temperature and added with potassium sodium tartrate tetrahydrate 330 µL. The absorbant was measured at 575 nm wavelength in spectrophotometry. Total carbohydrate was counted in the standard formula, with the absorbance as value *x*.

The protein analysis applied a method designed by Bradford (Luo et al., 2021) with some modifications. As much 5 µL of sample was added with 45 µL of distilled water and 950 µL of Bradford, which was then incubated for 15 minutes. The absorbent value was measured in spectrophotometry with 595 nm wavelength. Total protein was solved using Bovine Serum Albumin (BSA) which is the standard in mg BSA/gr sample.

For antioxidant concentration analysis, 0,5 mM was firstly prepared which was solved in methanol (Teğin et al., 2018). The supernatant was extracted with the amount of 100 µL and added with 100 µL of methanol and 800 µL of 50 mM DPPH. This solution was incubated for 20 minutes and the absorbent was measured in 517 nm wavelength. The percentage value immersion of radical DPPH in the sample extract was determined by following this formula (Gálvez et al., 2005):

$$\text{Immersion percentage} = \frac{\text{ABS control - ABS sample}}{\text{ABS control}} \times 100$$

Note: Immersion of radical DPPH optimal for every extract sample in mg GAE/mL.

The phenolic compound was measured based on the method adopted from Taiga et al. (1984), using gallic acid as standard phenolic compound measurement (Yildiz et al., 2011) with amount of 50 µL in each extract as it was dissolved in 2 mL 2% Na2CO3. Then, it was followed by adding reactor by 100 µL of 50% Folin-Cicalteau Reagent. They should mix it until it is homogenous. It was left for 30 minutes at room temperature, and the absorbent value was measured in spectrophotometry U-2001 with 750 nm wavelength.

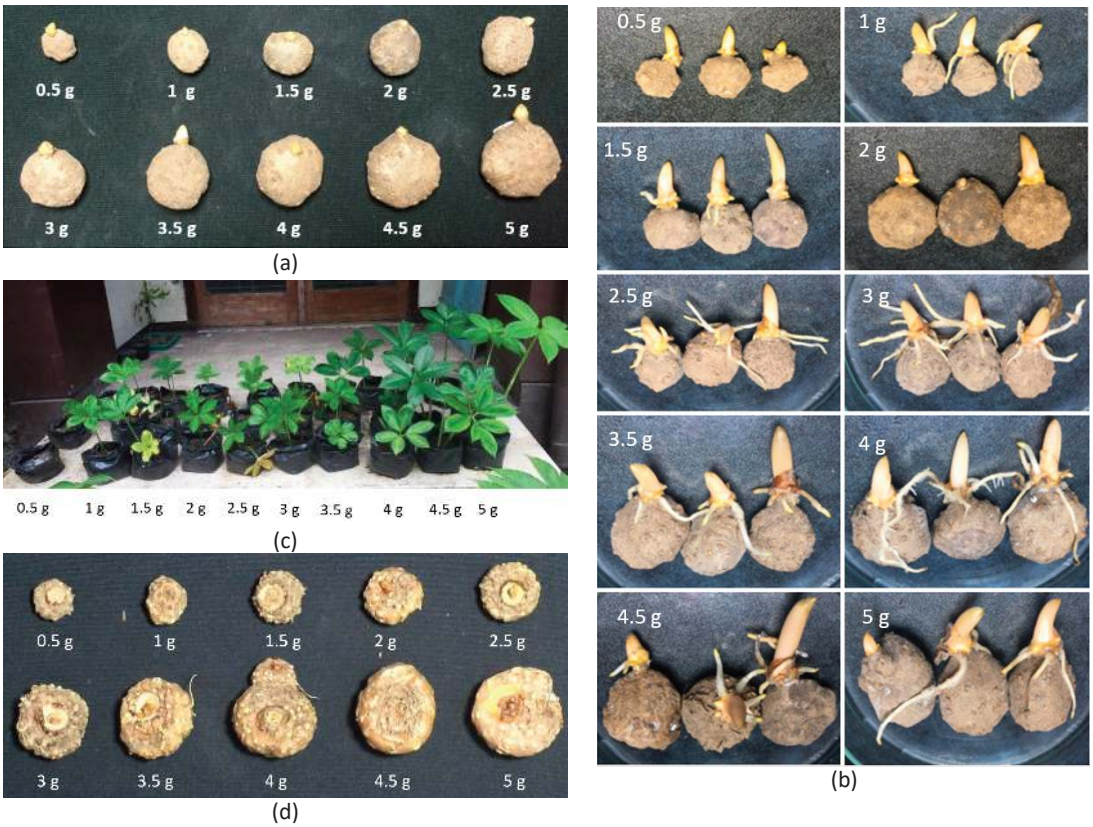


Figure 1. Bulbil of porang: (a) before planted, (b) in 2 weeks, (c) after growing to be porang plant, (d) after harvest

Saponin compound was detected using Thin Layer Chromatography (TLC). The first step was the preparation of maceration samples with 95% of ethanol at room temperature for a day. It was followed by preparation of eluen solution composition; chloroform: methanol: aquades subsequently 14 : 6 : 1 (Minarno, 2016). Sample that was dissolved then dripped on TLC, while TLC at low part was dyed in eluen solution. After a while, there would be new spots appearing. Saponin compounds had an Rf value range from 0.275 until 0.375. All phytochemical data were collected and analyzed in Microsoft Excel for comparative purposes.

RESULTS AND DISCUSSION

The observation of growth as a representative in morphology change has quantified. The parameters measured in height; canopy diameter; stem diameter; tuber weight; tuber diameter, for three months (Fig 1). The plant height, canopy diameter, and stem diameter were measured each month, while tuber weight, tuber diameter, and tuber height were observed at harvest. The data were analysed using PAST 4.03 software. The results analysis showed that parameters observed in the study, like plant height, canopy diameter, stem

Table 1. Statistical analysis of growth observation using PAST 4.03 software

Variable	F-value	P-value	Significance
Height (cm):			
Month 1	2.109	0.079	Non significance
Month 2	9.410	1.819×10^{-5}	Significant
Month 3	6.770	1.913×10^{-4}	Significant
Canopy diameter (cm):			
Month 1	6.199	3.444×10^{-4}	Significant
Month 2	6.711	2.030×10^{-4}	Significant
Month 3	6.038	4.087×10^{-4}	Significant
Stem diameter (cm):			
Month 1	4.312	0.003	Significant
Month 2	12.544	0.000	Significant
Month 3	3.624	0.008	Significant
Tuber weight (g)	1224.324	2.073×10^{-25}	Significant
Tuber diameter (cm)	9.228	2.107×10^{-5}	Significant
Tuber height (cm)	49.975	9.321×10^{-12}	Significant

Remarks: significant at α=0.05 probability levels by the F test.

Table 2. Data of growth variables

Variable	Sample									
	0.5 g	1 g	1.5 g	2 g	2.5 g	3 g	3.5 g	4 g	4.5 g	5 g
Plant Height [month 2] (cm)	8.90 a	13.83 ab	13.67 ab	14.67 ab	15 ab	15.33 ab	16.67 b	17.67 b	18.00 bc	24.33 c
Height [month 3] (cm)	13.83 a	15.17 a	15.83 a	16.17 a	17 a	17.33 a	17.67 a	18.67 a	19.67 ab	25.33 b
Canopy diameter [month 1] (cm)	7.67 a	12.67 ab	13.83 abc	13 abc	14.50 abc	15.83 bc	16.17 bc	20.33 c	18.67 bc	18.67 bc
Canopy diameter [month 2] (cm)	9.67 a	16 ab	18.33 b	18.33 b	19.33 b	19.67 b	20.00 b	21.33 b	21.67 b	22.67 b
Canopy diameter [month 3] (cm)	14.33 a	17.33 ab	19.67 abc	19.33 abc	20.33 bc	20.67 bc	21.67 bc	22.00 bc	22.67 bc	24.33 c
Stem diameter [month 1] (cm)	0.43 a	0.53 ab	0.60 ab	0.67 b	0.67 b	0.60 ab	0.67 ab	0.63 ab	0.70 b	0.73 b
Stem diameter [month 2] (cm)	0.43 a	0.57 ab	0.60 ab	0.63 bc	0.67 bcd	0.70 bcd	0.73 bcd	0.73 bcd	0.80 cd	0.83 d
Stem diameter [month 3] (cm)	0.47 a	0.63 ab	0.67 ab	0.70 ab	0.73 ab	0.83 ab	0.93 ab	1.00 b	1.03 b	1.07 b
Tuber weight (g)	1.21 a	2.32 b	3.37 c	4.36 d	5.48 e	6.92 f	9.90 g	13.44 h	14.52 i	17.32 j
Tuber diameter (cm)	1.70 a	1.90 ab	2.20 abc	2.43 abc	2.53 abcd	1.92 ab	3.27 bcde	3.47 cde	3.83 de	3.97 e
Tuber height (cm)	1.07 a	1.33 ab	1.53 bc	1.70 c	1.73 c	1.77 c	2.20 d	2.30 de	2.43 de	2.60 e

Remarks: Means followed by the same letters on the same line are not significantly different.

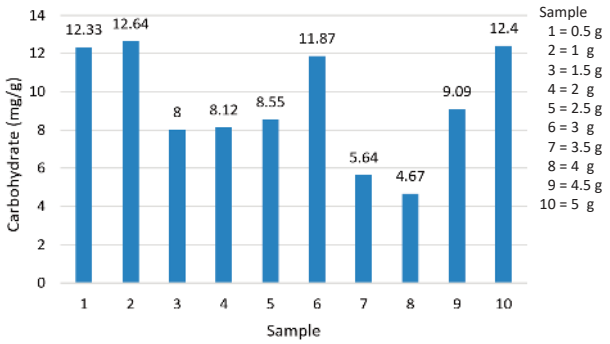


Figure 2. Graphic of carbohydrate value

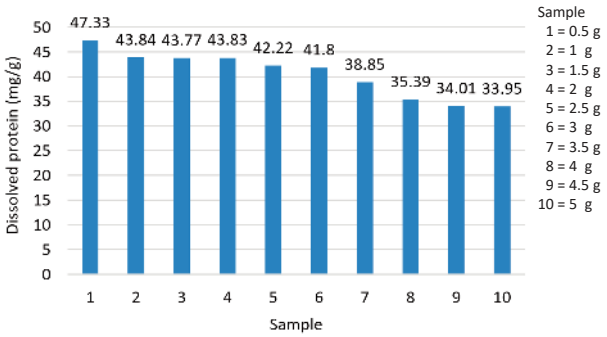


Figure 3. Graphic of dissolved protein value

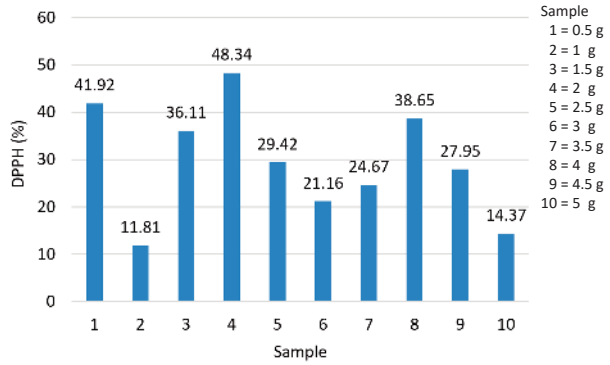


Figure 4. Graphic of antioxidant value

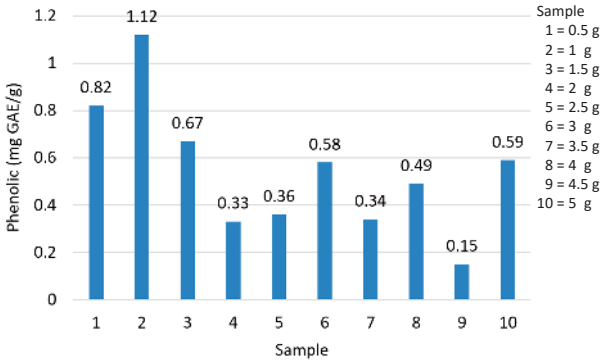


Figure 5. Graphic of phenolic value

diameter, tuber weight, tuber diameter and tuber height were significantly different in each category group (Table 1). However, plant height at one month after planting was not statistically different (Table 2). Turkey test results are presented in Table 2. Variables growth from various bulbil weight are shown in different results in each sample category. The tuber diameter that came from bulbil with weight of 1 g and 3 g were not statistically different. It was the opposite of the observation of the tuber weight.

The plant coming from 5 g bulbil weight were showing dominant value in plant height, canopy diameter, stem diameter, and heaviest tuber of all. This was in line with the experiment conducted by Sumarwoto and Maryana (2011) about growing several bulbils with various weights in different media. They stated that big-sized (± 10 g) and medium-sized (± 5 g) bulbil had an equal good response towards growth based on the parameters of canopy diameter, pseudo-stem diameter, tuber diameter, and tuber fresh weight. Moreover, Saefudin et al. (2021) also stated that bigger bulbil produced heavier fresh tuber weight than the smaller ones. The bigger bulbils have more nutrition and higher growing potential. Other research about tuber size of plant that affects growth were conducted by M. de Almeida et al. (2016), Ebrahim et al. (2018), and Dagne et al. (2019) about the seed size of potato tuber on its growth performance. Seeds with large tuber size were resulted in better growth. Large seeds represent more cells that support the plant growth. In addition, in large seeds more energy is stored and will be used when the enzymatic system begins to emerge for growth. However, in the third month, there was no significant change in tuber weight, tuber diameter, and tuber height, which is caused by normal activity of *porang* tuber that needs a long time for growing. Three months were considered a small time for *porang* long live time until flowering, which is about 4 years (Lontoh et al., 2019).

The results of identification of phytochemical properties such as carbohydrate, dissolved protein, antioxidant, phenolic, and saponin showed different responses than growth observation. Phytochemical properties were examined in the third month. The carbohydrate contents from highest to lowest were 1 g, 5 g, 0.5 g, 3 g, 4.5 g, 2.5 g, 2 g, 1.5 g, 3.5 g, and 4 g (Fig 2). Soluble proteins from the highest to lowest were obtained in samples of tuber grown from bulbils with the following weights of 0.5 g, 1 g, 2 g, 1.5 g, 2.5 g, 3 g, 3.5 g, 4 g, 4.5 g, and 5 g (Fig 3). Meanwhile, antioxidant content from the highest to lowest was found in tuber samples from bulbils with the following

Table 3. Saponin identification using TLC

Category (g)	Existence
0.5	0.314
1.0	-
1.5	-
2.0	0.348
2.5	0.345
3.0	0.333
3.5	-
4.0	-
4.5	-
5.0	-

weight order of 2 g, 0.5 g, 4 g, 1.5 g, 2.5 g, 4.5 g, 3.5 g, 3 g, 5 g, and 1 g (Fig 4). In addition, tuber samples were obtained from bulbils weighing 1 g, 0.5 g, 1.5 g, 5 g, 3 g, 4 g, 2.5 g, 3.5 g, 2 g, and 4.5 g. which produced phenolic content from the highest to the lowest (Fig. 5). The identification of saponin compound using TLC showed that only tuber sample from bulbils weight of 0.5 g, 2 g, 2.5 g, and 3 g resulted in spot/stain under UV light whereas others did not give the same result (Table 3). Phytochemical properties were examined in the third month. It is considered a short time compared to *porang* harvest time which was in four years. These phytochemical results indicated that the growth stage could affect before maturity. The research by Kirui et al. (2018) showed that potatoes in the un-mature phase (55 DAP) have different phytochemical contents compared to the semi-mature stage (95 DAP). Glycoalkaloids and phenolic content decreased along with the age. The same thing also happened to *porang* phytochemicals content caused by the vegetative stage of *porang* which created unstable phytochemicals content for growing buds to roots.

Warm temperature in dry season, still in May to July, makes the plant lose water faster. Based on the research by Liu et al. (2016) they found that environmental factors such as temperature and altitude can affect the active substance production and antioxidant activity in *Potentilla fruticosa* L. The phenolic compound in Irish species was found affected by environmental factors such as sunshine duration and precipitation (Mykhailenko et al., 2020) The same consideration was reviewed by Pant et al. (2021) about the environmental condition which affected secondary metabolites of medicinal plants. They stated that environment was an ecological barrier for plant to struggle to live that includes carbon dioxide, temperature, ozone, lighting, soil fertility, soil salinity, and soil water.

Destruction of sample must be done under phytochemical properties analysis for *porang* tuber. This made the tubers unable to be planted or grown again. New samples were in need for further research in different life phases of *porang* growth. It would be better if phytochemical properties were analyzed in the same process, but it is actually difficult to be done.

CONCLUSIONS

Porang growth coming from various bulbils weight at three months age were indicated that larger tuber has better growing. Larger tuber has more cells than smaller one for growing process. The phytochemical properties were more affected by growing stage than bulbils size. This growing stage is considered immature because it was still three months. Soluble protein content was inversely proportional to the size of the bulbils. The lightest bulbils have the highest dissolved protein. However, other phytochemical properties had results that were not in line with tuber weight. To ensure this, it is necessary to conduct further experiments on the response of tubers to environmental changes.

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