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***Asrianti ARIF*, HUSNA, Faisal Danu TUHETERU, ROSNAWATI*[1]**

SHOOTS CUTTINGS PROPAGATION OF ENDANGERED AND ENDEMIC TREE SPECIES *Kalappia celebica* Kosterm USING THE APPLICATION OF ROOTONE-F

ABSTRACT

Kalappia celebica Kosterm is a member of the Fabaceae family and is included as monotypic species. The tree is one of the most important timber because has a high quality of wood, with dark brown color, making *K. celebica* has great performance thus increasing its economic value. However, its population has decreased with the increase in logging and wood harvesting, mining, and housing. Natural regeneration is also limited due to infrequent flowering periods and fruiting. Therefore, to improve plant material for replanting, it is crucial to do vegetative propagation, such as cutting and accelerating their rooting and growth using a growth regulator. This study aimed to determine the effect of rootone-f as a plant growth regulator on the rooting and growth of *K. celebica* cuttings. The study was designed using a randomized block design which consisted of 4 (four) treatments. The treatments used were untreated cuttings (control), application of 100 ppm, 200 ppm, and 300 ppm/rootone-f solution. The data recorded were analyzed using an analysis of variance (ANOVA). The results showed that the application of rootone-f treatments had a significant effect. There is a different response between untreated and treated cuttings. The treatment of rootone f produced better rooting and growth than untreated ones. There was a significant increase in the total of primary and secondary roots, length of roots, shoots number, leaf area, shoots dry weight, and roots dry weight of *K. celebica* cuttings at eight weeks after planting. The highest rooting and growth were reached by 200-300 ppm treatment. Rootone f application with concentrations of 200 ppm is the optimal dose that could be considered to produce high-quality seedlings of *K. celebica* from cuttings. The success of shoots cutting propagation for *K. celebica* will provide primary practice of *K. celebica* cultivation, support juvenile stage bypass, and contribute to conservation efforts for valuable, endemic, and threatened tree species.

Keywords: Cuttings, endangered species, growth regulator rootone-f, *K. celebica* Kosterm, propagation

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[1] Asrianti Arif* (corresponding author: antyarf75@gmail.com), Husna, Faisal Danu Tuheteru, Rosnawati, Halu Oleo University, Kendari, INDONESIA

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INTRODUCTION

Kalappia celebica Kosterm, called kalapi (*local name*), is the local and endemic plant that is widely distributed to Sulawesi Island, especially in Malili, Each Sulawesi and Kolaka, Abuki, Konawe, etc., Southeast Sulawesi, Indonesia (Arif *et al*., 2016; Arif and Uslinawaty, 2017). *K. celebica* belongs to the Fabaceae family and is categorized as monotypic species (Trethowan *et al*., 2019). The tree is one of the most important timber because it has a high quality of wood, with dark brown color make *K. celebica* has great performance thus increasing its economic value. It is commonly used in house constructions such as door and window frames, house walls, light construction, boat materials, bridges, etc. (Whitmore *et al*., 1989). However, *K. celebica* was listed as vulnerable species on IUCN red list (Trethowan *et al*., 2019; IUCN, 2022), with significant threats being logging and wood harvesting, mining, and housing. In the regulation of Ministry of Forestry No P57/2008, the Indonesian Government has declared that *K. celebica* should be conserved (Mardiastuti *et al*., 2008). There is no accurate information about *K. celebica* cuttings and still limited reports about replanting activities. Therefore, propagation such as generative and vegetative and replanting of *K. celebica* should be done. Approaches to generative and vegetative propagation can save many endangered or extinct species (Dunsin *et al*., 2016; Susilowati *et al*., 2018; Hendalastuti *et al*., 2010; Darma and Priyadi, 2019).

In a natural forest, the *K. celebica* population has limited due to logging and wood harvesting, and regeneration is merely limited due to infrequent flowering periods and fruiting. In addition, seed collection posed some problems, as seeds have a hard coat, thus poor viability and germination. One vegetative propagation that should be done is cutting. This method is the strategy to produce many high qualities of seedlings and faster tree growth (Husen and Pal, 2007). Besides that, this is a valuable method to produce seedlings when seeds are limited. The propagation techniques depend on characteristics and plant type itself. Cutting propagation was applied to more plants species which improved tree yield, quality, and good seedlings in *Hopea gregaria* Slooten (Tuheteru *et al*., 2020), *V. paradoxa* (Yeboah et al. 2020), guava (Qadri *et al*., 2018), *Corchoorus sp*. (Alam *et al*., 2017), *K. celebica* Kosterm (Arif *et al*., 2015), *Eucalyptus pellita* F. Muell (Sulichantini *et al*., 2014), *E. ganitrus* (Rahman and Rohandi, 2012).

In order to accelerate and increase the rooting and growth of cuttings, the use of hormones is required. Hormones are chemical compounds synthesized into the plant tissues to support the roots and shoot development (Monteuuis, 2016), regulate and stimulate plant growth (Dunsin *et al*., 2016). It can be produced in the growing organ of the plant and meristematic tissues (endogenous), and some are synthetic hormones or growth regulator substances (exogenous). Growth regulator hormone also contains auxin that would improve the division and elongation of cells. Thus it will accelerate roots formation (Choi *et al*., 2000; Truemand and Richardson, 2008; Fadli *et al*., 2017; Mabizela *et al*., 2017).

Synthetic hormones (exogenous hormones) are also known as growth regulator substances, for example rootone-f, which contains active compounds such as NAA, NAD, and IBA as well as thiram (Sudomo *et al.,* 2013). Rootone-f is a synthetic hormone that plays an essential role in rooting and growth regulation. It is beneficial to stimulate root growth and promote plant root initiation. Rootone-f, a growth regulator, is very active in accelerating initial roots formation and provides a better root length. The growth increased because rootone-f has several elements that can encourage growth and are more stable than other hormones. Tuheteru et al. (2020) proved that rootone f increased the number of secondary roots of *H. gregaria*. A study on using rootone-f to enhance the rooting and growth of *K. celebica* shoots cuttings is not recorded yet. Therefore, this research aimed to determine the effect of rootone-f on increasing the rooting and growth of *K. celebica* cuttings.

MATERIAL AND METHODS

Materials and tools

Two years *K. celebica* seedling collected from Tanggetada district, Kolaka Regency was cut its shoots with the height of about 10 cm, rootone f- PT Rhone-Poulence Agrocard (a commercial product of synthetic hormone for rooting stimulator), rice husk charcoal, sterilized fine sand, and cow manure as growth media, a tray for cutting growing, cutting scissors, electrical scale, oven, digital thermometer and hygrometer, transparent plastic and styrofoam for covering (to maintain humidity). The cover was created from styrofoam with 80 cm length x 70 cm width, line height in the middle was 25 cm, and both sides of 20 cm in height.

Preparation and cuttings planting

Shoots were cut from stocks of seedlings using sharp scissors, approximately 1 cm below its node. Then, it was soaked immediately in water to keep them fresh. Leaves were removed from the seedling in approximately ¼ parts to reduce transpiration. Rootone f solution was applied on the cutting base using dipped method for 10 minutes. Next, cuttings were planted into growth media in a tray (sterilized sand: manure: rice husk charcoal : 2: 1: 1 v/v) (Arif *et al*., 2015). In which every tray contained ten cuttings. Trays were put and arranged on benches randomly according to the randomized block design in the greenhouse of Indonesia Mycorrhiza Association branched Kendari Indonesia. Each tray was covered using transparent plastic to maintain humidity and avoid dryness. Cuttings were grown for two months, and the environmental condition recorded was about 29.8^{o}C for an average minimum temperature and 33.87^{o}C for an average maximum temperature. For maintenance, cuttings were poured with water using the sprayer one time a day or depending on the humidity (humidity maintained about ± 90%), and weeds were removed manually.

Experimental design

The experimental design used was Randomized Block Design (RBD), which consisted of untreated cuttings/ control, 100 ppm rootone-f, 200 ppm rootone-f, and 300 ppm rootone-f solution, and each treatment was repeated three times. Each treatment consisted of ten cuttings, and thus, the total cuttings were 120. Cuttings were grown in the greenhouse of the Indonesia Mycorrhiza Association branch Kendari, Southeast Sulawesi, Indonesia.

Data Collection

Parameters measured were the number of primary and secondary roots, which were manually counted, roots length, leaf area, shoot number, shoots, and roots dry weight. Shoots and roots dry weight were determined after drying them using an oven with temperatures of 70 ºC for 2 x 24 hours, then balanced with electrical balance. Measurements of data were conducted at the laboratory of the Forestry Department, Forestry and Environmental Science Faculty, Halu Oleo University. Additional data such as temperatures and humidity were also collected and measured using a digital thermometer and hygrometer.

Measurement of roots length

The roots length was calculated using *Grid Intersect Method* (Rowell, 1995) with the formula:

$$R = \lambda \times N,$$

where:
R: roots length;
λ=0.786 (grid 1 cm);
N: number of roots intersection with gridline

Data analysis

Data were analyzed using a one-way analysis of variance (ANOVA). Comparisons of means were done using Least Significant Differences (LSD) Test at the 5% probability level when the F values were significant. All statistical analyses were conducted using SAS 9.1.3 portable statistical software (Mattjik and Sumertajaya, 2002).

RESULTS AND DISCUSSION

The results showed that rootone f improved the rooting and growth of shoots cutting of *K. celebica* aged eight weeks. Rootone f treatments significantly affected the number of primary and secondary roots, roots length, shoot number, leaf area, shoots, and roots dry weight. Data showed that maximum rooting was produced by rootone f treated cuttings. In spite of rooting was also observed on untreated cuttings, but growth was lowest. Based on the LSD test, 200 and 300 ppm treatments were more and similarly effective than others (Table 1). Treatments of 200-300 ppm were increased about seven times on primary roots number, thirteen times on secondary roots number, and nine to fourteen times on roots length, respectively, than control.

Table 1. Effect of treatments on rooting growth of *K. celebica* cuttings after 8 weeks

Rootone-F (ppm) Treatments	Number of primary roots	Pi (%)	Number of secondary roots	Pi (%)	roots length (cm)	Pi (%)
Untreated	0.60±0.256 c	-	2.07±1.112 b	-	1.0±0.577 c	-
100	2.07±0.646 b	245	10.03±2.723 b	385	3.83 ± 1.123 bc	284
200	4.87±0.649 a	711	30.03±5.636 a	1353	10.35 ± 1.967 ab	939
300	5.07±0.719 a	745	29.73±5.136 a	1338	15.01±2.999 a	1407
Pr>F	**		*		*	

Average values followed by unequal letters in the same column were significant differences at the 0.05 LSD test level. Percentage of increasing (Pi), significant (*), very significant (**)

The use of rootone f also improves the growth of cutting shown in table 2. Treatments of rootone f 200 and 300 ppm increase shoot number and leaf area at eight weeks. Treatments of rootone f 300 ppm increased shoot number, which was different from other treatments. In the leaf area, treatments of 200 and 300 ppm rootone f were more and similarly effective in stimulating the leaf area. In contrast, untreated cuttings (control) were the poorest treatments that differed from others. Treatment of 300 ppm increased shoots number about six times than control, while treatment of 200 ppm improved leaf area about four times than control (Table 2).

Table 2. Effect of Rootone F treatments on shoots growth (number of shoots and leaf area) of *K. celebica* cuttings, after 8 weeks

Rootone-F (ppm) Treatments	Shoots number	Pi (%)	Leaf area (cm^2)	Pi (%)
Untreated	0.07± 0.046 c	-	0.81±0.357 b	-
100	0.23± 0.078 b	250	2.42±0.234 ba	200
200	0.33± 0.087 b	400	4.95±0.305 a	514
300	0.50±0.114 a	650	4.10±0.390 a	408
Pr>F	**		*	

Average values followed by unequal letters in the same column were significant differences at the 0.05 LSD test level. Percentage of increasing (Pi), significant (*), very significant (**)

Better growth was also presented by treatments of 200 and 300 ppm rootone f on the dry weight. It is shown that the treatment of 200 ppm is the best treatment with increasing shoots and roots dry weight, but no significant differences with 300 ppm treatment. In contrast, untreated cuttings (control) produced the lowest value (Table 3).

Table 3. Effect of Rootone f treatments on plant dry weight *K. celebica* cuttings after 8 weeks

Rootone-F (ppm) Treatments	Shoots dry weight (g)	Pi (%)	Roots dry weight (g)	Pi (%)
untreated	0.116±0.011 b	-	0.001±0.0005 c	-
100	0.174±0.015 b	49	0.006±0.001 b	408
200	0.258±0.020 a	120	0.014± 0.002 a	1106
300	0.257±0.24 a	119	0.013±0.002 a	1012
Pr>F	**		**	

Average values followed by unequal letters in the same column were significant differences at the 0.05 LSD test level. Percentage of increasing (Pi); significant (*); very significant (**)

There were significant differences in the number of primary and secondary roots, roots length, shoot number, leaf area, and plant dry weight due to the different treatments applied in the rooting of *K. celebica* cuttings. Rootone-f, as a growth regulator substance, stimulates and accelerates roots growth. The effect would be better when using the proper hormone concentration and suitable for plant species. This experiment showed that the addition of 200-300 ppm treatments produced better rooting and growth. It stimulated elongation and cell growth, thus increasing roots and shoot growth of *K. celebica* cuttings.

According to Small and Degenhardt (2018), hormones can enhance cell division and development of cells, including the structure and functions. Giving hormone in an optimal concentration will stimulate the growth and development of the roots (Akhtar *et al*., 2015; Okao *et al*. 2016; Maggioni *et al*., 2020). In addition, there was a correlation between endogenous auxin content and the ability of cuttings to form rooting (Henselova *et al*., 2002). Thankamani *et al*. (2020) also noticed that auxin could increase the activity of meristematic tissues and carbohydrates transport to the base of cuttings, thus improving the rooting. The presence of roots will give better growth for plants. Roots are crucial parts of a plant that play essential roles in soil nutrients and water uptake. In which nutrients and water supply are translocated to the leaves, which are involved in plant metabolism activities such as photosynthesis to produce carbohydrates (Lambers *et al*., 2008). In line with some research, *Duabanga mollucana* Blume cuttings (Supriyanto and Prakasa, 2011), *Anthocephalus cadamba* (Putra *et al*., 2014), and *H. gregaria* (Tuheteru *et al*. 2020) better rooting and growth was also achieved when cutting were treated with rootone f. Similarly, Sudomo *et al*. (2013) found that using rootone f 100 ppm increased shoots, roots length, and the number of roots on *Manglietia glauca* BI at three months old. Furthermore, cutting material taken from the juvenile seedling of *K. celebica* has produced better rooting. Danu *et al*. 2011 found that shoots cutting material obtained from seedling gave the highest survival than obtained from the young tree or mature tree.

Figure 1. Cuttings of *K. celebica* after soaked into rootone-f solution based on treatments, planted on media and covered with transparent plastic

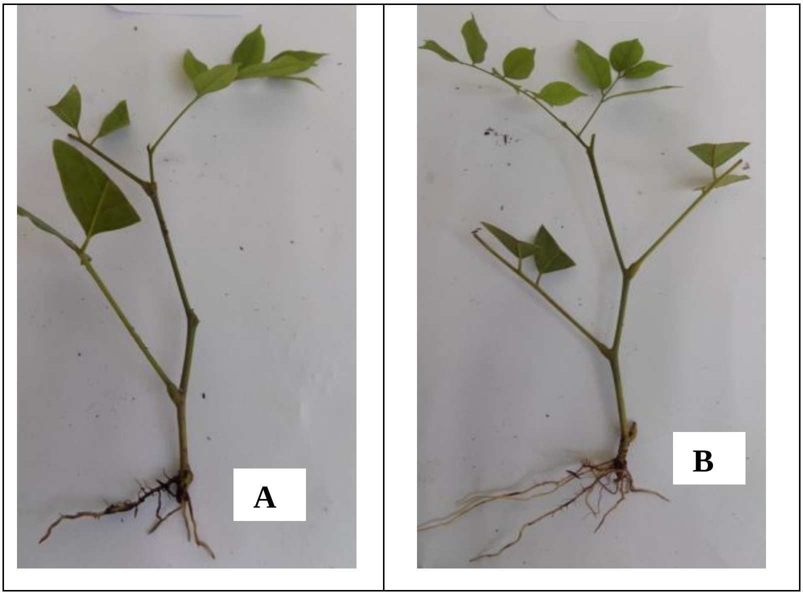

Figure 2. Appearance of *K. celebica* cuttings after 8 weeks of growth.
A: 200 ppm and B: 300 ppm of rootone-f

In contrast to untreated cuttings, the rooting and growth were the lowest. It was assumed that internal hormone is not adequate to promote the development of roots. According to Rahayu & Riendriasari (2016) that the sea bidara plant did not affected by rootone-f application (exogenous auxin) due to the adequacy of endogenous auxins. However, a high dose of the hormone also can bring adverse effects. This case was proved by Truemand and Richardson (2008) found that the highest amount of 8 g IBA/ kg powder caused leaf abscission and reduced leaf area or shoot dry weight on *C. torreliana* and *C. citriodora*. High concentrations of hormones have a negative impact, like oxidative stress, that effected in death of cells (Flasinski and Hac-Wydro, 2014).

Cuttings achieved the highest shoots number, leaf area and plant dry weight when applied 200-300 ppm of rootone-f. Rootone f stimulates cell division, resulting in growth and increased shoots number and leaf area. It will impact the rate of photosynthesis. An increase in growth will occur, indicated by an increase in plant dry weight. Enhancing the leaf area will also improve stomata for CO_2 fixation, and then the CO_2 assimilation rate would be better. Chlorophyll concentration also would be enhanced as increasing of the leaf area. Lambers *et al*. 2008 also noticed that adequate absorbed light was caused by improving leaf surface and chlorophyll concentration. Therefore, the photosynthetic rate would be increased, and the production of carbohydrates would be sufficient for the growth of roots and shoots. Devi et al. 2016 found that there was rising in leaves number (18.74), leaf area (3.76 cm^2), and content of chlorophyll (39.76%) of *Grewia asiatica* cuttings when treated with IBA 300 ppm and highest than other treatments. In addition, the presence of bud and shoots on cuttings, including meristematic tissues, will stimulate the production of hormones (Small and Degenhardt, 2018) and then circulate to the bottom or basal to form the roots.

Efforts to conserve *K. celebica* have been carried out, such as planting on gold mining land and propagation through root cuttings, seed germination, and shoot cuttings. The obstacle faced in supporting conservation efforts is the shortage of seedlings for planting. For this reason, shoot cuttings are a solution to overcome the problem of limited seeds by providing seedlings from cutting for planting in the field.

CONCLUSIONS

Application of Rootone-f has improved rooting and growth of *K. celebica* cuttings aged eight weeks. Using rootone-f as a growth regulator at concentrations of 200 ppm has been more efficient and could be considered to support *K. celebica* propagation and conservation program. The success of shoots propagation for *K. celebica* will provide the primary practice of *K. celebica* cultivation, support bypassing the juvenile stage, and contribute to conservation efforts for endemic and threatened tree species.

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