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Effect of pre-sowing treatment on the germination and early growth of *Kalappia celebica* Kosterm.: an endemic and vulnerable tree species of Sulawesi, Indonesia

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Abstract. *Tuheteru FD, Husna, Albasri, Arif A, Basrudin, Nurdin WR, Arman E, Agustin DI, Saribadu J, Rahmat, Dermawansyah A, Daliana, Lody LP, Deri AS, Safitri I, Sudrajat DJ. 2022. Effect of pre-sowing treatment on the germination and early growth of* Kalappia celebica *Kosterm.: an endemic and vulnerable tree species of Sulawesi, Indonesia. Biodiversitas 23: 4297-4302.* Kalapi (*Kalappia celebica* Kosterm.) is an endemic tree species of Sulawesi and is threatened with extinction, so it requires conservation efforts to preserve the tree species. The research aims to determine the effects of seed pre-sowing methods on the germination of *K. celebica*. Matured seeds of *K. celebica* were collected from several mother trees at the Lalona Village, Kolaka Regency, Southeast Sulawesi, Indonesia. The seeds were treated with six pre-germinations treatments (control, nicking, nicking and soaking in cool water (20°C) for 12 h, nicking and soaking in cool water for 24 h, soaked in hot water (80°C) for 24 h till the water-cooled and soaked in cool water for 48 h. Three replication and each replication using 25 seed were used in this research, so the total seed were 450 seeds. The randomized completely design was used to test the effect of pre-germination treatments on seed germination parameters at the greenhouse. The germination parameters calculated were the first day of germination, last day of germination, germination period, germination capacity, mean germination time (MGT), and mean daily germination (MDG). The results showed that pre-germination treatment affected all seed germination parameters, which significantly increased the germination capacity. The germination capacity with the nicking was 94,67%, nicking and soaking in cool water (20°C) for 12 h (94,67%) followed by nicking and soaking in cool water (20° C) for 24 h (90,67%). In all treatments, germination started 5 to 10 days after sowing and was completed from 4 to 16 days later. The seed treatment by nicking and soaking in cool water for 12 d exhibited the lowest MGT (5.56 days), the highest MDG (0.95 germinate day^{-1}), the highest seedling collar diameter (1.64 mm), and the highest leaf number (1.95). Therefore, the study recommends nicking followed by soaking in cool water for 12 h as an ideal pre-germination treatment to promote germination and seedling growth of *K. celebica* tree species for supported conservation activity.

Keywords: *Kalappia celebica*, scarification, seed germination, reforestation

INTRODUCTION

Kalapi (*Kalappia celebica* Kosterm.) is an endemic and endangered tree species from the family Fabaceae (Trethowan 2019) which is naturally distributed in Malili in South Sulawesi and Konawe and Kolaka in Southeast Sulawesi (Arif et al. 2016; Trethowan et al. 2019). This species has a height of up to 40 meters with a trunk diameter of 90 cm and has buttresses up to 3 m. The bark is brownish. *K. celebica* is a tropical tree species whose wood is used for construction, furniture, bridges, handicrafts, ship and buildings. Wood also has become a high-value export commodity (Sosef et al. 1998) due to it has durable class II and strong class II. Based on field observations, *K. celebica* population is threatened by overexploitation, habitat conversion, unidentified flowering and fruiting seasons, low natural regeneration, and very limited knowledge of species cultivation. Therefore, this tree species needs to be conserved from the threat of extinction in its natural habitat through in situ conservation approaches (maintaining natural populations and their habitats) and the development of ex situ conservation areas.

Kalappia celebica is listed as Vulnerable (VU) under criteria B1+2c by IUCN Red List (IUCN 1998). To support these efforts, the Government of Indonesia has established *K. celebica* as one of the priority tree species of conservation in Indonesia (the Minister of Forestry Regulation No. P.57/Menhut/II/2008). The regulation has directed to examine aspects of the cultivation of *K. celebica* trees to support conservation efforts and their sustainable use. Several studies on *K. celebica* have reported on several research aspects, such as ecology and natural distribution

(Trethowan et al. 2019), mycorrhizal symbiosis (Arif et al. 2016; Husna et al. 2021), seed handling (Arif et al. 2018), and vegetative propagation (Arif et al. 2022). Conservation of the species facing a high threat of extinction in nature requires effective and comprehensive efforts to remain sustainable and utilized for human welfare.

Efforts to save endemic and threatened tree species are often constrained by the availability of quality seeds and seedlings and have become a major bottleneck. The condition can increase operational costs and delay the success of conservation of the endemic and threatened tree species in the framework of forest restoration in various countries (Duguma et al. 2020; Bosshard et al. 2021; Di Sacco et al. 2021). One of the causes is the inhibition of seed germination and the low percentage of seedling survival (Jalonen et al. 2018; Ribeiro et al. 2019). Similar conditions also occur in the conservation of *K. celebica*. The success of producing *K. celebica* seedlings is still often hampered by the low germination capacity of the seeds, so research related to seed germination techniques is needed to provide high quality and quantity seedlings. An increase in seed germination will increase the success of seedling production, which is the main component in the *K. celebica* conservation effort, both through in situ (reintroduction) and ex situ conservations.

Kalappia celebica seeds have a hard and thick layer of seed coat that causes physical dormancy. It will inhibit seed germination. Physical dormancy is generally found in tropical tree species, especially species from the Fabaceae family, which are orthodox seeds and generally have hard seed coats so that water and air cannot enter the seeds (Dumroese et al. 2016). To overcome dormancy and increase seed germination, it is necessary to do pre-treatment before seed germination. Seed pre-treatment has the dual purpose of ensuring that the seeds will germinate and that germination will be faster and uniform (Syamsuwida et al. 2020). Initial treatment of seeds with physical dormancy can be conducted by scarification (piercing, nicking, chipping, filing, or burning with the aid of a knife, needle, file, hot-wire burner, abrasion paper), hot water, and acid pre-treatment (Dumroese et al. 2016). Early germination treatment has been reported to be a very effective method in increasing seed germination of tropical tree species (Azad et al. 2012, 2013; Nongrum and Kharlukhi 2013; Raji and Siril 2018). The response of seeds to seed pre-treatment varies depending on the characteristics of the seeds of each species, so trials of various treatments are needed to improve seed viability and vigor.

Inappropriate seed germination techniques for native species are often the main factor in the failure to provide adequate quality seedlings in nurseries. In general, the failure to provide seedlings in the nursery for the endemic or native species has become a serious problem in various forest and land restoration programs (León-Lobos et al. 2020; Bosshard et al. 2021). This is because research on seed germination techniques for local and endangered tree species is very limited (Iralu et al. 2019). Knowledge of seed germination is very important for the provision of quality seeds in order to support various conservation and ecological restoration programs to save endangered *K. celebica*. Thus, the purpose of this study was to test some seed germination pre-treatments that could increase the germination of *K. celebica* seeds.

MATERIALS AND METHODS

Materials

Kalappia celebica seeds were collected manually from several mother trees in March 2022 at the Lalona Village, Kolaka Regency, Southeast Sulawesi Province, Indonesia. The Lalona Village is situated about 76-118 m above sea level, slope 20-40%, soil type cambisol, and geographic position between 03°49'8.51" S and 121°15'28.87" E. Seed collection is done by picking physiologically ripe fruit on the forest floor, which is characterized by brownish fruits color. The seeds were then extracted by dry extraction method and the seeds were manually extracted 4-5 days after collection. The collected seed was checked to remove damaged seeds. Healthy dried seeds were used for the experiment. We measured the length, width and thickness of the fresh seeds.

Seed treatment and experimental design

Seeds were surface sterilized by 5.25% NaClO (hypochlorite of sodium), with a concentration of 1 ml NaClO dissolved in 1 liter of water. Seeds are soaked in the solution for 2 minutes to remove bacterial and fungal contaminants and rinsed thoroughly in distilled water. The seeds were treated by six treatments, namely control (A), nicking-scarified near micropyle using nail clipper (B), nicking and soaking in cool water (20°C) for 12 h (C), nicking and soaking in cool water for 24 h (D), soaked in hot water (80°C) till the water-cooled for 24 h (E) and soaked in cool water (20°C) for 48 h (F). A randomized complete design with three replications was used to test the effect of the pre-sowing treatments on the germination parameters of *K. celebica* seed. Six treatments with 3 replications and each replication using 25 seed were used in this research, so the total seed were 450 seeds.

The seed germination test was conducted in the greenhouse of the Indonesian Mycorrhizas Association, Southeast Sulawesi Branch, Kendari, Indonesia. The study area is situated about 40 m above sea level in the Kendari (03°57'55.9" S; 121°31'51.4" E). The temperature was recorded as 28-40° C and relative humidity ranged from 60-83% during the experiment. The germination test was carried out by sowing the seed in the germinator box (26 cm x 26 cm x 10 cm). The sowing media was a mixture of sand and rice husk charcoal (2:1, v/v). The seeds were sown at a depth of 0.5-1 cm and watering was lectured manually once a day. The experiment was done in April-May 2022.

Measurement of seed germination parameters

The seed germination in each treatment was observed every day by calculating normal seedlings. Criteria for normal seedlings are characterized by the appearance of a pair of healthy leaves (Sudrajat et al. 2015; 2018). The germination parameters calculated were the first day of germination, last day of germination, germination period, germination capacity, mean germination time, and mean daily germination. The germination capacity (GC) was calculated by the formula:

$$GC = \frac{number\ of\ seeds\ germinated}{number\ of\ seeds\ sown}\ x\ 100\%$$

Mean germination time (MGT) was calculated by the formula:

MGT = Σtini/Σni,

Where; t is the day required to germinate and n = number of germinating seeds at the end of the observation. The average seed germination per day or mean daily germination (MDG) was calculated by the formula: MDG = N/t, where N is the total number of seeds that germinate at the end of the observation and t is the number of days of observation (Al-Ansari and Ksiksi 2016; Sudrajat et al. 2022).

Seedling growth performance

Seedling growth was measured on total seedling height, root collar diameter, leaf length, and width at the end of the study (25 days after seed sowing). Seedling height was measured using a ruler, while root collar diameter was measured using a digital caliper.

Data analysis

Analysis of variance (ANOVA) was used to examine the effect of seed pre-sowing treatments on the germination parameters (the first day of germination, last day of germination, germination period, germination capacity, mean germination time, and mean daily germination) of *K. celebica* seeds. Duncan Multiple Range Test (DMRT) at the 95% confidence level is used when the results of the analysis of variance have a significant effect.

RESULTS AND DISCUSSION

Seed morphology

The fruits of *K. celebica* contain 1-5 seeds. The average length, width, and length-width ratios of the fruit were 8.38 ± 0.12 cm, 2.81 ± 0.04 cm, and 3.00 ± 0,05, respectively. The *K. celebica* seeds were dark reddish brown in color; their seed was disk-like with cotyledons thin (Figure 1). The average length, width, and thickness of the seeds were 13.20±0.111 mm, 10,82±0.192 mm, and 1.23±0.028 mm, respectively. Healthy and pure seeds were used for the experiment.

Seed germination

Seed pre-germination treatment had a significant effect on all tested seed germination parameters (Table 1). Pre-germination treatment of seeds was able to accelerate the process of seed germination and increase the vigor of *K. celebica* seeds. Seed germination started earlier in several seed treatments, such as nicking, nicking and soaking in cool water (20°C) for 12 h, nicking and soaking in cool water for 24 h than the seed germination in control. The seed germination in nicking, nicking and soaking in cool water (20°C) for 12 h, nicking and soaking in cool water for 24 h occurred faster than germination in control with a germination period of 4,67, 4,67 and 5.67 days, respectively. While germination was in control, soaked in hot water (80°C) for 24 h till the water-cooled, and soaked in cool water (20°C) for 48 h occurred longer with a germination period of 15, 16.33, and 16 days, respectively (Table 1 and Figure 1).

DMRT showed that the pre-germination treatment was a significant difference in the all-germination parameters. Three treatments, namely nicking, nicking and soaking in cool water (20°C) for 12 h, nicking and soaking in cool water (20°C) for 24 h, resulted in the highest germination capacity, respectively 94.67%, 94.67%, and 90.67%. This treatment consistently also resulted in the best first day of germination, last day of germination, germination period, and mean daily germination. However, for the MGT parameter, only nicking and soaking in cool water (20°C) for 12 h gave the best MGT, which was 5.56 days (Table 1). Overall, the best pre-germination treatment to increase the viability and vigor of *K. celebica* seeds was given by nicking and soaking in cool water (20°C) for 12 h. The emergence of seedlings of *K. celebica* showed epigeal type which cotyledons spread above the soil.

Figure 1. Fruit and seed of *Kalappia celebica*

Table 1. Effects of pre-germination treatment on the germination parameters of *Kalappia celebica* seeds

Treatment	The first day of germination (days)	The last day of germination (days)	Germination period (days)	Germination Capacity (%)	MDG (germinate day^{-1})	MGT (days)
A	8.67±0.33 a	22.00±2.08 a	15.00±2.00 a	46.67±3.53 c	0.47±0.04 cb	15.60±0.98 a
B	5.67±0.33 b	8.67±0.33 c	4.00±0.00 b	94.67±3.53 a	0.95±0.04 a	7.39±0.29 bc
C	4.67±0.33 b	767±0.33 c	4.00±0.58 b	94.67±2.67 a	0.95±0.03 a	5.56±0.58 c
D	4.67±0.33 b	9.67±0.33 c	5.67±0.33 b	90.67±3.53 a	0.91±0.04 a	6.69±0.13 bc
E	5.33±0.33 b	16.67±3.67 b	16.33±3.71 a	57.33±11.39 b	0.57±0.11 b	8.59±1.69 b
F	9.67±0.88 a	24.67±0.33 ab	16.00±0.58 a	37.33±4.81 d	0.37±0.05 c	14.67±0.81 a
Pr>F	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

Notes: A: control, B: nicking-scarified near micropyle using nail clipper, C: nicking and soaking the seed in cool water (20°C) for 12 h, D: nicking and soaking the seed in cool water for 24 h, E: soaking the seed in hot water (80°C) for 24 h till the water-cooled, F: soaking the seed in cool water (20°C) for 48 h. Data are shown in mean ± SD. Different letters in the same column indicate a significant difference at $p < 0.05$

Table 2. Height and collar diameter growth and the total number of leaves of *Kalappia celebica* seedlings

Treatment	Height (cm)	collar diameter (mm)	Leaf number	Width leaf (cm)	Length leaf (cm)
A	6.92±0.54	1.33±0.02 b	1.37±0.23 b	1.73±0.03	4.19±0.10
B	7.77±0.36	1.57±0.02 a	1.99±0.09 a	2.03±0.12	4.88±0.36
C	7.66±0.22	1.64±0.06 a	1.95±0.05 a	2.19±0.12	5.17±0.20
D	7.09±0.12	1.57±0.05 a	1.93±0.05 a	1.99±0.06	5.09±0.07
E	6.88±0.31	1.43±0.02ab	2.02±0.02 a	1.94±0.11	4.49±0.30
F	7.87±0.25	1.32±0.14 b	1.61±0.20 ab	1.95±0.11	4.56±0.40
Pr>F	0.1912	0.0186	0.0295	0.1150	0.1552

Note: A: control, B: nicking-scarified near micropyle using nail clipper, C: nicking and soaking in cool water (20°C) for 12 h, D: nicking and soaking the seed in cool water for 24 h, E: soaking the seed in hot water (80°C) for 24 h till the water-cooled, F: soaking the seed in cool water (20°C) for 48 h. Data are shown in mean ± SD. Different letters in the same column indicate a significant difference at $p < 0.05$

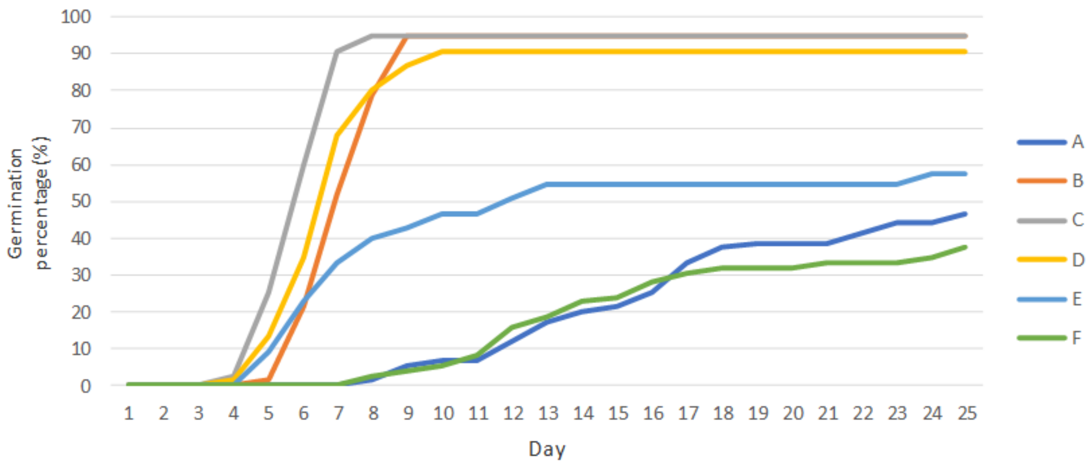

Figure 1. Cumulative seed germination (%) throughout the germination period of *Kalappia celebica* under six pre-germination treatments. Notes: A: control, B: nicking-scarified near micropyle using nail clipper, C: nicking and soaking the seed in cool water (20°C) for 12 h, D: nicking and soaking the seed in cool water for 24 h, E: soaking the seed in hot water (80°C) for 24 h till the water-cooled, F: soaking the seed in cool water (20°C) for 48 h

Seedling growth

Analysis of variance showed a significant difference in root collar diameter and leaf number of *K. celebica* seedlings ($p<0.05$), while other seedling growth parameters, such as seedling height, leaf width, and leaf length were not affected by seed pre-germination treatment. DMRT showed a significant difference in collar diameter and leaf number of *K. celebica* seedlings in the nicking, nicking and soaking seed in cool water (20°C) for 12 h, and nicking and soaking seed in cool water for 24 h treatments with other treatments. It also showed that there was no significant difference between the control and soaked seed in cool water (20°C) for 48 h. The trend of the best treatment for seedling growths was almost the same as the best treatment for seed germination parameters.

Discussion

The dimensions of the fruit and seeds of *K. celebica* collected from Lalonaha Village, Kolaka Regency are no different from the size of the fruits and seeds collected from the District of Abuki, Konawe Regency (Arif et al. 2018), and fruit and seed dimension information in the publications of Sosef et al. (1998). In this study, the length and width of the *K. celebica* fruit were 8.38 cm and 2.81 cm, respectively, while in the study of Arif et al. (2018) the average fruit length and width were 7.018 and 2.97 cm, respectively. The average length, width, and thickness of the seeds of *K. celebica* in this study were 13.2 mm, 10.82 mm, and 1.23 mm, respectively, compared to the study of Arif et al. (2018) namely 11.33 mm, 9.44 mm, and 1.39 mm and the publication of Sosef et al (1998), 10(-11) - 13 (-15) mm. There is no significant variation between the dimensions of the fruit and seeds of *K. celebica* at different locations, presumably because the fruit and seeds are controlled by genetic factors. For this reason, it is necessary to study molecular genetics for these *K. celebica* populations to determine their diversity.

The *K. celebica* seeds have an initial moisture content (fresh seed) ranging from 11.3%-13.1% (Arif et al. 2018) and the water content can still be lowered to <8%. As a member of the Fabaceae family, *K. celebica* has dry seed characteristics with hard seed coats and can be categorized as an orthodox seed (Jayasuriya et al. 2013; Syamsuwida et al. 2020), however, not all seeds from the Fabaceae family have such orthodox characteristics (Jayasuriya et al. 2012). In *K. celebica* seeds, the hard seed coat is a feature of physical dormancy and becomes an obstacle for the entry of water into the seed tissue, thereby inhibiting germination.

In this study, the pre-germination treatment had a significant effect on all germination parameters (the first day of germination, last day of germination, germination period, germination capacity, mean daily germination, and mean of germination time). These results indicated that the germination of *K. celebica* seeds could be increased through pre-germination treatment. Mechanical scarification (nicking, nicking, and soaked in cool water for 12 d and nicking and soaking in cool water for 24 d) was very effective in increasing seed germination and shortening the seed germination period. Scarification treatment on *K. celebica* seeds was able to increase water absorption by seeds which were previously hampered by the thickness and hardness of the seed coat so that water could not enter the seed tissue (Dumroese et al. 2016). Several previous studies showed the same thing that seed scarification could increase and accelerate the germination of forest plant seeds, such as *Albizia procera* (Azad et al. 2012), *Albizia chinensis* (Nongrum and Kharlukhi 2013), *Tamarindus indica* (Azad et al. 2013), and *Faidherbia albida* (Fredrick et al. 2017).

Pre-germination treatment by immersion in cold water showed less than optimal results in increasing the germination of *K. celebica* seeds. This treatment resulted in lower seed germination with a longer germination period than scarification of seeds with nicking treatment. This indicates that the seeds of *K. celebica* have a fairly strong physical dormancy with the characteristics of the seeds being hard, dense, and inflexible. Likewise, the treatment of hot water (80°C) immersion is also not suitable for increasing and accelerating the germination process of *K. celebica* seeds. Similar results were reported by Singh et al. (2019) which stated that the high-temperature range of water (53-55°C) reduced seedling emergence of *Capsicum annuum*. The probable reason for reduced seedling emergence might be attributed to the fact that increased temperature affected the physiological activities of the embryo of seeds. However, several studies reported that hot water treatment could improve the germination of seeds and several other plant species (Azad et al. 2012; El-Sayed et al. 2013; Das 2014; Suleiman et al. 2018; Seng and Cheong 2020).

The nicking treatment combined with soaking the seeds in cold water for 12 h was the best pre-germination treatment for *K. celebica* seed germination. The effectiveness of nicking treatment and cold-water immersion for 12 h in increasing and accelerating seed germination was also reported in several seeds from the Fabaceae family, such as *Acacia stenophylla* (Khan and Sahito 2017), *Vachellia eroloba* (Odirile et al. 2019), and *Pterocarpus erinaceus* (Peter et al. 2021). This pre-germination treatment is quite practical and suitable for smallholder tree growers in nurseries because it is simple and effective in increasing and accelerating seed germination. This technique also increased the growth of *K. celebica* seedlings (Table 2), especially the root collar diameter and leaf number. This is due to the seeds being treated with nicking and soaking in cold water, resulting in faster germination so that the initial growth of the seedlings will be faster. Seedlings with large root collar diameters generally have a higher sturdiness quotient (the ratio of the height of the seedling to the root collar diameter), so the seedlings are more vigor which will improve adaptation during seedling transplanting at the nursery and out planting in the field (Budiman et al. 2015) thus increasing the success of seed provision and forest and forest activities. Landscape restoration. Thus, the pre-germination treatment method with nicking in combination with cold water immersion for 12 h is suitable for breaking seed dormancy to support seedling production in nurseries for the domestication and conservation of *K. celebica* in tropical Indonesia.

In conclusion, *K. celebica* seeds can be categorized as orthodox seeds with the characteristics of a hard, dense and inflexible seed coat. Pre-germination treatment is very important to improve germination and shorten the germination period of *K. celebica* seeds. Based on the study findings, nicking and soaking cold water for 12 h could increase germination, speed up germination time, and increase vigor and seedling growth (diameter and number of seedlings), so this treatment was recommended as the pre-germination treatment for *K. celebica* seeds.

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