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THE PROPAGATION TECHNIQUE AND CULTIVATION POTENTIAL BY SEEDS OF *MELIENTHA SUAVIS* PIERRE IN PHU THO PROVINCE, VIETNAM

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Abstract

Melientha suavis Pierre is a small tree species listed in the Vietnam Red Book and is used as a forest vegetable with high nutritional value. However, studies on the propagation and cultivation of this species are very limited. We conducted the experiments on propagating by seeds in the nursery and sowing seeds directly under *Acacia mangium* plantation. The results showed that the mean radicle protrusion time of *M. suavis* seeds was 24 days, and its rate was 54%. The survival rate of seedlings in the nursery was very high (over 97%) but direct sowing in the field was low (less than 33%). In the nursery, the growth was strongest from the first to the second month and then slowed down, while in the field the growth was strongest after the second month. The removal of the cotyledons for plants at one month of age did not have a significant effect on the growth of seedlings. At 4 months of age, sowing seeds directly in the field but without fertilizing in advance (T6) exhibited the seedling growth not different from the remaining treatments in the nursery. From the obtained results, we propose that (1) *M. suavis* can be propagated by seeds; (2) Cultivation of *M. suavis* by direct seeding in the field may be a potential solution to preserve and develop this valuable plant.

Keywords: *Cultivation, propagation, Phu Tho, Melientha suavis, nursery.*

1. Introduction

Melientha suavis Pierre is one of two species of the genus Melientha, family Opiliaceae, only recorded and distributed in Indochina. In Vietnam, this small tree species is listed in the Red Data Book as Endangered (VU B1+2e) [1]. In nature, the species flowers from March to April, the fruit ripens from June to August and flowers usually appear along the stem. Local people often take the young shoots and flowers to cook soup, which is sweet, fragrant, rich in precious amino acids, and beneficial for health [2]. *M. suavis* is a unique genetic

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source that not only improves biodiversity in the forest ecosystem but also is a plant providing delicious and high nutritional value vegetables for humans (such as: amino acid, protein, lipid, fiber etc.) [3, 4].

Although having a wide distribution, the population of *M. suavis* is fragmented, together with the negative impact of humans, leading to a decreasing number of individuals. In Xuan Son National Park, *M. suavis* is naturally distributed and grows scattered in tropical evergreen forests on limestone mountains [5]. However, the species is still illegally exploited and even cut down the trees to collect young shoots and flowers easily. Currently, local people harvest in the wild and there are no measures to conserve and cultivate for long-term use. Therefore, the natural supply is more and more decreasing and unable to meet the increasing demand. The source of seedlings for planting is still very limited [6]. On the other hand, the studies on the propagation and cultivation of this species are very limited. In some places in Vietnam and Thailand, the species has been propagated and planted but it is very difficult to grow due to unsuitable to the soil and sensitivity to mechanical care methods [7-9].

Propagation techniques and trials of planting of *M. suavis* have been initially carried out and proposed by some researchers or local people. In the North of Thailand, various propagations have been tried, and the propagation by seed is suggested [7]. At Rom Pho Thong community forest of Tha Takiap district, Chachoengsao province, Thailand, there are three propagation methods applied: by seeds, by cuttings, and by digging seedlings from the forest to plant [8]. However, these authors also pointed out many limitations and suggested that more studies and trials are needed to propagate and grow this plant. In Vietnam, there are very few studies on the propagation and cultivation of *M. suavis*. Some authors and information websites also provide instructions on how to propagate and grow the species [6, 9, 10]. However, further trials and studies are needed to verify the extent to which results have been obtained from this information.

Therefore, it is very necessary to study the technique of creating seedlings and the ability to grow *M. suavis*, contributing to the conservation and development of this plant in the future. The objectives of this study were: (1) To determine the technical measures to create seedlings of *M. suavis* from seeds in polybags containing potting mixture; (2) To evaluate the potential for cultivation of *M. suavis* by sowing seeds directly under plantation shade conditions.

2. Materials and Methods

2.1. Materials

The ripe yellow fruits of *M. suavis* were collected from healthy mature mother trees distributed naturally in Xuan Son National Park, Phu Tho province. Seed treatments and experimental designs were carried out at the Experimental Garden of Hung Vuong University, Phu Tho. Ripe fruits were harvested in Summer (July) and the propagation experiments were conducted during the Autumn-Winter crop season (August-December).

2.2. Seed treatments

Ripe fruits were collected and incubated for a few more days to ripen evenly. The exocarp of ripened fruits was rubbed and washed out to collect mature seeds. Seeds were immersed in water for 15 minutes at room temperature, then washed and incubated in clean sand to a depth of at least 0.5 cm. The sand was kept moist regularly to help the radicle come out of the seed coat. To evaluate the percentage of seeds with radicle protrusion, seeds were divided into three replications (200 seeds each). Seeds with protruded radicles were counted and removed daily. For each replication, the first day of radicle protrusion, the mean time, and the percentage of seeds with radicle protrusion were recorded (see Figure 1ab).

2.3. Experimental design in the nursery

Germinated seeds with protruded radicle lengths of from 20 to 30 mm were used for experimental design. There were four treatments (T1, T2, T3, and T4) conducted in the nursery by Completely Random Design (CRD) with 3 replicates (30 seeds each). The seeds with protruded radicles were transplanted into polybags (12x16 cm). Each seed was transplanted into a polybag by putting down a radicle into the soil. All treatments were placed under 60 percent shade condition on average and watered sufficiently to keep the soil moist (one time per day for the first month, then gradually reduced). The survival and height of seedlings were measured monthly for four months. The detailed treatments were as follows:

+ T1: Transplanting seeds with protruded radicles into polybags (12x16 cm) which were filled with the potting mixture in the ratio by volume of 85% topsoil: 14% farmyard manure: 1% NPK.

+ T2: Repeating T1. After one month of transplanting seeds, the cotyledons enclosing the plumule were removed.

+ T3: Transplanting seeds with protruded radicles into polybags (12x16 cm) which were filled with the potting mixture in the ratio by volume of 85% topsoil: 9% farmyard manure: 1% NPK: 5% powdered lime.

+ T4: Repeating T3. After one month of transplanting seeds, the cotyledons enclosing the plumule were removed.

The reason why we conducted T2 and T4 by removing the cotyledons was originated from the personal experience when investigating in 2014 at Xuan Son National Park [6]. Observing after about one month of germination, the radicle grew downward into the soil, and the hypocotyl (the embryonic stem) elongates, raising the cotyledons, plumule, and remains of the seed coat aboveground. At that time, the testa was cracked and the small plumule had a sign of coming out of cotyledons. However, these very thick cotyledons still enclosed the small plumule, and it took a certain time for the plumule to protrude out of the cotyledons (see Figure 1c). Our hypothesis was that seedlings would grow faster if the cotyledons were removed.

With T3 and T4, 5% lime powder was added. The reason was that *M. suavis* is a plant species naturally distributed in limestone mountains where have highly alkaline soils and have very high calcium content [11]. Thus, we tested the addition of lime in the media to compare with the rest treatments.

2.4. Experimental design in the field

Like above design, the seeds with 20-30 mm protruded radicle lengths were used for experiments. Two treatments (T5, T6) were in the field (under *Acacia mangium* plantation) by sowing the germinated seeds in 30 holes (three seeds each). The treatments were also placed under 60 percent shade condition on average and watered sufficiently to keep the soil moist (one time per day for the first month, then gradually reduced). The survival and height of seedlings were measured monthly for four months. The detailed treatments were as follows:

+ T5: Sowing seeds with protruded radicles directly under *Acacia mangium* plantation (60% canopy cover). The plantation site was flat, well-drained. Before sowing seeds 15-20 days, the weeding partly around planting holes with a diameter of 2 m was applied. The hole size was 40 × 40 × 40 m. Each hole was added with 1.2 kg of farmyard manure + 0.05 kg of powdered lime + 0.1 kg of NPK and mixed with topsoil before filling the hole.

+ T6: Repeating T5 but only adding fertilizers when seedlings had 3-4 true leaves.

The treatments T5 and T6 were tested by direct seeding. The reason came from the findings of Khamyong and Serarnethakun [7] and Soontornwong et al. [8] that the *M. suavis* seedlings from seeds replanted in the field were stunted and grew very slowly. The authors believed that the roots of this species were easily broken during transporting and planting. Sowing seeds directly in the field might limit the mechanical impact on the roots of seedlings. Moreover, the above authors also suggested the trials of planting in the open and in an environment like forest shade conditions. On the other hand, during the investigation in Xuan Son National Park, we noticed that some local people collected seeds and sew directly in their gardens, initially realizing that the trees could grow [6].

2.5. Data analysis

Data were calculated by Excel and SPSS 20.0 sofwares. The Mann-Whitney test (Sig. < 0.05) was used to assess the statistically significant difference in height of seedlings for each pair of experimental treatments.

3. Results and discussion

3.1. Impact of the seed treatments on the time and percentage of M. suavis *seeds with radicle protrusion*

Regarding *M. suavis* seeds incubated in the sand, the mean radicle protrusion time of *M. suavis* seeds was 24 days. The first seed protruding radicle was noted after 4.3 days, and the number of seeds of radicle protrusion was highest on day 14. After 24 days the number of seeds protruding radicles was decreasing and was negligible after 36 days. The seed percentage of radicle protrusion was 54% of the total seeds (Table 1).

To improve the the germination rate of *M. suavis*, other seed treatment techniques should also be further studied. For instance, Anousack (2020) suggested the seed priming technique which enhanced high and fast seed germination and good seedling growth in *M. suavis* [12].

Table 1. Time and percentage of *M. suavis* seeds with radicle protrusion

Replication	First day of radicle protrusion (day)	Mean time of radicle protrusion (day)	Seed percentage of radicle protrusion (%)
1	4	26	62
2	4	24	53
3	5	20	46
Mean	4.3	24	54

Figure 1. Morphology and seedling of *M. suavis*: (a) Fruits, (b) Seeds with protruded radicles, (c) Seeds after 1 month in the nursery with cotyledons attached, (d) Seeds after 1 month in the field - not sprout, (e) A seedling 4 months old from the treatment T4, (f) A seedling 4 months old from the treatment T6 (Photos by Long N.T.)

3.2. Impact of planting media and tending techniques on the growth of M. suavis seedlings in the nursery

For the survival rate of seedlings in the nursery, the study revealed a very high percentage. At the age of from one to three months, the survival rate reached almost 100%. After 4 months this rate decreased slightly, but still over 97%. In general, the seedlings of *M. suavias* cared for in the nursery had a high survival rate and there was no difference among treatments. Thus, most of the seeds with protruded radicles have successfully germinated in the nursery to become complete seedlings (Table 2).

Table 2. The survival rate of *M. suavis* seedlings in six treatments

Treatment	Survival rate (%) at different ages			
	1 month	2 months	3 months	4 months
T1	100	100	100	97,8
T2	98.9	98.9	98.9	98.9
T3	100	100	100	98.9
T4	100	100	100	100
T5	-	32.2	32.2	32.2
T6	-	23.1	23.1	23.1

Note: "-" not measured due to most plumules of seedlings not yet coming out of cotyledons.

Figure 2 shows that with four treatments in the nursery (T1, T2, T3, T4), the height growth of seedlings was strongest in the period from one to two months old. From two to four months old, they grew more slowly.

The results in Figure 3 show that after 1 month of sowing, there was no difference in height growth among four treatments in the nursery. At 2 months of age, the difference was a little significant, in which T4 (liming and cotyledon removal) showed the best height growth (13.6 cm) compared to the other three treatments. However, at 3 and 4 months, height growths were not statistically different among four treatments. The addition of lime in the potting mixture has not yet proved the obvious superiority, but it may be a useful method when propagating the seeds of *M. suavis*.

3.3. Impact of direct seed sowing techniques on the growth of M. suavis seedlings in the field

The direct seeding in the field gave low survival rates, attaining 32.2% in T5 and 23.1% in T6 (Table 2). One of the reasons may be the fact that seedlings lived in natural conditions and were not cared for adequately, planting media were not treated as well as in the nursery. Observing in the field, we found that many seeds sown in T5 and T6 did not die, but they could not sprout for certain reasons (Figure 1d).

The treatments of direct sowing in the field (T5, T6) in the first month, most the plants have not yet sprouted; only after the first month the plants started to sprout and grew strongly from the second month onwards (Figure 2).

The results in Figure 3 show that T6 (adding fertilizers when seedlings had 3-4 true leaves) achieved significantly higher growths in height than T5 at two, three and four months of age. At 3 and 4 months, seedlings in T6 grew substantially; Compared to seedlings in T1, T2, T3, and T4, even they exhibited no significant difference.

From above results, it showed that the direct seeding in the field but without fertilizing at the first stage (T6) gave very positive results in terms of height growth.

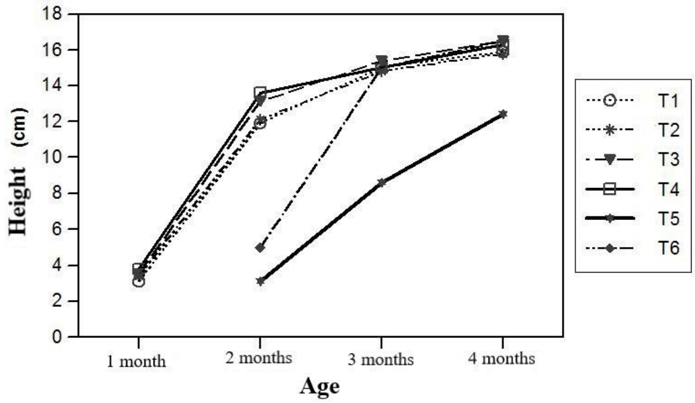
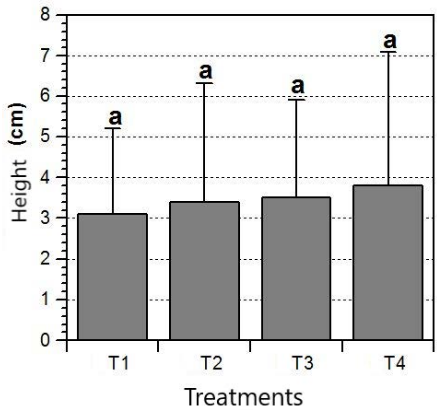
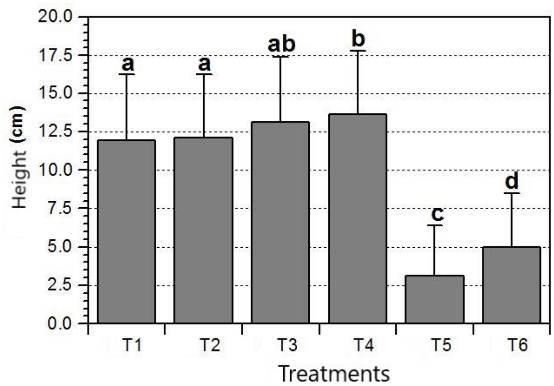


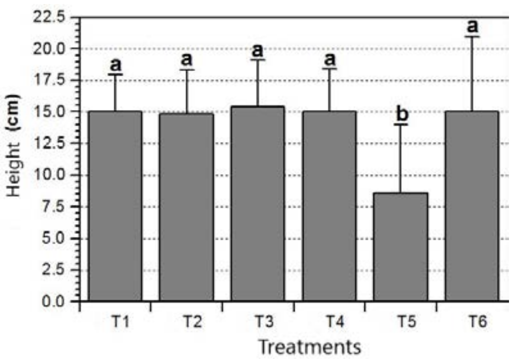
Figure 2. Effect of different treatments on the height growth of *M. suavis* seedlings



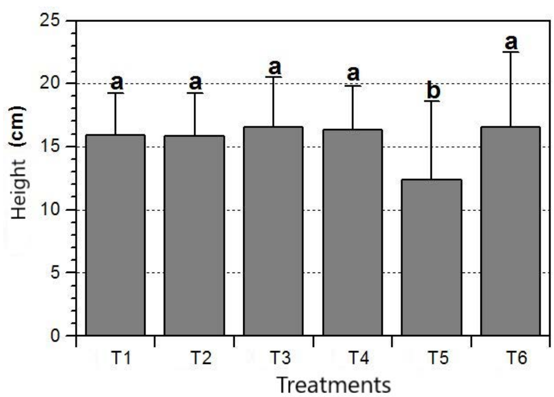
One month



Two month



Three month



Four month

Figure 3. Effect of different treatments on the height growth of *M. suavis* seedlings. Letters a, b, c, and d indicate significant differences in the Mann-Whitney test (Sig. < 0.05) for each pair of experimental treatments

4. Conclusions and recommendations

4.1. Conclusions

- The mean radicle protrusion time of *M. suavis* seeds after incubation was 24 days. The percentage of radicle protrusion was 54% of total seeds.

- In the nursery, after 4 months of age, the seedlings had a very high survival rate (over 97%); Most of the seeds with protruded radicles have successfully germinated in the nursery to become complete seedlings. The height growth of seedlings was strongest in the period of from one to two months old. From two to four months old, they grew more slowly. The removal of the cotyledons for plants at one month of age did not have a significant effect on the growth of seedlings.

- Sowing seeds directly in the field gave a survival rate very low (under 33%). Only after the first month the plants started to sprout and grew strongly from the second month onwards. The direct seeding in the field but without fertilizing at the first stage (T6) gave very positive results in terms of height growth.

4.2. Recommendations

From the obtained results, we suggest that *M. suavis* can be propagated by seeds. Cultivation of *M. suavis* by direct seeding in the field may be a potential solution to preserve and develop this valuable plant.

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KỸ THUẬT NHÂN GIỐNG VÀ TIỀM NĂNG GÂY TRỒNG CÂY RAU SẮNG (*MELIENTHA SUAVIS* PIERRE) BẰNG HẠT TẠI TỈNH PHÚ THỌ, VIỆT NAM

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Tóm tắt

Rau sắng là loài cây gỗ nhỏ có tên trong sách đỏ Việt Nam, làm rau ăn có giá trị dinh dưỡng cao. Tuy nhiên, các các nghiên cứu về nhân giống và gây trồng loài này còn rất hạn chế. Chúng tôi đã tiến hành nghiên cứu nhân giống bằng hạt trong vườn ươm và thử nghiệm gieo hạt thẳng tán dưới rừng Keo tai tượng. Kết quả cho thấy thời gian xuất hiện rễ mầm của hạt Rau sắng bình quân 24 ngày, tỷ lệ là 54%. Tỷ lệ sống của cây con trong vườn ươm khá cao (trên 97%) nhưng gieo thẳng ngoài thực địa lại thấp (dưới 33%). Trong vườn ươm cây sinh trưởng mạnh nhất từ tháng thứ 1 đến tháng thứ 2 và sau đó chậm dần, trong khi ngoài thực địa sinh trưởng mạnh nhất sau tháng thứ 2. Việc bóc bỏ lá mầm cho cây ở thời điểm 1 tháng tuổi không có ảnh hưởng rõ rệt đến sinh trưởng của cây con. Tại thời điểm 4 tháng tuổi, gieo hạt thẳng ngoài thực địa nhưng không bón phân giai đoạn đầu (CT6) cho sinh trưởng cây con không khác biệt so với các công thức còn lại trong vườn ươm. Từ kết quả đạt được, chúng tôi đề xuất: (1) Rau sắng có thể được nhân giống bằng hạt; (2) Việc gây trồng Rau sắng bằng cách gieo hạt thẳng ngoài thực địa có thể là giải pháp tiềm năng để bảo tồn và phát triển loài cây đặc sản có giá trị này.

Từ khóa: *Gây trồng, nhân giống, Phú Thọ, Rau sắng, vườn ươm.*