Propagation Trials with Application of Pre-Germination Treatments to Seeds and Rooting Hormones to Stem Cuttings of *Beltik* *(Syzygium sub-caudatum* (Merr.) Merr.) and *Itsa/Tsa-a (Ehretia microphylla* Lam.)

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ABSTRACT

Propagation trials for *Beltik (Syzygium sub-caudatum* (Merr.) Merr.) and *Itsa (Ehretia microphylla* Lam.) using seeds and stem cuttings were conducted at the nursery of the College of Forestry, Benguet State University, La Trinidad, Benguet. The trials were used to determine the planting stock production techniques for *Beltik* and *Itsa* for their efficient propagation and establishment of plantations for economic development and environmental protection. Specifically, the study aimed to determine the germination rate and period, survival rate of the stem cuttings, and average quantity of roots that develop in the rooted stem cuttings of the species. Results showed that *Beltik* can be propagated using seeds and stem cuttings but entails intensive care, including partial shading of the newly germinated seeds in the former, and the "double cutting" procedure, also with intensive care, for the latter. Seed germination rates averaged 91.1% for the extracted seeds and 2.54% for the un-extracted seeds. These were not significantly affected by the applied sun-drying and air-drying treatments. The planted hardwood cuttings produced sprouts but did not produce roots. On the other hand, *Itsa* can be propagated using stem cuttings but may be difficult to propagate using seeds. The survival rates of the planted stem cuttings averaged 65.74% and were not significantly affected by the stem cutting sections used and the applied hormonal treatments. Furthermore, the stem cutting sections used and the applied hormonal treatments did not significantly affect the quantity of roots developed in the rooted stem cuttings but their interaction did.

Keywords: *propagation, stem cuttings and sections, pre-germination and hormonal treatments, double cutting procedure and germination rate and period*

INTRODUCTION

The Philippines ranks 8th in the world list of endemic plants and is among the 17 countries that hold the greatest number of species of living organisms. It is considered a megabiodiversity area but was declared a biodiversity hotspot due to fast forest denudation (Ong, *et al*., 2002).With the continuing conversion of forests into farms and other uses, indigenous forest trees and shrubs continue to be depleted. Based on the extent of forests in the Cordillera in 1997 and in 2003 as reported by the DENR, about 19,000 ha of forests have been lost yearly. As of 1987, the DENR reported that 50% of endemic flora have become extinct. Hilton-Taylor (2000) reported that 216 plant species in the country: two bryophytes, four conifers, eight monocots and 202 dicots are endangered. In this research, studying the propagation techniques that would produce planting stocks for artificial planting hopes to conserve our native teas and other species.

Beltik and *Itsa/Tsa-a* are among the 18 indigenous shrub and tree species used as tea in Benguet as reported by seventy nine key informants in the municipalities of Atok and Tuba. Except for the report of the key informants that these species can be propagated using stem cuttings and wildlings, no other report as to their artificial propagation can be found.

Beltik or Daniwdiw (Figures 1-3) belongs to Family Myrtaceae. It is a small tree with brown bark. The leaves have a mild scent, and

Figure 1. A *Beltik* plant

Figure 2. *Beltik* flowers

Plate 3. *Beltik* fruits

are oppositely arranged, lanceolate with entire margin and sub-caudate apex, pinnately veined, of smooth surfaces, and about 6-6.5 cm long and 2-3 cm wide. The petiole measures about 0.4- 0.5 cm long and about 1.5mm in diameter. Flowers are white. Fruits are globular, green when young, turning reddish-violet when fully matured and about 0.5-0.8 cm in diameter. Representative plants are located in areas with elevation ranges of 1,872 – 2,325 masl and with soil of the clay – silty clay loam type and with an average pH of 3.9 – 4.7 (moderately-strongly acidic). The fruiting months as reported by key informants are March – May in Brgy. Topdac, August – September in Brgy. Cattubo, and November – December in Brgy. Abiang, in the municipality of Atok, and from December – February in Barangays Poblacion and Tadiangan in the municipality of Tuba, Benguet.

Itsa/Tsa-a as locally known (Figures 4-5) is a shrub of the Family Boraginaceae. Its local common name is *Kalimonog*. Its other scientific names are *Carmona microphylla* (Lawn) G. Don or *Carmona retusa* (Vahl) Masam (Rojo, 1999). The leaves are simple, white, axillary, solitary, 1 or 4 on a common stalk. Fruits are yellow when ripe, about 4-5 mm in diameter, fleshy and with four seeds.

In this study, two representative trees were located within forest openings with elevations of 789 and 207 masl. Soil was found to be moderately to highly acidic (pH of 3.10 – 4.65), and of the silty clay and silty clay loam types. Matured fruits were collected on May 21, 2014 in Ansagan, Tuba, and in February 2015 in Benengbeng, Sablan, both in the Province of Benguet. The plants observed in September 2014 at Legleg, Kibungan, Benguet had immature fruits.

Propagation techniques. The use of seeds is the most common propagation method for forest trees, followed by the use of stem cuttings. Seeds, when used, are usually extracted from the fruits, and pre-germination treatments are applied to induce earlier germination. This is to avoid uneven germination, especially for those with thick or horny seed coat (PCARRD, 1992).

Figure 4. Branch of *Itsa* with foliage and immature fruits

Figure 5. *Itsa* flower

Non-extraction of seeds have affected seed germination in certain species due to fermentation as reported by Dickens (2011), Woessner and Mcnabb (1979), Amunnidin and Zakaria (1980) and Ogunnika and Kadeba (1993). The pre-germination treatments to promote germination include soaking in water, nicking, scarifying, removal of seed coat and heat treatment. In this study, air-drying (drying under the shade) and sun-drying were applied, with the latter, a form of heat treatment. The former was found to promote the germination of *Annona squamosa* (Banful, *et al*. 2015) and *Annona cherimola* (Duarte, *et al*. 1974).

To obtain exactly the same genetic characteristics of the parent plant and/or to propagate species that are difficult to reproduce using seeds or seedless types, asexual propagation methods are applied (Agpaoa, *et al.*, 1975). The use of stem cuttings is the most common asexual propagation method applied to some forest trees. Propagation of tea species by stem cuttings is advantageous as this will not only augment planting stocks for larger scale plantation establishment but also produces shorter and smaller plants that can ease harvesting and maintenance operations. Stem cuttings of some species fail to develop or have difficulty developing roots. This may be remedied by the application of root-inducing hormones that include Andole Butyric Acid (IBA) and Alpha Naphthalene Acetic Acid (ANAA). In this study, ANAA and "Hormex" (combination of Indole Butyric Acid – 0.013%, Alpha Napthalene Acetic Acid – 0.24%, and Thiamine hydrochloride – 0.25%) were used as these are readily available commercially.

Objectives of the Study

The study aimed to know the planting stock production techniques for *Beltik* and *Itsa* towards their efficient propagation and the establishment of plantations for economic development and environmental protection. Specifically, the propagation trials for *Beltik* and *Itsa* were conducted to determine the following: (1) germination rate and period of germination of their seeds as affected by pre-germination treatments such as seed extraction, and sun and air-drying, and (2) survival rate of stem cuttings and the quantity of roots that develop as affected by the stem sections (base, middle and tip) and the application of root-inducing hormones (ANAA and Hormex).

METHODOLOGY

Time and Place

The study was conducted at the nursery of the College of Forestry, Benguet State University, La Trinidad, Benguet from February 2013 – August 2014 for the propagation of *Beltik*, and from February, 2015 – January, 2016 for the propagation of *Itsa*.

Experimental Designs

Germination trials. For *Beltik*, germination trials on Petri dishes using Completely Randomized Design (CRD), and on seedbed, using Randomized Complete Block Design (RCBD) were conducted. The following were the treatments applied: EF = extracted fresh seeds as Control, ESD = extracted seeds, sun-dried from 8am – 12 noon for two days, and EAD = extracted seeds, air-dried for two days. The air-dried, sun-dried and freshly extracted seeds were placed in Petri dishes overlain with tissue paper (Figure 6). Fifty seeds were used for each treatment, each with three replications. For the trials on seedbed, un-extracted seeds from fully-ripened fruits were used.

Similarly, sun-drying from 8am–12nn and 10 days air-drying were the treatments applied, with the fresh fruits as Control, with

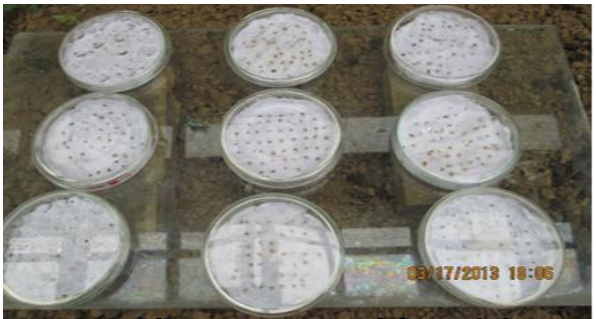

Figure 6. Seed germination trial for *Beltik* using Petri dishes

three replications. Three hundred each of air-dried, sun- dried and fresh fruits (100 fruits per replication) were used.

For *Itsa*, Completely Randomized Design was used with three replications, each for the following treatments: T_0 = whole fruit, T_1 = fleshy skin removed, T_2 = fleshy skin removed and air-dried for three days, and T_3 = fleshy skin removed and sun-dried for three days.

Use of stem cuttings. For *Beltik*, the Split-split Plot Design was used with three hormonal treatments as the main plot factors: T_0 = Control (no treatment), T_1 = Hormex (15 minutes dipping the base of cuttings in a solution of 6.5 ml Hormex and 500ml distilled water), and T_2 = ANAA (15 minutes dipping the base of stemcuttings in a solution of 9 ml ANAA and 500 mldistilled water); three stem diameter ranges assub-plot factors (≤ 1 cm, >1-2 cm, and > 2-3 cm); and three stem sections (base, middle and tip) as sub-sub plot factors. The level of concentration of the hormones applied followed the recommendation printed on their container labels.

For *Itsa/Tsa-a*, the Split Plot Design was used with three stem sections (base, middle and tip) as the main plot factors and the following hormonal treatments as sub-plot factors: T_0 = Control (no treatment), T_1 = Hormex (15 minutes dipping the base of cuttings in a solution of 6.5 ml Hormex and 500 ml of distilled water) and T_2 = ANAA (15 minutes dipping the base of stem cuttings in a solution of 9 ml ANAA and 500 ml distilled water). About 30 cm of the upper part of selected small branches were collected and cut into three sections each. Each section consisted of at least four nodes (Figure 7). A total of 108 cuttings were used. There were three cuttings for each treatment, with each treatment having 4 replications.

Figure 7. Prepared Itsa stem cuttings: base and middle (left), base and tip (right)

The cuttings of both species were planted in a mixture of 50% river sand and 50% top soil, (placed in black plastic bags) after treatment with hormones The set-up for *Itsa* was enclosed with polyethylene plastics with a clearance of three feet high from the planting media. The enclosure was maintained until the 3rd month from planting. The planted cuttings were watered every other day for one month and then twice a week thereafter.

The number of cuttings that survived and the number of roots that developed were recorded one year from planting. For the counting of the developed roots, a representative rooted

Figure 8. Representative of rooted cuttings used to determine the number of roots developed

cutting from each treatment per replication (a total of 36 plants) were selected systematically and their roots were exposed by careful removal of the plastic bags and washing off the soil (Figure 8).

RESULTS AND DISCUSSION

Germination Trials

Beltik (Syzygium sub-caudatum)

Extracted seeds. Results showed that air-dried seeds had higher mean germination rate (94%) than those of the sun-dried and fresh seeds, both of which had 89.33%. However, these treatment means did not significantly differ from each other (Table 1). This result differs from the findings of Banful, *et al.* (2015) that air drying and sun drying the seeds of *A. squamosa* for three consecutive days resulted to significantly higher germination, and that of Duarte, *et al.* (1974) that drying the seeds of *Annona cherimola* under shade resulted to significantly higher germination rates than those of the fresh seeds. The results of this study as compared with the above reports show that response to pre-germination treatments varies with species.

Un-extracted seeds. Results showed an average germination rate of only 2.54%, and that unextracted seeds from fresh fruits (UF) had higher mean germination rate (4.0%), followed in descending order by the unextracted seeds from the sun dried and air-dried fruits, respectively. The differences, however, are statistically insignificant (Table 2). This result differed from that of the first trial wherein air-dried seeds had higher mean germination rates than those of the sun-dried and fresh seeds. The insignificant differences in the 1st trial and in this trial indicate that the treatments applied do not significantly affect the germination rate of *Beltik*. It also differed from the first trial as the germination rates were very low which can be attributed to the overcrowding of the seeds. These were sown unextracted from the fruits, and there was probable loss of viability due to fermentation in the fruits. The germinating seeds underlain by other seeds/fruits could have been completely obstructed, thus were unable to emerge above the seedbed. According to Dickens (2011), the viability of *Irvingia wombolu* seeds were lost when fermented beyond one week. Similarly, Woessner and Mcnabb (1979) reported that green fruits of *Gmelina arborea* have only 10% germination rate when sown directly (without removing the pulp), while Amunnidin and Zakaria (1980) reported that the products of pericarp fermentation in *Gmelina arborea*

Table 1. Analysis of Variance for Data on the Germination Rate of *Beltik* Seeds

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Computed	F- Tabular Values	
					5%	1%
Treatment	2	56.88	28.44	1.78^{ns}	5.14	10.92
Error	6	95.94	15.94			
Total	8	152.82				

cv = 6.21% ns = not significant

Table 2. Analysis of Variance from Data on the Germination Rate of Unextracted Seeds from Sun-dried (USD), Air-dried (UAD) and (UF) Fruits of *Beltik*

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Computed	F- Tabular Values	
					5%	1%
Treatment	2	1.769	0.884	2.28^{ns}	6.94	18.00
Block	2	0.523	0.2615			
Error	4	1.235	0.388			
Total	8	3.527				

cv= 24.55% ns = not significant

contributed to loss of viability. Furthermore, Ogunnika and Kadeba (1993) reported a sharp drop in germination of *Gmelina arborea* when the fruits were allowed to ferment by piling up after collection.

Raised Seedling

The germinated seeds were transplanted to potted soil and maintained under partial shade as the young seedlings were observed to be sensitive to full exposure to sunlight. Some of the resultant seedlings are shown in Figure 9.

Figure 9. *Beltik* seedlings grown from the germination trial in seedbeds

Germination period. The number of days that *Beltik* seeds started to peak and complete germination are shown in Table 3. Data show that extracted *Beltik* seeds required 19–26 days from sowing (average of 22.33 days) to start germination, and 44–54 days or an average of 48.33 days to complete germination. Extraction of seeds from fruits hastened germination by about 20 days and shortened the period to complete germination by about 42 days (based on the germination data from fresh seeds). A similar result was observed in the study on the germination of *Sarcandra glabra (Gipas).* Seed extraction from the fruits (removal of the fleshy part) hastened their germination (Tacloy 2000). In *Gmelina arborea*, sun and air dried extracted seeds led to early germination (26 days) while the control (fresh and unextracted) resulted to non-germination (Banful, *et al.* 2015).

An important observation is the germination of a few seeds from undiscarded sown seeds in fruits (unextracted from fruits) 230 days after sowing. This indicates that *Beltik* seeds in dried fruits may remain viable within 230 days.

Itsa/Tsa-a

There were no seeds germinated from the fruits sown for this trial. Recovered five months after sowing, the fruits were rotten. There were a few which were not deformed but were empty of seeds inside (Figure 10). The viability of the seeds in the fruits sown for this trial were not ascertained, hence the inconclusive result.

Figure 10. Uncovered fruits of *Itsa*, either rotten or intact but empty

A possible reason for the non-germination could be the loss of viability of the seeds inside the fruits due to chemical reactions. In the study Woessner and Mcbnabb (1979), non-extraction of seeds from the pulp resulted to only 10% germination rate of *Gmelina arborea*. They suspected that chemicals associated with fermentation may have inhibited the germination.

Table 3. Germination period of *Beltik* seeds

EXPERIMENTAL SET-UP AND TREATMENTS		PERIOD OF GERMINATION (DAYS) START	PEAK	END
*1st set-up (in petri-dish):	EF	22	26	44
	ESD	26	30	47
	EAD	19	30	54
	MEAN	**22.33**	**28.67**	**48.33**
**2nd set-up (in seedbed):	UF	42	60	86
	UAD	25	69	69
	USD	25	46	69
	MEAN	**30.67**	**53.33**	**74.67**
	GRAND MEAN	**26.5**	**43.5**	**61.5**

* = Extracted seeds ** = Un-extracted seeds (seeds in fruits)

Propagation Using Stem Cuttings

Beltik (Syzygium sub-caudatum)

The stem cuttings used produced shoots starting on the 17th day from sticking but the shoots eventually wilted, and no callus or sign of initial root formation was observed at the base of the cuttings (Figure 11). Another trial using RCBD was set up on December 24- 25, 2013 but similarly did not succeed. The treatments

applied were the following: T_0 (Control/No treatment); T_1 (dipping base of cutting for 20 minutes in a solution made of 10 ml Hormex diluted in 4 liters of water); and T_2 (dipping base of cuttings in 12hrs in solution made of 4 tbs of ANAA dissolved in 4 liters of water). Related to this result are the observations of Reily and Shry, Jr. (1983) that growth on tips and sides of cuttings is normal but does not always indicate that the cuttings have rooted. The report of Danthu, *et al.* (2002) showed that receptiveness to propagation by cuttings varies with the species. Results showed that the sub-genus *Sycomorus* of genus *Ficus* had no capacity to propagate from cuttings while sub-genus *Urostigma* can be propagated by cuttings with varying degrees of difficulty. *F. thonningii, F. leprieurii* and *F. ovata* easily root while *F. platyphylla* and *F. elasticoides* were difficult to root. The report of Krishnamoorthy (1981) showed that stem cuttings of majority of large woody plants, including mango and eucalyptus, do not form roots even with the application of auxins. In addition, Hartman and Kester (1975) observed that softwood cuttings generally develop roots more easily and more quickly than hardwood cuttings. The failure of the *Beltik* cuttings used in this study to produce root could be due to their hardwood condition.

Figure 11. Experimental set-up of planted *Beltik* cuttings (left photo) and lifted planted cuttings showing the absence of initial callus formation (right photo)

Use of sprout from planted cuttings. Eighty four sprouts from planted cuttings were collected and planted. Fifty two of the planted sprouts (61.9%) successfully developed into planting stocks. This is referred to as "double cutting" procedure (Fig. 12). As observed, the base of the hardwood cuttings that sprouted were dried without any sign of callus formation. It is propable that the nutrients

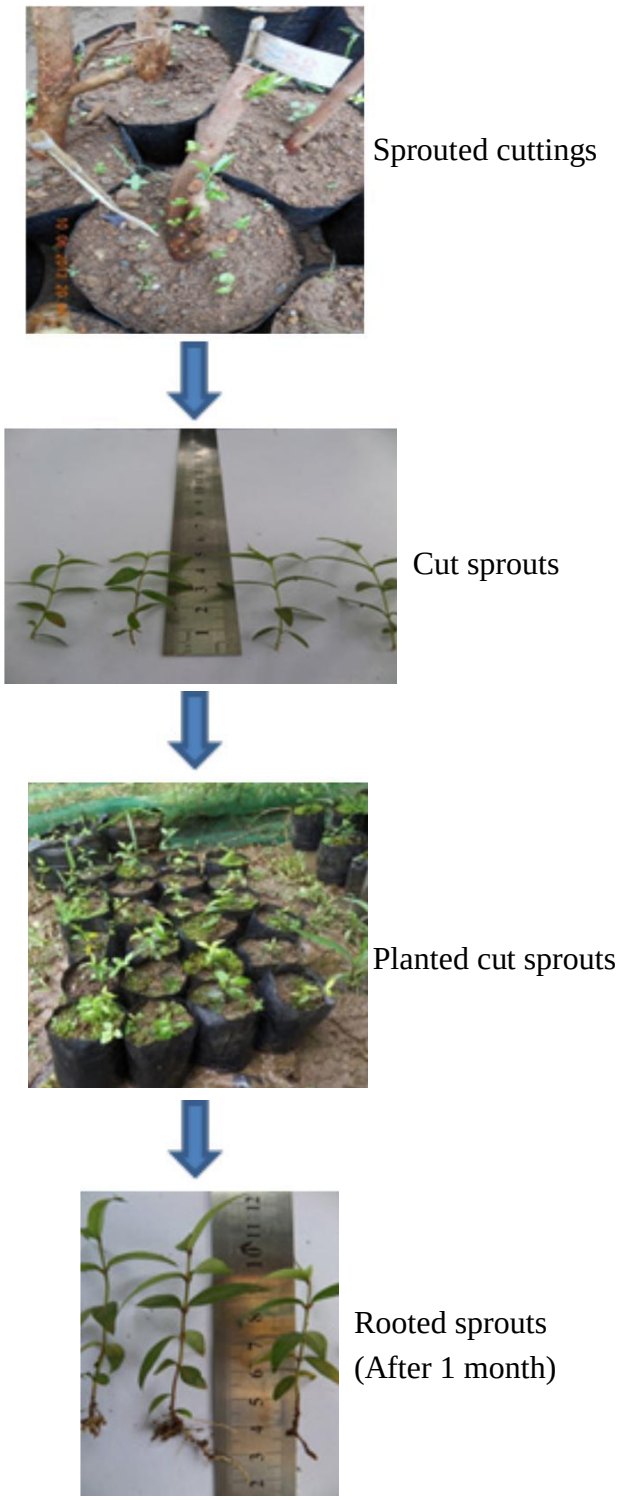

Figure 12. "Double cutting" procedure

and natural hormones were translocated to the dormant buds above the soil level to activate them to develop into sprouts, leaving very little or no nutrients to nourish cells at the base. On the other hand, the sprouts carry with them the translocated substances but once depleted, these may wilt due to the absence of nutrient replenishment.

Collecting the sprouts while they contain nutrient reserve and sticking them to growing

media usually result to rooting success due to the presence of newly developed cells containing natural hormones. As reported by Reily and Shry Jr. (1983), some plant cuttings root more easily than others due to natural hormones. Also, Sundaraiya, *et al.* (2003) found that terminal cuttings of *Calotropis procera* rooted well as compared to the basal and middle cuttings. This was due to their softwood and herbaceous nature.

Itsa/Tsa-a

Survival rates. As shown in Table 4, statistical analysis showed that the survival rates of the cuttings averaged 65.74% and were not significantly affected by the stem sections used and the hormonal treatments applied nor the interaction of the two factors (stem sections x hormonal treatments). Rooted stem cuttings are shown in Figure 13.

Based on the treatment total, the survival rates of "base" and "middle" cuttings without treatment (Control) are lower than those treated with hormones while the survival rates of untreated "tip" cuttings are higher than those treated with hormones. These results imply the occurrence of a slight interaction effect of the hormonal treatments and stem position on the survival rates of the planted *Itsa* cuttings. This result is similar to the findings of Ngiwas (2007) that the survival rate of 'Deguai' stem cuttings were not significantly affected by the applied Hormex and ANAA treatments and the stem sections used, nor was there an interaction between the two factors.

Figure 13. Rooted stem cuttings of *Itsa*: one month from sticking (left photo) and one year from sticking (right photo)

Roots developed. The number of roots developed from representative rooted cuttings were recorded one year from planting. Statistical analysis showed that the stem cutting sections used and the Hormex and ANAA treatments did not significantly affect the number of roots developed among the rooted cuttings. Their interaction, however, did (Table 5). Based on the treatment totals, there were more roots developed at the "base" and "middle" cuttings treated with hormones than those untreated while the untreated "tip" cuttings had more developed roots than those treated with hormones.

According to Devlin (1977), plant hormones in small amounts or in low concentrations may promote, inhibit, or otherwise modify certain physiological processes. Auxins, compounds having the capacity to induce elongation in shoot cells and affect other physiological processes, are concentrated at the growing tips of plants. The action of auxins in roots is similar to that in stems but concentration of auxin stimulatory to stem growth are inhibitory to root growth. In other words, there is a specific auxin level

Table 4. Analysis of Variance of Survival rate (%) of *Itsa* stem cuttings as affected by stem sections and hormonal treatments

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Computed	F- Tabular Values 5%	1%
Replication	3	6,759.61	2,253.2			
Stem Position (A)	2	987.68	494.84	0.80^{ns}	5.14	10.92
Error (a)	6	3,703.93	617.32			
Hormonal Treatment (B)	2	1,174.38	587.19	1.21^{ns}	3.55	6.01
A x B	4	4,197.30	1,049.33	2.17^{ns}	2.93	4.58
Error (b)	18	8,703.63	483.58			
Total	**35**	**25,527.37**				

Legend: cv (a)= 37.8%; cv (b)= 33.45%; ^{ns} = not significant

Table 5. Analysis of Variance of the number of developed roots of *Itsa* stem cuttings in a split-plot experiment with three stem sections as main plot factors and three hormonal treatments as subplot factors

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Computed	F- Tabular Values	
					5%	1%
Replication	3	5.44	1.81			
Stem Position (A)	2	0.67	0.335	0.48_{ns}	5.14	10.92
Error (a)	6	4.22	0.7			
Hormonal Treatment (B)	2	0.67	0.335	1.52_{ns}	3.55	6.01
A x B	4	4.16	1.04	4.73**	2.93	4.58
Error (b)	18	4.00	0.22			
Total	**35**	**27.00**				

cv (a)= 23.9%; cv (b)= 13.4%; ns = not significant; ** = highly significant

needed for root development which is different from what is needed for stem/shoot development. Thus, real stimulation of root formation and elongation may be achieved only if appropriate auxin concentration is used.

In this study, since auxin is more concentrated at the "tip" cuttings, the application of this may have increased above the optimum level for root induction. On the other hand, the application of hormone to the "middle" and "base" cuttings may have increased this to a level favorable for root induction. Successful root induction and development in the cuttings resulted to their survival and better growth.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Based on the results, *Beltik* can be propagated using seeds and sprouts from planted (hardwood) cuttings. Seed extraction from the fruit shortens the starting period and the completion of germination, while non-seed extraction and drying the fruit may prolong the viability of *Beltik* seeds. For *Itsa*, it can be concluded that it can easily be propagated using stem cuttings even without using root-inducing hormones. The interaction of stem sections with Hormex and ANAA have significantly affected the initial root development of planted stem cuttings.

Recommendations

To propagate *Beltik*, it is recommended that extracted seeds from fully matured fruits or double cutting technique be used. Young seedlings and newly planted sprouts should be kept under partial shade. To achieve earlier start and completion of germination, the seeds should be extracted. To prolong the viability of the seeds, the fruits need to be dried before storage. To propagate *Itsa*, it is recommended that stem cuttings with 3-4 nodes be used. If root-inducing hormones like ANAA or Hormex are used, it is best to apply them to "base" and "middle" cuttings. Trials using extracted fully developed seeds from fruits should be done.

Follow-up studies related to this need to be pursued towards the development of planting stock production technology for the species. Priority studies may include more seed germination trials for *Itsa* and the determination of the optimum and range of light intensity for raising planting stocks of *Beltik*.

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