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*Full Length Research Paper*

# Promotion of seed germination, subsequent seedling growth and *in vitro* propagation of korarima *Aframomum corrorima* (Braun) P. C. M. Jansen)

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**The germination of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) seeds faces certain problems. The study was carried out to explore the effects of different seed treatments on germination and seedling growth attributes of korarima. Seeds were subjected to seven pre-sowing treatments viz., control (no pretreatment) ($T_0$), soaking in tap water for 24 h ($T_1$), soaking in 50% sulfuric acid ($H_2SO_4$) for 60 min ($T_2$), soaking in 50% sulfuric acid ($H_2SO_4$) for 60 min, followed by soaking in 250 $mgL^{-1}$ gibberellic acid ($GA_3$) for 24 h ($T_3$), cold stratification at 4±1°C for one week ($T_4$), cold stratification at 4±1°C for two weeks ($T_5$) and cold stratification at 4±1°C for three weeks ($T_6$). Also micropropagation systems were developed for highland korarima cultivars viz., Mume, Wondogenet and Mesketo. The effective concentration of hormones for shoot multiplication and rooting in cultivar Mume were determined. Two explant sources, in vitro seedling shoot tips and field grown rhizome buds of cultivar Mume were evaluated. The study revealed that exposure of seeds to $T_3$ was the most effective treatment for enhancing germination (88.3%), followed by 81.7% germination obtained from $T_2$ after 6 weeks of sowing. Other treatments were less effective. Seed pretreatment had positive effects on seedling height, number of leaves, root number, root length and fresh weight. The highest number of shoots and percent survival was achieved in the cultivar Mume as compared to other two cultivars after five weeks of in vitro culture. Significantly higher shoots number (11.00), leaves number (15.33) and fresh weight (0.81 g) per explant of cultivar Mume was obtained when cultured on Murashige and Skoog (MS) medium containing 0.5 $mgL^{-1}$ thidiazuron (TDZ) compared to number of shoots (2.67), number of leaves (6.00) and fresh weight (0.25g) in the control. MS medium with 0.5 $mgL^{-1}$ TDZ and 3 $mgL^{-1}$ 6-benzyladenine (BA) also gave high shoot number (10.00) per explant. The growth parameters were significantly correlated. The highest percent survival and number of shoots were obtained from in vitro seedling shoot tips compared to rhizome buds. The indolebutyric acid (IBA) at 1 $mgL^{-1}$ significantly promoted the rooting (75.56%) over the control (33.33%).**

**Key words:** *Aframomum corrorima*, korarima, cultivar, seed germination, seedling growth, micropropagation, shoot formation.

## INTRODUCTION

Korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) is herbaceous, perennial and aromatic species classified in the monocotyledonous family Zingiberaceae, native to Ethiopia. The plant consists of an underground rhizome, a pseudostem, and several broad leaves and resembles *Elettaria* species morphologically. Mature korarima can reach a height of 1-2 m. It sets seed after 3-5 years of planting depending on the planting materials used and it continue to bear seeds for a number of decades.

Korarima occurs as a cultivated crop only in Ethiopia. The seed of korarima is mainly used as sources of spices in traditional Ethiopian dishes. It is a source of income for growers as its seeds fetches high prices in local and export markets. Korarima parts are used in traditional medicine for humans and cattle. Also korarima is important plant for soil conservation as the rhizomes and leaves spread on the ground covering and protecting the soil from erosion in hilly areas the year around (Eyob et al., 2008).

According to formal survey carried out in southern Ethiopia, recent attempts in Ethiopia to encourage farmers to cultivate the korarima plant have not been

successful due to several production constraints. Among all production constraints, farmers emphasized that lack of improved varieties with improved agronomic practices like propagation techniques had contributed to decrease in production (Eyob et al., 2009). The slow seed germination and growth of the subsequent seedlings were concerns of korarima growers. The germination of korarima seeds faces certain problems. There might be some kind of dormancy, possibly associated with the hard seed coat. Dormancy as a result of impermeable seed coat was reported from seeds of *Elettaria* species (Sulikeri and Kololgi, 1977). Low food reserve in the seed endosperm might be a reason for the very slow growth of the seedlings. Enhancement of korarima seed germination is important in propagation and breeding program as well as for testing and using germplasms. To make hard seed coats permeable to water or gases, some form of seed treatments are used in different plants (Bhattacharya and Khuspe, 2001; Polat, 1997; Taha, 1987). Although korarima is mainly propagated by vegetative method using one year old rhizomes, the need for bulk of rhizome as planting materials and slow multiplication rate of the rhizomes became another critical problem. Also the destructive harvestings of the rhizomes for vegetative propagation seems not to be feasible because there is always the possibility of losing the mother plant during this process. The cultivators could retain bulk of annual production for raising the following season crop and this requires much attention, space and transportation cost. Rhizomes are prone to damages due to different factors during transportation. Susceptibility to unknown diseases is some of the major problems faced by korarima growers presently in Ethiopia. Generally, these are some of critical problems for large scale production of the crop.

Micropropagation allows multiplication of plants with different chemical constituents (Echeverrigaray et al., 2003) and gave possibility of establishing a germplasm collection in *Cunila galioides* (Fracaro and Echeverrigaray, 2001). The efficiency of in vitro techniques is strongly genotype dependent, allowing the selection of cultivars with high performance (Pe´ros et al., 1998).

Despite the fact that korarima is a useful crop with a high potential as income source and other purposes, only limited efforts have been made to improve this crop using traditional and modern biotechnological approaches. To achieve such an improvement, proper agronomic and tissue culture procedures, which assure successful and efficient propagation, need to be developed. To date, only two tissue culture studies have been reported for korarima but have no reports on agronomic practices such as seed germination procedures. Tefera and Wannakrairoj (2004) investigated that addition of 5% coconut water to basal media and supplementation ofthe medium with 2 mg/l imazalil (IMA) in combination with 0.5 mg/l thidiazuron (TDZ) was effective in micropropagation of mid- altitude korarima cultivar Jimma Local growing in Western Ethiopia but *in vitro* studies have not been conducted on cultivars growing in southern high lands of Ethiopia. Considering inaccessibility of coconut water to korarima growers and negative residual effects of IMA, these studies focused on combined use of TDZ and 6-benzyladenine (BA) using high land cultivar Mume.

Therefore, the overall goal of the present study was to investigate the effects of different seed treatment methods, to evaluate *in vitro* performances of different cultivars, to determine various concentrations of hormones for shoot multiplication and rooting, and *in vitro* growth response of explant sources of korarima.

## MATERIALS AND METHODS

### Seed germination and seedling characteristics

#### Plant material

Mature fruits of the korarima cultivar Mume were collected in a private farm in the Chencha highland of southern Ethiopia and transported to the NORAD (Norwegian Agency for Development Cooperation) laboratory of Hawassa University.

#### Seed pretreatments

The seeds were extracted and immediately washed with tap water, divided into 7 groups (60 seeds each). Each group was divided into 3 replicates (20 seeds each) and subjected to one of the following treatments:

$T_0$: Control (no pretreatment)
$T_1$: Soaking in tap water for 24 h
$T_2$: Soaking in 50% sulfuric acid ($H_2SO_4$) for 60 min
$T_3$: Soaking in 50% sulfuric acid ($H_2SO_4$) for 60 min followed by soaking in 250 $mgL^{-1}$ gibberellic acid ($GA_3$) for 24 h
$T_4$: Cold stratification at 4±1°C for one week
$T_5$: Cold stratification at 4±1°C for two weeks and
$T_6$: Cold stratification at 4±1°C for three weeks.

For cold stratification the seeds were sown on a moist filter paper in Petri dishes.

#### Seed germination

After the pretreatments the seeds were sown in perforated plastic pots (0.22 cm) containing forest soil, sand and clay (1:1:2, v/v) and grown in the garden protected by plastic mesh. The pots were watered once a day. Two weeks after sowing, the germination percent was recorded for about six weeks. The number of days to 50% germination was recorded. Sixty days after sowing the seedlings were evaluated, and seedling length (cm), number of leaves and roots, root length (cm) and total fresh weight (g) were recorded.

### *In vitro* propagation

#### Plant material and disinfection

Three *in vitro* experiments were conducted. The first experiment was carried out to evaluate *in vitro* performances of three highland cultivars Mume, Wondogenet and Masketo using rhizome bud as explant sources and was terminated after five weeks. The second

**Table 1.** Effects of seed treatment on seed germination of korarima.

| Treatments | Days to first germination | Days to 50% germination | Germination % weeks from sowing | |
|---|---|---|---|---|
| | | | 5 | 6 |
| $T_0$ | 31.7 a | 41.3 a | 32.0f | 50.0e |
| $T_1$ | 26.3 b | 32.0 b | 50.0e | 51.7e |
| $T_2$ | 10.0 ef | 11.7 de | 81.7ab | 81.7ab |
| $T_3$ | 9.0 f | 9.3 e | 88.3a | 88.3a |
| $T_4$ | 12.0 e | 17.0 cd | 73.3bc | 76.7bc |
| $T_5$ | 15.0 d | 19.3 c | 66.7dc | 70.0dc |
| $T_6$ | 21.0 c | 29.7 b | 56.7de | 63.3d |
| LSD | 2.0 | 5.3 | 11.9 | 10.1 |
| SE | 0.7 | 1.9 | 4.2 | 3.6 |

LSD-least significant difference; SE-standard error.
Means values with the same letter in a column are not significantly different at $P < 0.05$ (Tukey's test).

experiment was carried out to study the effects of various concentrations of hormones on shoot multiplication and rooting of cultivar Mume. In the third experiment, rhizome buds and *in vitro* seedling shoot tips of Mume as explant sources were evaluated and the experiment was terminated after four weeks. Actively growing buds (2-5 mm) from the rhizomes keeping a little portion of the rhizome tissue were isolated and thoroughly washed under running tap water for 2 h, dipped in 70% ethanol for 30 s, and surface disinfected in 2.5% (W/V) sodium hypochlorite with 2 ml $L^{-1}$ Tween-80 for 15 min, followed by three rinses with sterile double distilled water for 3, 10 and 15 min. The seeds of cultivar Mume in the third experiment were disinfected as above. Ca 1-2 mm shoot tips were excised in a laminar flow hood and used for culture.

#### Medium and culture conditions

The basic nutrient medium (BM) consisted of MS (Murashige and Skoog, 1962) medium including vitamins and 2 ml $L^{-1}$ plant preservative mixture (ppm), 3% sucrose, and 0.7% agar. The pH was adjusted to 5.7 with 0.1 N KOH or 1 N HCl before autoclaved for 15 min at 121℃. The cultures were cultivated in a growth chamber at 25 ± 2℃ under 16 h cool-white fluorescent light (Philips TL33) at 40 $\mu molm^{-2}s^{-1}$.

#### Shoot proliferation

Cultures from second experiment were transferred to fresh medium after four weeks. The shoots were sub-cultured on BM with BA @ 3 mg $L^{-1}$ and kinetin @ 1 mg $L^{-1}$ for the first four weeks and then transferred to BM with no hormones or TDZ 0.25 mg $L^{-1}$or 0.5 mg $L^{-1}$ with or without BA 3 mg $L^{-1}$. Shoot number, shoot length (cm), leaf number, fresh weight (g) and dry weight (g) per explant were recorded after six weeks.

#### Rooting

Actively growing shoots 1-2 cm long grown on TDZ 0.5 mg $L^{-1}$ were used for rooting. The rooting medium consisted of BM with indolebutyric acid (IBA), 0 mg $L^{-1}$, 0.5 mg $L^{-1}$, 1 mg $L^{-1}$ or 1.5 mg $L^{-1}$. After six weeks percent rooting, root number and root length were recorded.

#### Statistical analysis

The data were statistically analyzed by one-way ANOVA using SAS 9.1 statistical soft ware and Tukey's test was applied at $\alpha = 0.05$ significance level. Correlation analyses on the growth characteristics were carried out.

## RESULTS

### Seed germination and seedling characteristics

The seed germination was significantly affected by the seed pre-treatments (Table 1). After 6 weeks of sowing, $T_3$ and $T_2$ significantly affected seed germination and resulted in higher germination percent than the other treatments and $T_0$. The highest germination percentage (88.3%) was obtained from $T_3$ in six weeks period after sowing. Also $T_2$ gave good germination (81.7%). Increasing the cold stratification period more than 1 week did not increase the germination percentage but increased the time to 50% germination. Both the number of days to first germinated seed and the time to 50% germination were highest in $T_0$ and lowest for treatment with $T_3$ or $T_2$. Seed germination was not observed in $T_3$ or $T_2$ treatments after five weeks, while it was not observed in any other treatments after six weeks.

Generally, $T_3$ and T2 significantly improved the seedling length, leaf number, roots number, root length and total fresh weight (Table 2). Increasing the cold stratification period more than 1 week remarkably reduced the seedling growth. $T_3$ significantly enhanced total fresh weight (2.86 g) compared to $T_0$ (0.99 g).

### *In vitro* propagation

After 5 weeks of culture incubation in the plant growth medium, the growth responses of the three korarima cultivars were different. The highest survival percent (55%) was achieved in the cultivar Mume (Figure 1a),

**Table 2.** Effects of seed treatment on subsequent seedling growth of korarima 60 days after sowing.

| Treatments | Seedling height (cm) | Leaf No | No of Roots | Root length (cm) | Fresh weight (g) |
|---|---|---|---|---|---|
| $T_0$ | 1.37 e | 1.11 c | 2.56 b | 1.03 e | 0.99 e |
| $T_1$ | 1.73 de | 1.67 c | 3.11 b | 1.43 de | 1.13 ed |
| $T_2$ | 4.27 b | 3.67 a | 5.44 a | 2.27 bc | 1.97 b |
| $T_3$ | 5.10 a | 3.78 a | 5.99 a | 2.97 a | 2.86 a |
| $T_4$ | 3.13 c | 2.44 b | 3.78 b | 2.77 b | 1.72 bc |
| $T_5$ | 2.37 d | 1.78 bc | 3.22 b | 2.10 bcd | 1.50 cd |
| $T_6$ | 1.90 de | 1.44 c | 2.78 b | 1.70 dce | 1.30 ed |
| LSD | 0.69 | 0.68 | 1.54 | 0.69 | 0.39 |
| SE | 0.25 | 0.24 | 0.55 | 0.25 | 0.14 |

LSD-least significant difference; SE-standard error.
Means values with the same letter in a column are not significantly different at P<0.05 (Tukey's test).

followed by Wondogenet (45%) and Mesketo (30%). Mume, Wondogenet and Mesketo gave visible green buds after 16.6, 27 and 32.7 days of culture, respectively (Figure 1b). The highest mean shoot number (4.18) was obtained in the cultivar Mume (Figure 1c).

In the second experiment, rhizome bud explants were cultured on MS solid medium supplemented with varying levels of TDZ alone or in combination with BA. Visible green buds were observed after two weeks of culture. MS medium with different concentrations of TDZ alone or in combination with BA resulted in varying degree of multiple shoots (Table 3). Proliferation and elongation of the shoots obtained in 0.5 mg /l TDZ seems to be better when compared with the control and other treatments. The maximum number of shoots, leaf number and fresh weight per explant were obtained when grown on medium containing 0.5 mg $L^{-1}$ TDZ, with an average of 11 shoots per explant, compared to those exposed to other treatments. Supplementation of 0.5 mg $L^{-1}$ TDZ with 3 mg $L^{-1}$ BA also increased shoots number (10) per explant but the shoots were short. The longest shoots were obtained in the control (3.73 cm), and followed by 3 mg $L^{-1}$ BA (3.07 cm). The highest and significantly different dry weight was recorded in 0.25 mg $L^{-1}$ TDZ and the lowest was in the control (0.037 g), and 0.25 mg $L^{-1}$ TDZ + 3 mg$L^{-1}$ BA (0.036 g). Days to shoot formation were shorter in all treatments than in the control. The effect of 3 mg $L^{-1}$ BA + 1 mg$L^{-1}$ kinetin with respect to shoot number (7.67) was significantly higher than the control (2.67) but its effect was significantly less than 0.5 mg $L^{-1}$ TDZ (11 shoots) and 0.5 mg $L^{-1}$ + 3 mg$L^{-1}$ BA (10 shoots). Correlation analysis on the growth parameters revealed that there were significant relationships at 5% probability level but the relationship of dry weight with other characteristics were not significant (Table 4). Positive and higher significant correlation (r =0.893) was obtained from shoot number and leaf number, and followed by leaf number and fresh weight (r = 0.816).

In the third experiment, the highest percent survival and shoot number per explant were obtained from *in vitro* grown seedling shoot tips as compared to rhizome buds of field grown cultivar Mume after four weeks of culture (data not shown).

Excised shoots on MS medium supplemented with 0.5 mg $L^{-1}$ TDZ were transferred to rooting media. Rooting was formed by all applied treatments (Table 5). In the absence of any growth regulator, relatively fewer roots were formed. The optimal medium for rooting contained 1 mg $L^{-1}$IBA. A further increase of IBA and below this concentration decreased the rooting percentage, root number and length. Generally, better rooting was observed in all the IBA supplements when compared with the control (Figure 2).

## DISCUSSION

### Seed germination and seedling characteristics

The results of this study show that korarima seeds display dormancy that can be released by seed pretreatment for a certain period. Both $GA_3$ and $H_2SO_4$ pretreatments were more effective in breaking dormancy and stimulating germination of korarima seed. There was about a 3 fold reduction in days to 50% germinated seeds by both pretreatments. El-Dengawy (2005) found that $GA_3$ treated loquat seeds gave significantly higher germination percentage than the control when recorded after eight weeks of sowing. It has been observed in conifer species seed that $GA_3$ treatment caused an appreciable shortening of the germination period by 10 days than control (Rawat et al., 2006). The superiority of the sulfuric acid treated seeds compared with the other tested treatments on germination percentage was reported from tamarind seeds by Muhammad and Amusa (2003). On the other studies concentrated sulfuric acid treatments were more effective than hot water treatments in breaking the dormancy of *Calligonum benghalensis* aerial seeds

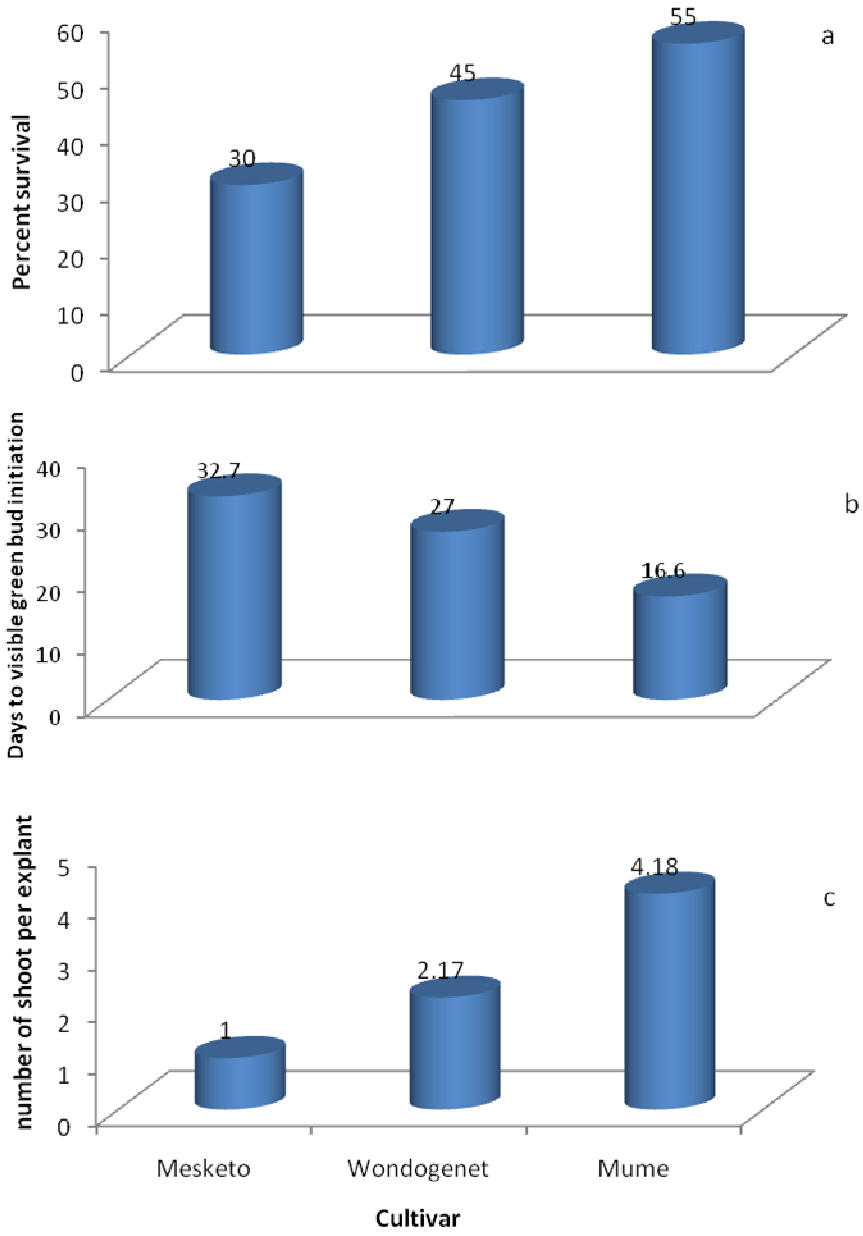


**Figure 1.** Effect of korarima cultivars on percent survival, days to visible green bud initiation and number of shoots per explants after five weeks of culture on BM without any hormones.

(Kim et al., 1990). Ren and Tao (2004) reported that sulfuric acid and cold stratification treatments significantly promoted overall germination in *Calligonum* species. Sulfuric acid scarification reduced mean germination times in the forage grass species (Usberti and Martins, 2007). The effect of sulfuric acid on promotion of seed germination might be due to the highly desiccant effect of the acid on the seed coat there by allowing easier water uptake and oxygen diffusion. The inhibitory effects on germination and growth of seedling due to high constituents of hydrocarbon monoterpenes were investigated in different plants (Kordali et al., 2007). Eyob et al. (2007) reported the higher contents of monoterpene from korarima seed. This might be another reason for poor germination and seedling growth in korarima. From the present results it can be concluded that soaking of korarima seeds in $T_3$ and $T_2$ may be recommended to promote the germination process and enhance growth characteristics of the seedlings of korarima.

### *In vitro* propagation

Korarima cultivars exhibited different level of *in vitro*

**Table 3.** Effect of hormone concentrations on the growth of the korarima cultivar Mume after six weeks of culture.

| BA (mg $L^{-1}$) | TDZ (mg $L^{-1}$) | Kinetin (mg $L^{-1}$) | Shoot No. explant $^{-1}$ | Shoot length (cm) explant $^{-1}$ | Leaf No. explant $^{-1}$ | Fresh weight (g) explant $^{-1}$ | Dry weight (g) explant $^{-1}$ |
|---|---|---|---|---|---|---|---|
| 0 | 0 | o | 2.67 d | 3.73 a | 6.00 c | 0.25 d | 0.037 c |
| 3 | 0 | 1 | 7.67 c | 2.10 c | 11.67 b | 0.49 bc | 0.056 bc |
| 3 | 0 | 0 | 4.67 d | 3.07 ab | 10.67 b | 0.34 cd | 0.070 ab |
| 3 | 0.25 | 0 | 8.00 bc | 1.43 dc | 11.00 b | 0.43 bcd | 0.036 c |
| 3 | 0.5 | 0 | 10.00 ab | 1.10 d | 12.33 b | 0.49 bc | 0.053 bc |
| 0 | 0.25 | 0 | 9.67 abc | 2.20 bc | 13.00 ab | 0.62 ab | 0.081 a |
| 0 | 0.5 | 0 | 11.00 a | 1.17 d | 15.33 a | 0.81 a | 0.061 ab |
| LSD | | | 2.19 | 0.91 | 2.65 | 0.19 | 0.02 |
| SE | | | 0.79 | 0.33 | 0.95 | 0.07 | 0.01 |

LSD-least significant difference; SE-standard error.
Means values with the same letter in a column are not significantly different at P<0.05 (Tukey's test).

**Table 4.** Correlation coefficients (r) among growth parameters.

| Growth characteristic | Shoot number | Shoot length (cm) | Leaf number | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|---|
| Shoot number | 1.000 | | | | |
| Shoot length (cm) | -0.876* | 1.000 | | | |
| Leaf number | 0.893* | -0.754* | 1.000 | | |
| Fresh weight (g) | 0.797* | -0.664* | 0.816* | 1.000 | |
| Dry weight (g) | 0.287 | 0.032 | 0.502* | 0.443* | 1.000 |

*Significantly different at P < 0.05.

**Table 5.** Effects of IBA on rooting percent, root number and root length of the korarima cultivar 'Mume' after six weeks of culture.

| IBA (mg $L^{-1}$) | Rooting (%) | No. of roots | Root length (cm) |
|---|---|---|---|
| 0 | 33.33 b | 3.33 b | 0.15 b |
| 0.5 | 55.56 ab | 9.33 ab | 0.18 b |
| 1 | 75.56 a | 13.67 a | 0.72 a |
| 1.5 | 44.44 ab | 8.00 ab | 0.24 b |
| LSD | 36.98 | 7.04 | 0.27 |
| SE | 14.15 | 2.69 | 0.10 |

LSD-Least significant difference; SE-standard error.
Means values with the same letter in a column are not significantly different at P<0.05 (Tukey's test).

(1998) in Vitis vinifera cultivates. The best response for the cultivar Mume was achieved from TDZ followed by BA. In the other study Chand et al (1999) found more vigorous growth in taro explants obtained from culture medium supplemented with TDZ at 0.6 mg $L^{-1}$ than BA, even at concentrations above 3.0 mg $L^{-1}$. The highest shoot multiplication from *Nothapodytes foetida* w responces in the similar way as the findings of Pe´ros et al. (1998) concentration of 2.2 µM as compared to that of BA at similar concentrations or more (Rai, 2002), which is in conformity with our present finding. In the present study shoot number showed increasing trend with the increased use of TDZ concentration in the culture medium. However, shoot length showed an opposite trend corresponding to report of Tefera and Wannakrairoj (2006) in the med-altitude growing korarima cultivar Jimma local. The same authors reported enhanced effects obtained by combined use of TDZ and BA on shoot proliferation of cultivar Jimma local. BA increased shoot multiplication in ginger (Nasirujjaman et al., 2005; Balachandran et al., 1990; Inden et al., 1988). The positive and significant correlations obtained from growth characteristics in this

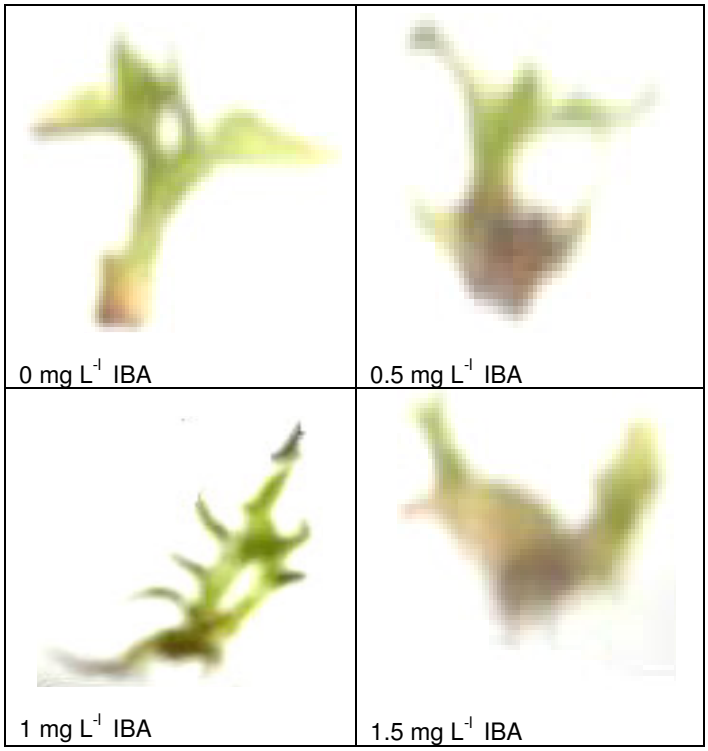


**Figure 2.** Rooting of shoots in various concentration of IBA after four weeks of culture.

study indicated that application of TDZ alone or in combination with BA might be beneficial to enhance multiplication of shoot and consequently for increase in other growth parameters.

The results confirmed that different explant sources exhibited different level of shoot formation. This finding confirms investigation of Yildiz et al. (2002) stating that *in vitro* grown seedlings were found to be more suitable than greenhouse grown plants as an explant sources in Flax (*Linum usitatissimum* L.). The efficient shoot formation of korarima achieved from *in vitro* germinated seedling shoot tips in this study will be useful for mass propagation where rhizome buds are not available for planting.

The results from present study on the rooting of korarima agrees with finding of Ali et al. (2004) that 100% rooting was achieved in turmeric by transferring an individual shoot to MS medium containing auxin. The rooting was found to be best in MS medium plus 0.5 to 2.4 µM of indolebutyric acid in *Cunila galioides* (Fracaro and Echeverrigaray, 2001).

## Conclusion

Treatment of korarima seeds with $T_3$ or $T_2$ may be recommended to promote the germination process and improve growth characteristics of the subsequent seedlings. It was apparent that responses of cultivars had significant impact on regeneration of explants and shoot formation. It is also possible to induce multiple shoot growth and complete plant development in highland korarima cultivar Mume by using different concentrations of TDZ alone or in combination with BA. Shoot tips obtained from *in vitro* seedlings showed better performance with respect to percent survival and shoot induction than rhizome buds obtained from crop grown under field condition. IBA at 1 mg $L^{-1}$ was best to induce rooting from *in vitro* shoots of korarima cultivar Mume.

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## REFERENCES

Ali A, Munawar A, Siddiqui FA (2004). *In vitro* propagation of turmeric, *Curcuma longa* L. Int. J. Biol. Biotechnol. 1: 511-518.

Balachandran SM, Bhat SR, Chandel KPS (1990). *In vitro* clonal multiplication of turmeric *(Curcuma Spp.)* and ginger (*Zingiber*

*officinale* Rosc.). Plant Cell Rep. 8: 521-529.
Bhattacharya J, Khuspe SS (2001). *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. Sci. Hort. 91: 39-49.
Chand H, Pearson MN, Lovell PH (1999). Rapid vegetative multiplication in *Clocasia esclulenta* (L) Schott (taro). Plant Cell Tiss. Org. Cult. 55: 223-226.
Echeverrigaray S, Fracaro F, Santos ACA, Paroul N, Wasum R, Atti-Serafini L (2003). Essential oil composition of south Brazilian populations of *Cunila galioides* and its relation with the geographical distribution. Biochem. Syst. Ecol. 31: 467-475.
El-Dengawy EFA (2005). Promotion of seed germination and subsequent seedling growth of loquat (*Eriobotrya japonica*, Lindl) by moist-chilling and $GA_3$ applications. Sci. Hort. 105: 331-342.
Eyob S, Tsegaye A, Appelgren A (2009). Analysis of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) indigenous production practices and farm based biodiversity in southern Ethiopia. Genet. Resour. Crop Evol. 56: 573-585.
Eyob S, Appelgren M, Rohloff J, Tsegaye A, Messele G (2008). Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen*)* from southern Ethiopia. S. Afr. J. Bot. 74: 181-185.
Eyob S, Appelgren M, Rohloff J, Tsegaye A, Messele G (2007). Chemical composition and physical properties of essential oils from fresh plant parts of korarima (*Aframomum corrorima*) cultivated in the highland of Southern Ethiopia. J. Essent. Oil Res. 19: 372-375.
Fracaro F, Echeverrigaray S (2001). Micropropagation of *Cunila galioides*, a popular. medicinal plant of south Brazil. Plant Cell, Tiss. Organ Cult. 64: 1-4.
Inden H, Hirano A, Asahira T (1988). Micropropagation of ginger. Acta Hortic. 230: 177-184.
Kim SY, DeDatta SK, Mercado BL (1990). The effect of chemical and heat treatments on germination of *Commelina benghalensis* L. aerial seeds. Weed Res. 30: 109-116.
Kordali S, Cakir A, Sutay S (2007). Inhibitory Effects of Monoterpenes on Seed Germination and Seedling Growth. Z. Naturforsch. 62: 207-214.
Muhammad S, Amusa NA (2003). Effects of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L). Afr. J. Biotechnol. 2: 276-279.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.
Nasirujjaman K, Uddin MS, Zaman S, Reza MA (2005). Micropropagation of turmeric (*Curcuma longa* Linn.) through *in vitro* rhizome bud culture. J. Biol. Sci. 5: 490-492.
Pe´ros JP, Torregrosa L, Berger G (1998). Variability among *Vitis vinifera* cultivars in micropropagation, organogenesis and antibiotic sensitivity. J. Exp. Bot. 49: 171-179.
Polat AA (1997). Determination of germination rate coefficients of loquat seeds and their embryos stratified in various media for different durations. Turk. J. Agric. For. 21: 219-224.
Rai VR (2002). Rapid clonal propagation of *Nothapodytes foetida* (Wight) sleumer-a threatened medicinal tree. *In vitro* Cell Dev. Biol. Plant 38:347-351.
Rawat BS, Sharma CM, Ghildiyal SK (2006). Improvement of seed germination in three important conifer species by Gibberellic acid (GA3). Lyonia 11: 23-30.
Ren J, Tao L (2004). Effects of different pre-sowing seed treatments on germination of 10 *Calligonum* species. Forest Ecol. Manage. 195: 291-300.
Sulikeri GS, Kololgi SD (1977). Seed viability in Cardamom (*Elettaria cardamomum* Maton). Curr. Res. 6: 163-164.
Taha FA (1987). Effect of plant growth regulators on seed germination and seedling characters of persimmon root-stock (*Diospyrus kaki* L.). Egypt. J. Hort. 14: 15-20.
Tefera W, Wannakrairoj S (2004). Micropropagation of korarima (*Aframomium corrorima* (Braun) Jansen). Sci. Asia 30: 1-7.
Tefera W, Wannakrairoj S (2006). Synergistic effects of some plant growth regulators on *in vitro* shoot proliferation of korarima (*Aframomum corrorima* (Braun) Jansen). Afr. J. Biotechnol. 5: 1894-1901.
Usberti R, Martins L (2007). Sulphuric acid scarification effects on *Brachiaria brizantha, B. humidicola* and *Panicum maximum* seed dormancy release. Rev. Bras. Sementes 29: 143-147.
Yildiz M, Ozcan S, Celal ER (2002). The Effect of Different Explant Sources on Adventitious Shoot Regeneration in Flax (*Linum usitatissimum* L.). Turk. J. Biol. 26: 37-40.