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Effect of Different Concentrations and Combinations of BAP, KN, and IBA on *In Vitro* Regeneration of *Cissus discolor* Blume and *Cissus repens* Lam. (Ta-Bin-Taing-Mya-Nan)

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Abstract

Cissus is also known as Ta-Bin-Taing-Mya-Nan in Myanmar, is a climbing vine used for medicinal purposes. In vitro micropropagation has been widely applied for the rapid production of many plant species, particularly medicinal plants, to generate high-quality planting material through tissue culture. In this study, stem segments from in vitro-germinated plantlets of two different *Cissus* species were cultured on Murashige and Skoog (MS) basal medium supplemented with varying concentrations and combinations of plant growth regulators: BAP (0.05, 0.1, 0.15, 0.2 mg/L), Kn (0.25 mg/L), and IBA (0.125, 0.25, 0.375, 0.5 mg/L). The results showed that, in the fourth data collection, the highest average shoot number per explant for *Cissus* discolor Blume was observed at 0.05 mg/L BAP, 0.25 mg/L Kn and 0.125 mg/L IBA while the lowest was recorded at 0.15 mg/L BAP, 0.25 mg/L Kn and 0.375 mg/L IBA For *Cissus* repens Lam. The highest shoot average per explant was achieved with 0.1 mg/L BAP, 0.25 mg/L Kn and 0.25 mg/L IBA whereas the control treatment yielded the lowest results. In root induction experiments, 0.05 mg/L BAP, 0.25 mg/L Kn and 0.125 mg/L IBA was most effective for *Cissus* discolor Blume, whereas 0.1 mg/L BAP, 0.25 mg/L Kn and 0.25 mg/L IBA performed best for *Cissus* repens Lam. The lowest root induction in *Cissus* discolor Blume was observed in the control treatment, while for *Cissus* repens Lam. the lowest response occurred at 0.2 mg/L BAP, 0.25 mg/L Kn and 0.5 mg/L IBA.

Keywords: *Cissus*, Ta-Bin-Taing-Mya-Nan, BAP, Kn, IBA, Shoot, Multiplication, Micropropagation.

INTRODUCTION

Cissus, Ta-Bin-Taing-Mya-Nan is a major source of medicinal plants. Recently there has been a tremendous increase in the use of plant-based health products in developed countries resulting in an exponential growth of herbal products globally [1]. According to WHO more than 80% of the world's population relies on traditional herbal medicine for their primary health care. Cissus spp. are dispersed throughout the tropical and subtropical regions in Central and South America, Asia, and Africa, with over 2,000 species recorded [2]. *Cissus discolor* Blume and *Cissus repens* Lam., are in a genus in the flowering plants family Vitaceae which is one of the ten largest angiosperm genera, are ornamental and medicinally valuable plant species widely distributed in tropical and subtropical regions [3]. *Cissus* spp. are attractive perennial herbs with soft, succulent stems and white, pink, red, orange or yellow flowers [4]. This family consists of 5 genera and 920 true species, majority of which belong to the genus *Cissus* [5]. This is a large genus and countless hybrids have been introduced which largely fall into 8 distinct groups: cane-like, rex-cultorum, rhizomatous, semperflorens, shrub-like, thick-stemmed, trailing or scandent and tuberous [6].

Traditional methods of propagation are often limited by seasonal dependency, low multiplication rates and susceptibility to diseases [7]. *In vitro* micropropagation has been extensively applied for the rapid production of many plant species and cultivars especially medicinal plants [8]. Micropropagation studies often involve examining a wide range of treatments, plant growth regulators (PGRs), and dosages to develop an effective propagation protocol [9]. The success of the micropropagation method depends on several factors like genotype, media, plant growth regulators (PGRs) and type of explants [10]. Using stems, petioles, or other explants and Benzyladenine (BA), 6-Benzylaminopurine(BAP), Kinetin (Kn), 1-Naphthaleneacetic Acid (NAA), 2,4-Dichlorophenoxyacetic Acid (2,4-D), and Indole-3-butyric acid (IBA) as plant growth regulators (PGRs), *Cissus* spp. can be micropropagated using a tissue culture approach. The technique used leaves as explants and varying concentrations of 6-Benzylaminopurine

(BAP) and 1-Naphthaleneacetic Acid (NAA) as plant growth regulators (PGRs) [11]. Through commercial cultivation, more than 200 species have been introduced. Generally, *Cissus* spp. were propagated by stem and leaf cuttings [12]. The propagation of *Cissus* could be done with a stem, shoot and leaf cuttings and plant tissue culturing, which is a technical method to produce a large number of plants in a short time [13]. Micronutrients of Heller medium and macronutrients of Murashige and Skoog medium (MS) supplemented with 6-Benzylaminopurine (BAP) and Kinetin (Kn) induced shoots in *Cissus* spp. whilst supplementing with indole-3-butanoic acid (IBA) or without any growth regulator supplement, induced roots [14].

Tissue culturing of Cissus spp. leaf parts can promote 90% of shoots with a maximum number of 132 shoots per piece, in MS medium with 6-Benzylaminopurine (BAP) and Kinetin (Kn) induced [15]. The petiole provided 82% of shoots, with a maximum number of 33 shoots per piece in MS medium with 6-Benzylaminopurine (BAP) and Kinetin (Kn) induced [16]. For root induction, MS medium with indole-3-butanoic acid (IBA) can induce roots within 3 months. The maximum number of shoots (44.33 shoots per piece) of Begonia rex Putz. was induced by MS medium with 6-Benzylaminopurine (BAP) and Kinetin (Kn). Also, MS medium with 6-Benzylaminopurine (BAP) and Kinetin (Kn) induced 20.8 shoots in *Cissus* spp. with the highest average shoot length of 4.0 cm [17]. For root induction, it was found that MS with indole-3-butanoic acid (IBA) produced root growth [18]. Shoots can be induced to root and can be transplanted in conditions of high humidity systems with 100% of the organisms surviving [19]. The success of tissue culture is influenced by several factors, such as medium components, light, temperature and suitable environments [20] Micropropagation has become an indispensable tool in modern agriculture, serving as the primary means of generating true-to-type plants on a commercial scale [21]. This technique provides a highly efficient alternative to traditional propagation methods. It is based on the culture of plant explants such as somatic cells, tissues, or organs in a sterile, artificial environment [22] By carefully controlling nutrients, hormones, and other conditions, micropropagation allows for the reliable and rapid production of a vast number of genetically identical plantlets (clones) derived from a single superior stock plant [23,24]. To produce *Cissus* spp. as a commercial ornamental plant in the future, tissue culture of *Cissus* spp. need to investigate the suitable concentration of growth hormones for shoots and roots induction [25].

The research objective was to investigate the effect of 6-Benzylaminopurine (BAP), Kinetin (Kn) and indole-3-butanoic acid (IBA) at different concentrations for asexual propagation of *Cissus* spp. by the plant tissue culture technology [26].

Scientific Classification of *Cissus discolor* Blume and *Cissus repens* Lam.

Kingdom: *Plantae*
Clade: *Tracheophytes*
Clade: *Angiosperms*
Clade: *Eudicots*
Clade: *Rosids*
Order: *Vitales*
Family: *Vitaceae*
Genus: *Cissus*
Species: *C. discolor*
C. repens

MATERIALS AND METHODS

Plant Materials and Source of Explants

Stems of two *Cissus* varieties were used as the source of explants for *in vitro* micropropagation. Stem segments (1.5–2 cm in length) were excised from cultured plantlets under the laminar flow cabinet in the Plant Tissue Culture Laboratory of the Department of Biotechnology Research, Kyaukse, in August 2024. Aseptic cultures of Ta-Bin-Taing-Mya-Nan varieties were subsequently established via *in vitro* plantlet regeneration.

Preparation of Culture Media

The Murashige & Skoog (MS) medium was used as basal media. It was added with 30 g/L of glucose and 6 g/L of agar. Then hormones (BAP and Kn) at different concentration and combination levels, i.e. BAP (0.05, 0.1, 0.15, 0.2 mg/L), and Kn (0.25 mg/L) were added to the medium for shoot induction. And then for root induction IBA (0.125, 0.25, 0.375, 0.5 mg/L) were added to the medium. The pH of the medium was regulated with HCl or Na OH until it reached 5.8 culture medium and then was poured into sterile culture bottles 20ml of media each and autoclave at 121 °C for 20 min at 15 psi subsequently they were kept to cool before the explant inoculation.

Cultural Environment and Culture Condition

All cultures were maintained under continuous cool white fluorescent tube light (1500 Lux) at a temperature of 27±1 °C, controlled by an air conditioning system. Culture observations were recorded throughout the shoot proliferation trials, which continued as the cultures were subcultured every four weeks onto fresh medium. Micropopagation efficiency was evaluated after the 4th subculture, while rooting studies were assessed at the end of 4 months.

Acclimatization of the Tissue Culture Plantlets

After four months of incubation in the culture room, the plantlets were removed from their bottles, and the agar was gently washed from their roots. For *ex vitro* acclimatization, they were transplanted into a seed bed containing a mixture of sand, biofertilizer, and soil in a 1:1:2 ratio. The seed bed was housed in a clear polyhouse. After about ten days, the plantlets were transferred to small polybags within a net house and frequently watered to maintain high humidity. Three weeks later, they were moved to large polybags, which were also kept in a net house. After two months, they were fully exposed to external environmental conditions and watered once a day to support hydration and growth.

Data Analysis and Evaluation of *In Vitro* Regeneration

The effects of different concentrations and combinations of BAP, Kn, and IBA on the *in vitro* regeneration of *Cissus discolor* Blume and *Cissus repens* Lam. were analyzed using appropriate statistical tools (e.g., ANOVA). In shoot development studies, the number of shoots, the number of leaves and shoot length (cm), were evaluated. The number of shoots per plant determines the overall growth and density of the vegetation [27] Shoot length varies depending on environmental factors and genetics of the plants [28].

Additionally, a higher number of leaves generally indicates healthy photosynthetic activity in plants [29,30]. However, leaf number can be influenced by nutrient availability and plant species. Similarly, the number of roots is directly proportional to the plants ability to absorb water and nutrients [31]. While root length plays a critical factor for anchoring the plant and accessing deeper nutrients [32].

RESULTS AND DISCUSSIONS

The experiment examined shoot and root formation, and full plantlet regeneration in mature *Cissus* spp. plants using stem segments. MS media supplement with different concentrations and combinations of BAP, Kn, and IBA were employed under *in vitro* laboratory conditions. After approximately four weeks of culture, shoots initiated from the explants across all tested concentrations of BAP, Kn, and IBA.

The study revealed during the fourth data collection that the highest average shoot per explant was 12.33 ± 4.93 for *C. discolor* Blume was achieved using the MS medium supplement with formulation of 0.05 mg/L BAP, 0.25 mg/L Kn, and 0.125 mg/L IBA. For *C. repens* Lam., the highest shoot average (22.33 ± 8.50) was obtained with MS medium containing 0.1 mg/L BAP, 0.25 mg/L Kn, and 0.25 mg/L IBA**.** In contrast, the lowest shoot average for *C. discolor* Blume (8.000 ± 1.000) was recorded in Treatment 4 (T4)**,** while for *C. repens* Lam., the lowest shoot

count occurred in the absence of BAP, Kn, and IBA (Control) with value of (11.667 ± 1.528) (Table 1).

The shoot development in *Cissus spp.* is enhanced when cultured on MS media supplemented with plant growth regulators (PGRs), such as 6-Benzlaminopurine (BAP), kinetin (Kn), and indole-3-butanoic acid, (IBA). Their study showed that MS medium containing 0.05 mg/L BAP, 0.25 mg/L Kn, and 0.125 mg/L IBA) resulted in the highest shoot multiplication rate in *C. discolor* Blume. (Figure 1) Shoot proliferation occurred when explants were cultivated on multiplication media with varying concentrations and combinations of PGRs. (Figure 2)

Both *C. discolor* Blume and *C. repens* Lam. exhibited their highest average shoot lengths when treated with the same hormone concentrations (0.05 mg/L BAP, 0.25 mg/L Kn, and 0.125 mg/L IBA) as shown in Table 2.

Specifically, *C. discolor* Blume reached an average shoot length of 11.47 ± 6.05 cm, while *C. repens* Lam. showed a significantly greater average length of 31.5 ± 18.1 cm under these conditions. (Figure 3) In contrast, the lowest shoot lengths were recorded in *C.discolor* Blume **(**5.710 ± 0.785 cm**)** at Treatment 3 (T3) and in *C. repens* Lam. **(**6.78 ± 3.29 cm**)** at Treatment 4 (T4)**,** indicating that varying hormone levels significantly influenced shoot elongation in both species. (Figure 4)

In this study, *C. discolor Blume* exhibited the highest average number of leaves (11.47 ± 6.05) in the treatment containing BAP 0.05 mg/L + Kn 0.25 mg/L + IBA 0.125 mg/L as shown in Table 3.

In contrast, *C. repens* Lam. reached its maximum leaf count (29.67 ± 13.20) in Treatment 3 (T3). (Figure 5) On the other hand, the lowest leaf production for *C. discolor* Blume (10.67 ± 1.53) was observed in Treatment 4 (T4), while *C. repens* Lam. showed minimal leaf growth (9.67 ± 1.53) in the control group, as found in (Figure 6).

As illustrated in Table 4 (representing the fourth data collection period), the highest root development was observed in *C. discolor* Blume under Treatment 2 (T2), with an average measurement of 19.67 ± 7.37**,** and in *C. repens* Lam. under Treatment 3 (T3), which recorded an average of 37.7 ± 19.1 as found in (Figure 7). Conversely, the lowest root development was noted in *C. discolor* Blume within the control group, averaging 12.00 ± 3.00**,** while *C. repens* Lam. exhibited its poorest root growth under Treatment 5 (T5), with a mean value of 8.33 ± 5.03**.** These findings could be seen in (Figure 8), highlighted significant variations in root development across different treatments and species, suggesting that certain conditions may either promote or inhibit root growth.

Rooted tissue cultured *Cissus* seedlings were transferred to the hardening bed consisting of sand, biofertilizer, and soil in a 1:1:2 ratio. In this hardening substrate, the plantlets grew robustly, had broad leaves, and exhibited the best growth (Figure 9). Therefore, the composition of sand: biofertilizer: soil (1:1:2) was considered as the optimal cultivation medium for hardening and transplanting of *Cissus* tissue culture seedlings.

Table 1: Effect of Different Hormone Concentrations and Combinations on Number of Shoots in Two *Cissus* spp

Treatments	Hormone Combinations (mg/l)	Number of Shoots	
		***Cissus discolor* Blume**	***Cissus repens* Lam.**
T1	Control	10.333 ± 1.528	11.667 ± 1.528
T2	BAP 0.05+Kn 0.25+IBA 0.125	12.33 ± 4.93	20.33 ± 4.04
T3	BAP 0.1+Kn 0.25+IBA 0.25	10.667 ± 0.577	22.33 ± 8.50
T4	BAP 0.15+Kn 0.25+IBA 0.375	8.000 ± 1.000	12.00 ± 3.00
T5	BAP 0.2+Kn 0.25+IBA 0.5	10.33 ± 3.21	13.333 ± 1.528

Data presented are means ± standard deviation

Table 2: Effect of Different Hormone Concentrations and Combinations on Shoot Length in Two *Cissus* spp

Treatments	Hormone Combinations (mg/l)	Shoot Length (cm)	
		***Cissus discolor* Blume**	***Cissus repens* Lam.**
T1	Control	6.58 ± 2.37	7.21 ± 1.83
T2	BAP 0.05+Kn 0.25+IBA 0.125	11.47 ± 6.05	31.5 ± 18.1
T3	BAP 0.1+Kn 0.25+IBA 0.25	5.710 ± 0.785	30.49 ± 14.81
T4	BAP 0.15+Kn 0.25+IBA 0.375	6.157 ± 0.717	6.78 ± 3.29
T5	BAP 0.2+Kn 0.25+IBA 0.5	6.920 ± 0.862	8.46 ± 2.99

Data presented are means ± standard deviation

Table 3: Effect of Different Hormone Concentrations and Combinations on Number of Leaves in Two *Cissus* spp

Treatments	Hormone Combinations (mg/l)	Number of Leaves	
		***Cissus discolor* Blume**	***Cissus repens* Lam.**
T1	Control	13.333 ± 1.528	9.667 ± 1.528
T2	BAP 0.05+Kn 0.25+IBA 0.125	11.47 ± 6.05	24.00 ± 8.72
T3	BAP 0.1+Kn 0.25+IBA 0.25	13.67 ± 2.52	29.67 ± 13.20
T4	BAP 0.15+Kn 0.25+IBA 0.375	10.667 ± 1.528	10.67 ± 3.79
T5	BAP 0.2+Kn 0.25+IBA 0.5	15.00 ± 5.29	15.67 ± 3.79

Data presented are means ± standard deviation

Table 4: Effect of Different Hormone Concentrations and Combinations on Root Development of Two *Cissus* spp.

Treatments	Hormone Combinations (mg/l)	Root Development	
		***Cissus discolor* Blume**	***Cissus repens* Lam.**
T1	Control	12.00 ± 3.00	10.33 ± 4.51
T2	BAP 0.05+Kn 0.25+IBA 0.125	19.67 ± 7.37	19.00 ± 10.82
T3	BAP 0.1+Kn 0.25+IBA 0.25	13.67 ± 3.06	37.7 ± 19.1
T4	BAP 0.15+Kn 0.25+IBA 0.375	15.33 ± 5.13	10.67 ± 3.79
T5	BAP 0.2+Kn 0.25+IBA 0.5	15.00 ± 5.57	8.33 ± 5.03

Data presented are means ± standard deviation

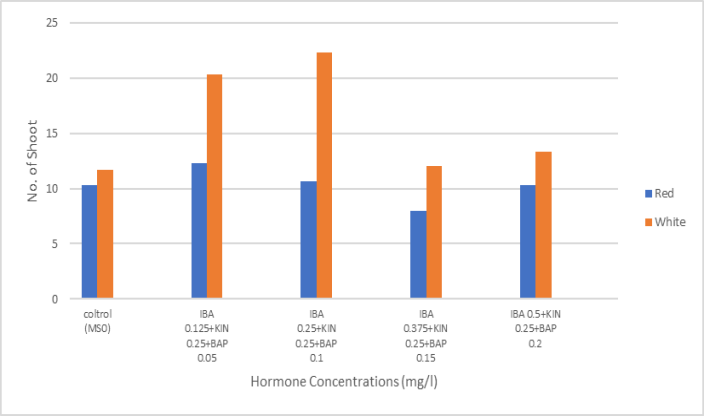

Figure 1: Comparison of the effect of Different Hormone Concentrations and Combinations on Number of Shoots in Two *Cissus* spp

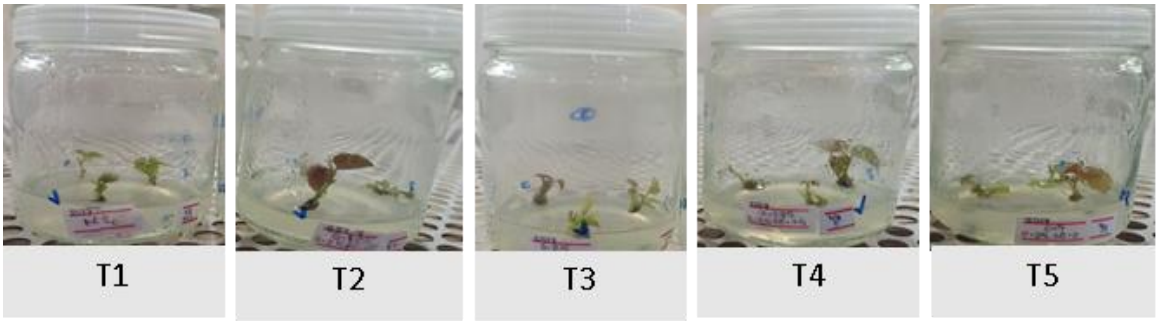

Figure 2: Shoot multiplication on MS media supplemented with BAP, Kn and IBA of *Cissus discolor* Blume after one month of culture

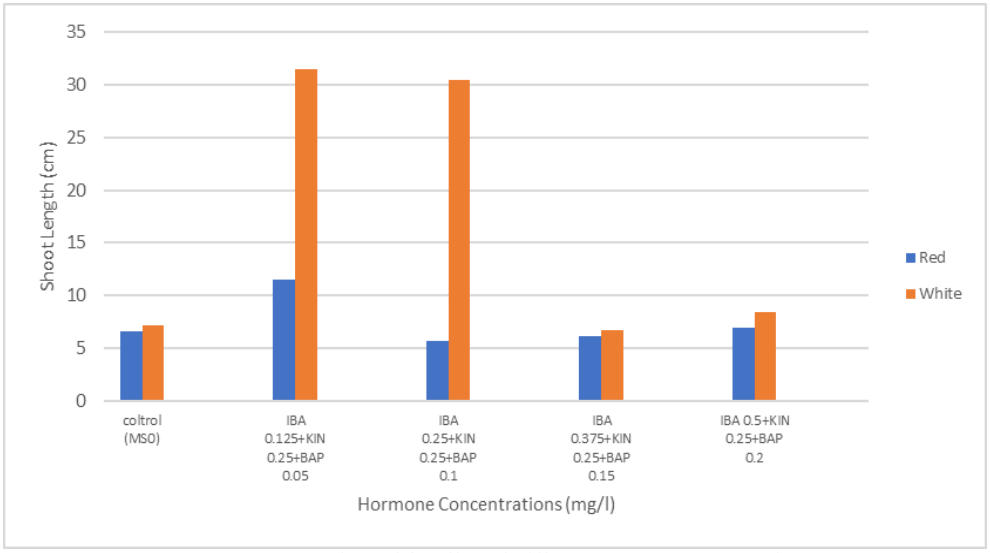

Figure 3: Comparison of the effect of Different Hormone Concentrations and Combinations on Shoots Length in Two *Cissus* spp

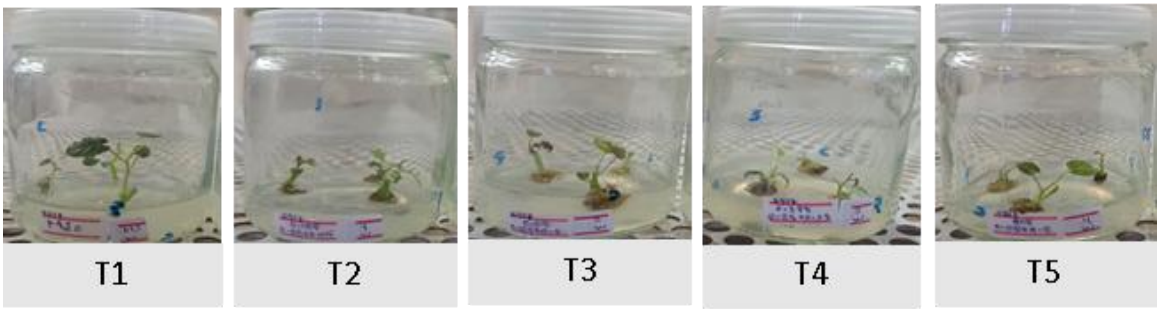

Figure 4: Shoot multiplication on MS media supplemented with BAP Kin and IBA of *Cissus repens* Lam. after one month of culture

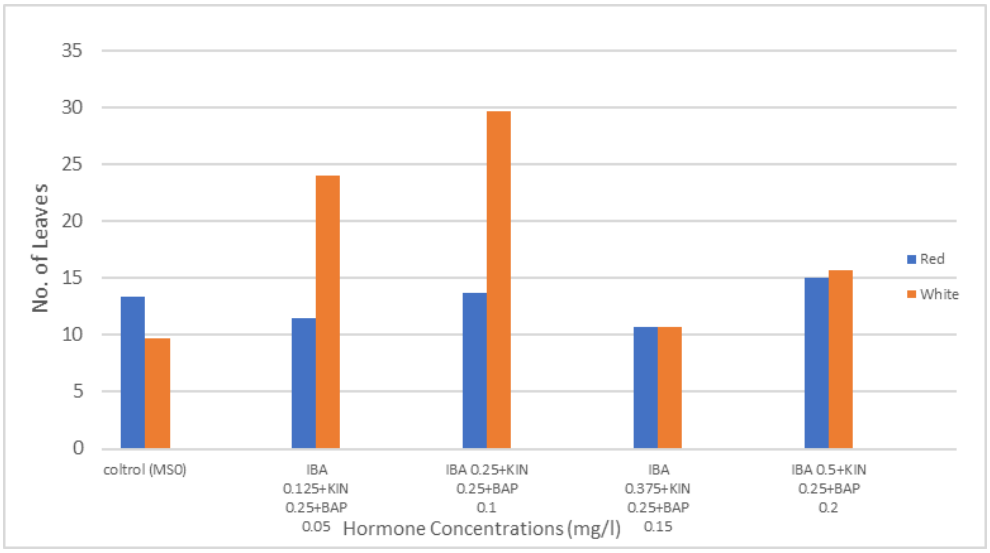

Figure 5: Comparison of the effect of Different Hormone Concentrations and Combinations on Number of Leaves in Two *Cissus* spp

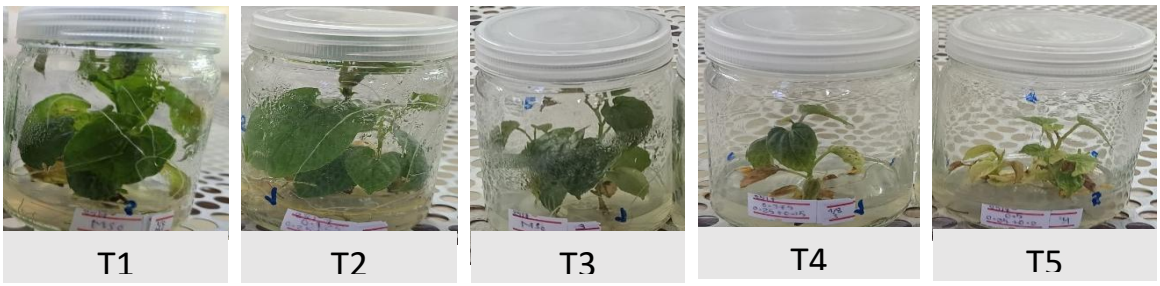

Figure 6: Shoot and root development of *Cissus discolor* Blume after four months of culture

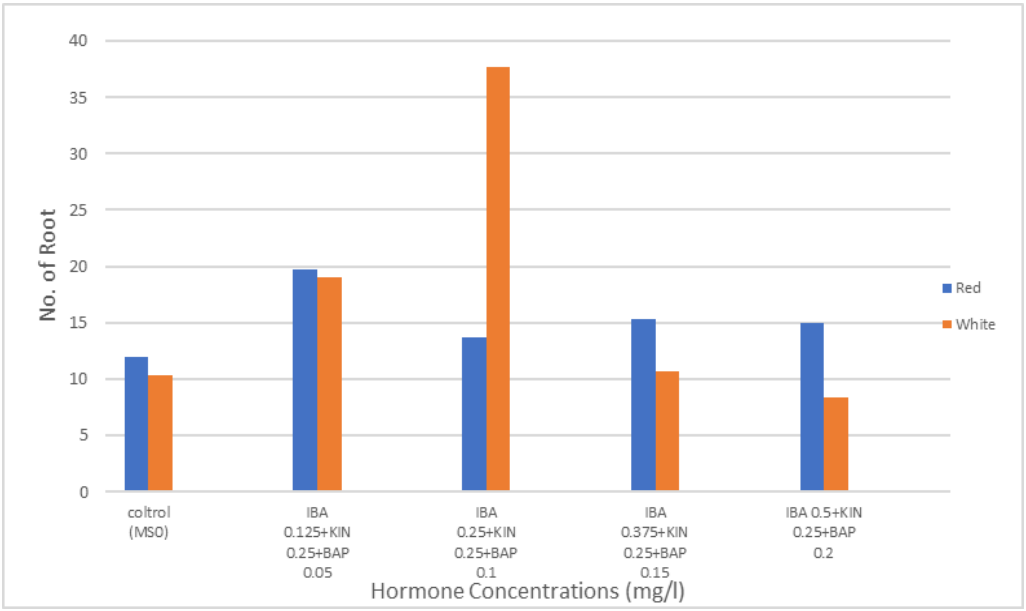

Figure 7: Comparison of the effect of Different Hormone Concentrations and Combinations on Root Development in Two *Cissus* spp

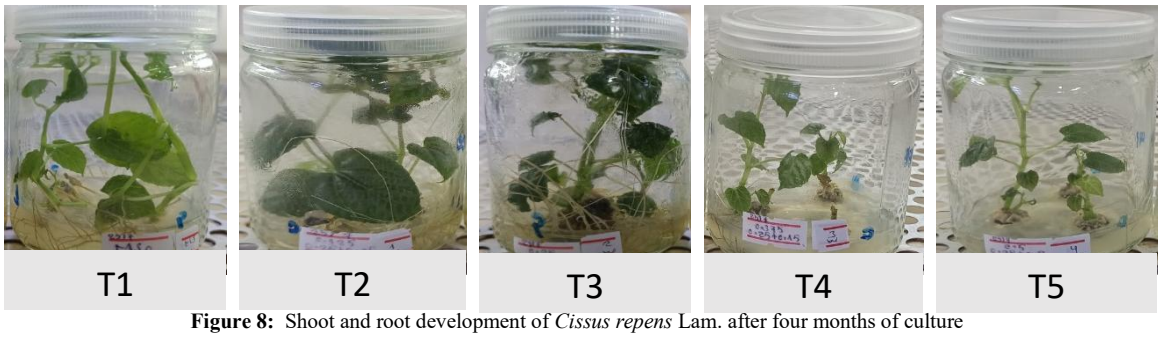

Figure 8: Shoot and root development of *Cissus repens* Lam. after four months of culture

Figure 9: Hardening of *Cissus discolor* Blume and *Cissue repens* Lam

CONCLUSION

The study demonstrated that the *in vitro* regeneration of *Cissus discolor* Blume and *Cissus repens* Lam. is highly responsive to specific concentrations and combinations of BAP, Kn, and IBA. Optimal shoot induction, elongation, leaf production and root development were achieved with hormone treatments such as BAP at (0.05, 0.1, 0.15, 0.2 mg/L), Kn at 0.25 mg/L, and IBA at (0.125, 0.25, 0.375, 0.5 mg/L). The optimal hormone combination for all measured parameters, number of shoots, number of leaves, shoot length (cm), and root development, in *Cissus discolor* Blume was 0.05 mg/L BAP, 0.25 mg/L Kn, and 0.125 mg/L IBA. For *Cissus repens* Lam., the optimal combination for the number of shoots, number of leaves, and root development was 0.1 mg/L BAP, 0.25 mg/L Kn, and 0.25 mg/L IBA. In contrast, the best shoot length (cm) for this species was achieved with 0.05 mg/L BAP, 0.25 mg/L Kn, and 0.125 mg/L IBA. These findings highlighted the importance of precise hormonal regulation for efficient micropropagation of these species. The established protocols can serve as a foundation for large-scale propagation, conservation, and utilization of *Cissus* spp., with further research needed to enhance acclimatization and field establishment.

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Conflict of interest

There is no conflict of interest.

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