

THE EFFECT OF DIFFERENT PLANT GROWTH REGULATORS ON SHOOT INDUCTION OF *Orthosiphon aristatus* Boldingh.

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Abstract

Among herbal plants which had been commercialized recently include *Orthosiphon aristatus* Boldingh. or better known as Java Tea and “misai kucing”. The plant’s leaves are believed to be able to help prevent and eliminate bladder stone apart from some other purposes when used alone or in combination with other plants. The production of the plant using tissue culture technique is hoped to reduce the time for growth under normal cultivation and to produce in a large quantity from a small source of explant. Sources of explant for this study were derived from leaves and stems for comparison. Each of the explants was grown under various plant growth regulators at various concentrations and combinations to determine the best hormone concentration that give adventitious shoots. Explants from stems were found to produce the best overall result with 0.2 mg/L BAP and 0.5 mg/L NAA but at a slower rate. The best result obtained from leaves explants was with 0.2 mg/L BAP which induces shoot faster than stem explants but at a smaller quantity. In comparison to the results from leaves explants, BAP with the same concentration using the stem explants gave a higher number of adventitious shoots. Stems are found to be a better explants source for regeneration of *O. aristatus*.

Keywords: *Orthosiphon aristatus*, stem, leaves, BAP, NAA, adventitious shoots.

Introduction

A native to the tropical Asia, *Orthosiphon aristatus* Boldingh comes from Lamiaceae family. It is an herbal plant whereby the entire plant can be used for medical purposes. Known as Java Tea or “Misai Kucing”, the name was thought to originate from its bluish flowers which resemble cat’s whiskers. The leaves have long been used as a tea to treat urinary tract infection especially for eliminating gall stones. Among its other uses is to treat diuretic and anti-inflammatory in arthritis (Gupta, 1994, Muhamad & Mustafa, 1994). Two types of flavones have been discovered in Java Tea; 5-hydroxy-6, 7, 3, 4-tetramethoxyflavone and 4, 5, 6, 7-tetramethoxyflavone, together with a compound called isosinensetin (Muhamad & Mustafa, 1994). All these were found to contribute to Java Tea’s properties as a medicinal plant.

Plant tissue culture allows the culture and maintenance of plant cells or organs in sterile, nutritionally and environmentally supportive conditions from a small amount of explants source. Plantlets obtained from direct shoot organogenesis are generally

preferred as they are more genetically stable than those regenerated through callus. Adventitious shoot may also be obtained apart from multiple shoot, as the initiation of adventitious shoot might promote the formation of meristematic regions that may be advantageous in plant transformation. Furthermore, preservation of genetic stability in germplasm and micropropagation via apical or axillary meristem culture permits recovery of true to type progeny.

The establishment of *Orthosiphon stamineus*, another variety of Java Tea for cell growth through cell suspension culture had been initiated (Lee & Chan, 2004). However, to date, there has been no regeneration system for Java Tea through adventitious shooting. Hence, a preliminary study of the adventitious shoot regeneration system is being conducted to determine if an efficient regeneration system could be established.

Materials and Methods

Plant Materials and Media

Leaves and stems of *O. aristatus* were used as explants. The explants were washed under running tap water for 10 minutes blotted with gauze to remove dirt. Surface sterilization was done with 70% ethanol for 20-30 seconds. Explants were transferred into 3% sodium hypochloride with a few drops of Tween 20 for 5 to 10 minutes with occasional shaking, rinsed 6 times with sterilized distil water and blotted dry. Before inoculation onto Murashige and Skoog (MS) medium, the explants were cut to smaller pieces.

The Effect of Various Hormones Treatment on Shoot Induction

Both leaves and stems were tested on various types of hormones with different concentrations and incubated for 2 to 8 weeks. Explants were subcultured to a new media with the same hormone treatment after 4 weeks in culture.

Leaves: Without hormone

BAP (6-Benzylaminopurine) → 0.2 – 0.5 mg/L

NAA(1-Napthylene acetic acid) → 0.5 – 4.0 mg/L

BAP : Kinetin → 1.0 : 1.0 mg/L

BAP : IBA (3-Indolebutyric acid) → 0.5 – 1.0 : 0.5 – 1.0 mg/L

2,4-D (2,4-Dichlorophenoxyacetic acid) : NAA → 0.5 – 2.0 : 0.2 – 4.0 mg/L

BAP : NAA → 0.2 : 0.2 – 0.5 mg/L

Stems: Without hormone

BAP → 0.2 – 0.5 mg/L

TDZ (Thidiazuron) → 0.2 – 2.5 mg/L

2,4-D → 0.2 – 2.0 mg/L

NAA → 0.2 – 0.5 mg/L

BAP : NAA → 0.2 : 0.2 – 0.5 mg/L

Results

The Effect of Various Plant Hormones on Shoot Induction from Leaf Explants

Explants were inoculated using various parts of the leaves from the young to the mature leaves and subjected to different types of hormones. The first shoot formation was observed as early as 2 weeks in culture for the young leaves segments in MS media supplemented with 0.2 mg/L BAP. After 6 weeks, most of the hormone treatment gave a positive result for shooting apart from auxins (Table 1). MS media supplemented with 0.2 mg/L BAP gave the highest number of shoot per explant (1.9) followed by MS media supplemented with 0.5 mg/L BAP (1.5). The shoots observed were found to be an adventitious shoots (Figure 1). Combinations of BAP and NAA together with BAP and IBA produced callus apart from adventitious shoot but callus growth were observed in explants not producing adventitious shoot. Most treatments produced roots especially for auxins such as NAA and 2,4-D. Both NAA and 2,4-D gave a high percentage for callus as well. At the end of the 8th week, the shoots obtained were transferred to rooting medium.

Table 1: Shoot Induction from Leaf Explants Using Various Plant Hormones

Hormones	Concentration (mg/L)	Average no. of shoot per explant	Shooting Percentage (%)	Shoot Morphology	Rooting Percentage (%)	Callus Percentage (%)
BAP	0.2	1.9	33	Adventitious shoot	20	0
	0.5	1.5	27	Adventitious shoot	13	0
NAA	0.5	0	0	-	20	30
	4.0	0	0	-	30	50
BAP: Kinetin	1.0:1.0	1.4	10	Adventitious shoot	0	0
BAP:IBA	0.5:0.5	1.0	10	Adventitious shoot	10	30
	1.0:1.0	1.3	20	Adventitious shoot	10	0
2,4-D: NAA	0.5:0.2	0	0	-	30	30
	2.0:4.0	0	0	-	40	40
BAP: NAA	0.2:0.2	0.6	20	Adventitious shoot	0	20
	0.2:0.5	0.3	10	Adventitious shoot	10	30

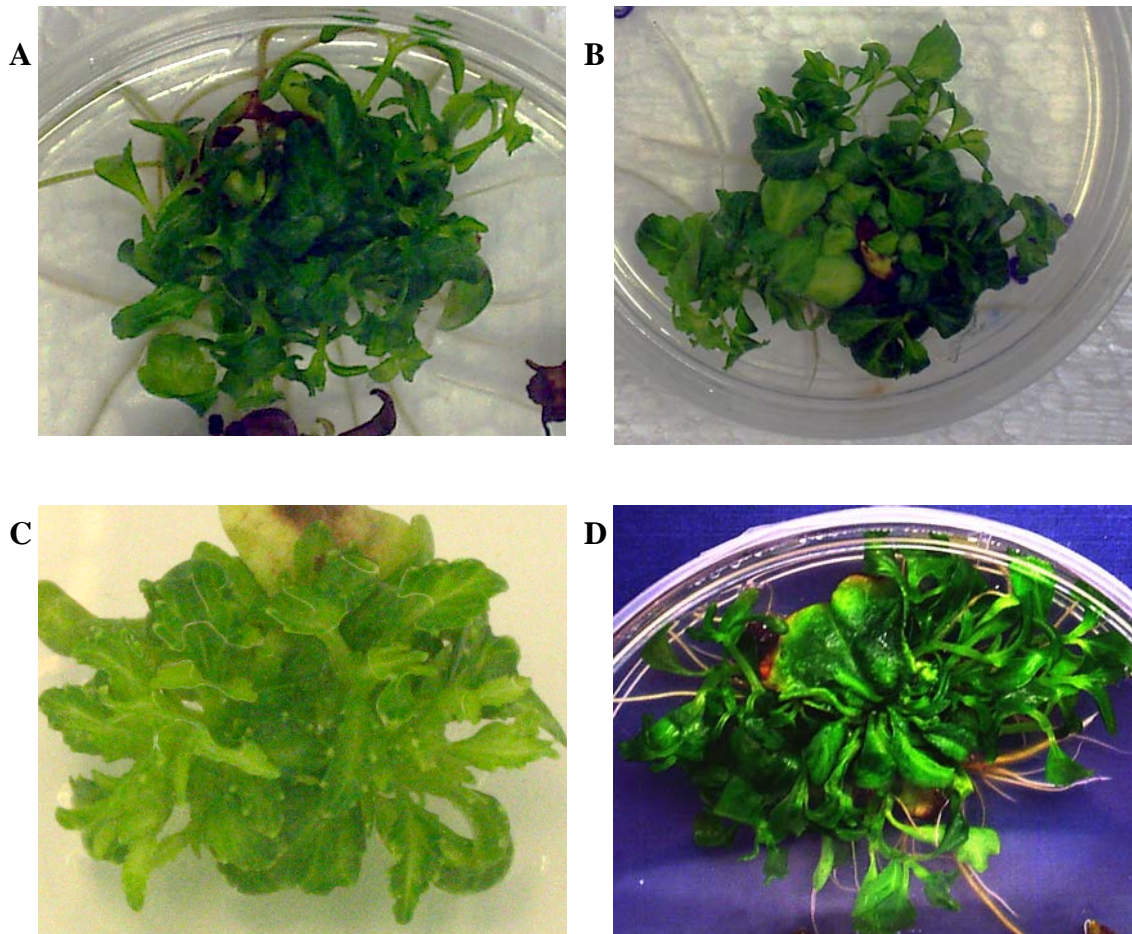


Figure 1: Shoot induction from leaf explants in MS media supplemented with various plant growth hormones after 8 weeks. A) 0.2 mg/L BAP. B) 0.5 mg/L BAP. C) 1.0 mg/L BAP and 1/0 mg/L Kinetin. D) 1.0 mg BAP and 1.0 mg/L IBA.

The Effect of Various Plant Hormones on Shoot Induction of Stem Explants

Initially various parts of the stems were used to test for the best source. Mature stems did not give as much shoots as young stems. However, young stems from the apex of the plant gave lower result than young stems from trimmed stems. After 6 weeks in culture, the highest number of shoots was obtained from explants in MS media containing 0.2 mg/L BAP and 0.5 mg/ NAA with 3.1 average number of shoot per explant followed by MS media containing 0.2 mg/L BAP (2.5) (Table 2). Only explants treated with NAA did not produce any shoot. The formation of shoots from stems were found to be slower than leaves but with a higher number of shoots. The shoots observed were also found to be an adventitious shoots (Figure 2). Most of the treatments produced roots with TDZ producing the highest percentage of rooting than other treatments. NAA treatment that did not produce any shoot produced callus at the highest percentage apart from roots. At the end of the 8th week, the shoots obtained were transferred to rooting medium.

Table 2: The Effect of Different Hormone Treatment on Shoot Induction from Stem Explants

Hormones	Concentration (mg/L)	Average no. of shoot per explant	Shooting Percentage (%)	Shoot Morphology	Rooting Percentage (%)	Callus Percentage (%)
BAP	0.2	2.5	67	Adventitious shoot	13	7
	0.5	0.4	20	Adventitious shoot	0	0
TDZ	0.2	0.6	40	Adventitious shoot	40	0
	1.5	1.0	80	Adventitious shoot	80	0
2,4-D	0.2	0.7	10	Adventitious shoot	10	0
	2.0	0.6	30	Adventitious shoot	30	0
NAA	0.2	0	0	-	20	60
	0.5	0	0	-	40	40
BAP: NAA	0.2:0.2	0	0	-	0	20
	0.2:0.5	3.1	67	Adventitious shoot	60	20

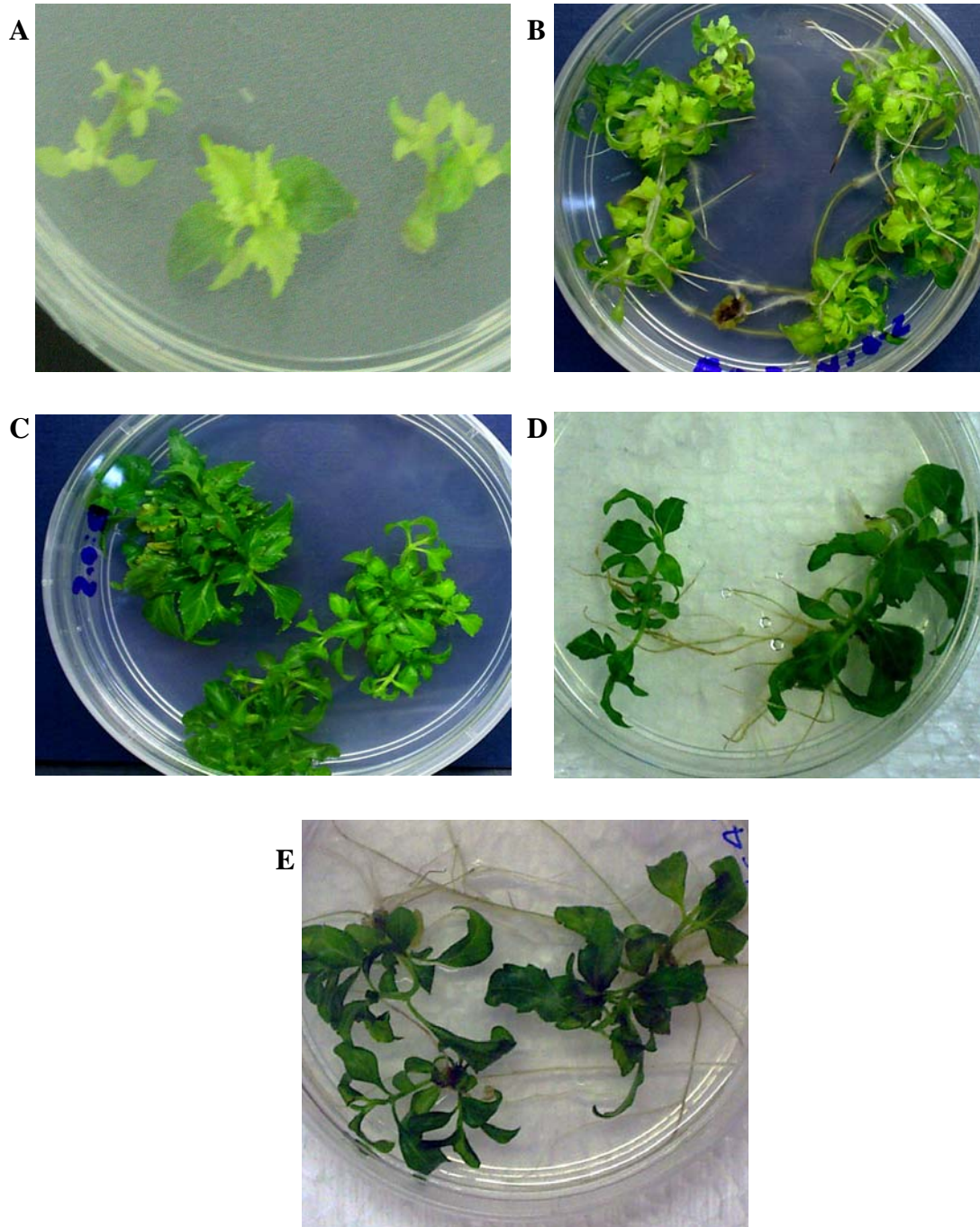


Figure 2: The effect of different plant growth hormones and concentrations on shoot induction from stem explants. A) 0.2 mg/L BAP and 0.5 mg/L NAA after 4 weeks. B) 0.2 mg/L BAP and 0.5 mg/L NAA after 8 weeks. C) 0.2 mg/L BAP. D) 1.5 mg/L TDZ. E) 2.0 mg/L 2,4-D.

Discussions

Comparison of the Source of Explants for Shoot Regeneration of O. aristatus

The findings from this study differ from those done by Lee and Chan in 2004. They established cell suspension culture of *O. stamineus*, another variety of *O. aristatus* sharing the same family while our study focused more on regeneration system. A comparison was made to determine the best source of explants from leaf, petiole and stem, and leaves were found to be the best source of explants with the most callus production (Lee and Chan, 2004). Callus produced by stem explants in the present study were found to be higher than those produced by leaves. The result contradicts with the findings done for *O. stamineus*.

Most studies on shoot regeneration without the use of seeds proved that leaves are a better source of explants. The studies include those done in regeneration of *Limonium perigrinum*, *Echinacea purpurea*, *Platanus acerifolia* and *Cajanus cajan* whereby leaves were used mainly to generate shoot (Seelye et al., 1994, Korocho et al., 2002, Liu & Bao, 2003, Dayal et al., 2003). These were in contrast to our findings whereby stem serves as the better explants for *O. aristatus* for shoot regeneration. A study for induction of shoot compared the source of explants between leaf and stem in *Nemesia strumosa* (Cui & Ezura, 2003). The findings proved that stems regenerate more shoot than leaves just as in our study.

The Effect of Different Plant Hormones on Shoot Induction between Leaf and Stem Explants

Based on the results obtained on shoot induction from both leaves and stems, different plant growth hormones were found to induce shoot at different rate for both of the explants. For leaf explants, BAP when used at a concentration of 0.2 mg/L was found to induce shoot at a faster rate as well as give the most average number of shoot per explant (1.9). While for stem explants, BAP at the same concentration gave a higher average number of shoot per explant (2.5) than the former although it was not the best hormone for shoot production. The combination of BAP and NAA at a concentration of 0.2 and 0.5 mg/L respectively were found to give the highest average number of shoot per explant (3.1).

For leaves explants, most studies shared high number of shoots formation when BAP is supplemented in the MS media either alone or in combination with IBA or Kinetin. Certain plants react well with low concentration of BAP that reached below 0.2 mg/L (Korocho et al. 2002), 1mg/L and above BAP (Liu & Bao, 2003, Dayal et al., 2003) or both high and low BAP concentrations (Hiregoudar et al., 2003). Our findings were in the lower range of BAP and combination of BAP with IBA or Kinetin was found to be consistent with other plants in producing shoots. In *Limonium perigrinum*, TDZ alone or in combination with NAA were reported to produce more shoots (Seelye et al., 1994).

Just as in leaves, stems were observed to utilize BAP much better than any other hormones. Low concentration provides the best shooting condition especially in conjunction with NAA. Combination of BAP and NAA usually result in callus as proved by theory and many studies as well as our findings. Higher NAA concentration than BAP will result in more roots as proved (Table 2). In the study of *Nemesia strumosa*, the stems were treated with TDZ either alone or in combination with NAA to produce shoots and high number was obtained using the combination of TDZ and NAA (Cui & Ezura, 2003). *O. aristatus* explants treated with TDZ alone also give a good shoot production but induces root as well. Although TDZ is capable of stimulating cell division, it is a synthetic cytokinin and thus explains why BAP serves as a better cytokinin for inducing shoot.

Conclusion

Shoots were generated well in MS media supplemented with 0.2 mg/L BAP for leaf explants while MS media supplemented with 0.2 mg/L BAP and 0.5 mg/L NAA provided the highest number of shoots for stem explants. From comparison between leaf and stem as the source of explants, stem served as a better explants based on its ability to generate more shoots than leaf explants. Shoots were generally induced much faster using leaf explants. Auxins when used alone or in combination with cytokinin would result in callus while the addition of auxins alone will not be able to generate shoots. Most treatments carried out produced roots.

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