Callus induction and identification of DNA variation in callus derived from *Etlingera elatior in vitro* culture using ISSR marker

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ABSTRACT

Etlingera elatior or torch ginger is a promising horticultural plant with various economic values that has been used for medicinal, culinary, and ornamental purposes in many countries. The extravagant and showy inflorescence has made *E. elatior* a valuable plant that can be used for cut flower and floral decoration. However, the plant itself lack of genetic variety with narrow genetic base due to its nature as an asexual propagated plant. To overcome this problem, somaclonal variation arises from the *in vitro* culture can be used to overcome the lack of variation in *E. elatior*. Moreover, early detection of the variation in callus stage using ISSR markers will help to examine the genetic variability of the induced callus. For this project, an optimum sterilization technique with contamination rate of 5% has been successfully developed. Furthermore, white friable calli were successfully developed from the innermost part of young closed buds. The results showed that the Murashige and Skoog medium supplemented with 30 g/L glucose, 3 mg/L 2, 4-D and 1.5 mg/L BAP has the highest percentage of callus induction (50%) after 20 weeks of culture. The calli were transferred into shoot induction media with different concentrations of BAP, NAA and TDZ. The calli from the 11 different media were evaluated for their genetic variations by seven primers of ISSR markers. A total of 72 bands were generated of which 51 were polymorphic with mean percentage of polymorphic bands was 72%. From our results, the calli were affected by various concentrations of auxin and cytokinin for callus and shoot induction treatments. In short, ISSR marker successfully revealed the occurrence of genetic variations in the induced callus. Even though, no *in vitro* shoots were successfully regenerated, this study revealed that there is a potential to generate new variants through *in vitro* culture.

INTRODUCTION

Importance

Medicinal use - treat disease, antibacterial agent.

Traditional use - culinary, ornamental, & floral arrangement purpose (Choon & Ding, 2016)

Economical value -Department of Agriculture Malaysia (2018) stated that planting area for *E. elatior* increase from 178.31 hectare in 2014 to 356.18 hectare in 2018.

Problem: Traditional propagation through asexual propagation method **lack of genetic diversity.**

Solution: Somaclonal variation through indirect regeneration.

Objective : To induce calli from *E. elatior* and evaluation of genetic variation of calli using inter simple sequence repeat (ISSR) marker.

METHODOLOGY

Eplant source and sterilization. → Callus induction on MS media and Callus Proliferation. → Shoot induction. → Genomic DNA extraction from calli. → DNA Quantification. → Genetic analysis of calli using ISSR marker.

Figure 1: *Innermost part of inflorescence as explants.*

Table 1:*Callus induction treatments.*

Treatments	MS Media Compositions	2,4-D (mg/L)	BAP (mg/L)
C1	Sucrose	0	0
C2	Sucrose	3	0
C3	Sucrose	6	0
C4	Sucrose	0	1
C5	Sucrose	3	1
C6	Sucrose	6	1
C7	Sucrose	0	1.5
C8	Sucrose	3	1.5
C9	Glucose	0	0
C10	Glucose	3	0
C11	Glucose	6	0
C12	Glucose	0	1
C13	Glucose	3	1
C14	Glucose	6	1
C15	Glucose	0	1.5
C16	Glucose	3	1.5

Table 2:*MS medium supplemented with various concentration combination of BAP, NAA, and TDZ for shoot induction treatments.*

Treatments	BAP (mg/L)	NAA (mg/L)	TDZ (mg/L)
T1	0	0	0
T2	1	0	0
T3	0	1	0
T4	0	0	1
T5	2	0	0.3
T6	3	0.5	0
T7	5	0.1	0
T8	3	0.5	0.3
T9	5	0.1	0.3
T10	2	0.5	0.3
T11	0	0.1	0.3

Table 3: *List of eight ISSR primers used in this study.*

No.	Primer Name	Sequence 5'to 3'	Annealing Temperature (°C)
1	UBC 808	AGAGAGAGAGAGAGAGC	52
2	UBC 809	AGAGAGAGAGAGAGAGG	52
3	UBC 811	GAGAGAGAGAGAGAGAC	52
4	UBC 830	TGTGTGTGTGTGTGTGG	54
5	UBC 855	ACACACACACACACACCT	52
6	UBC 880	GGAGAGGAGAGGAGA	52
7	UBC 888	CGTAGTCGTCACACACACACACA	52
8	UBC 891	ACTACGACTTGTGTGTGTGTGTG	54

RESULT

Callus Induction.

Table 4: *Result of callus induction of E. elatior after 20 weeks of culture.*

Treatments	Percentage of callus induction ± SE	Intensity of callus induction
C1	0	-
C2	16.67±8.33	+++
C3	0	-
C4	0	-
C5	0	-
C6	0	-
C7	0	-
C8	0	-
C9	0	-
C10	25±14.43	++++
C11	0	-
C12	0	-
C13	16.67±16.67	+++
C14	0	-
C15	0	-
C16	50±0	++

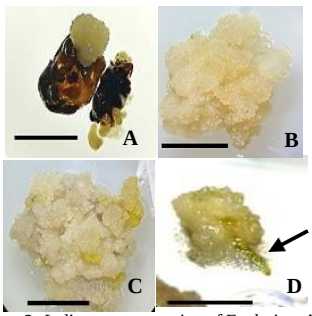

Figure 2: *Indirect regeneration of E. elatior. A) Treatment C13 explant produced friable calli after 20 weeks of culture B) Callus proliferation C) Green spot and globular organogenic calli shown in T4 after 8 weeks culture on shoot induction media D) Organ induction (arrow) shown in T11 after 12 weeks on shoot induction media.*

Genetic analysis of calli using ISSR marker.

Table 5: *List of primers, number of amplified products, polymorphic bands and polymorphism percentage.*

No	Primer	Optimized melting temperature (°C)	Total amplified products	Polymorphic bands	Monomorphic bands	Percentage of polymorphism
1	UBC 808	52	9	9	0	100
2	UBC 809	52	11	7	4	63.64
3	UBC 811	52	14	9	5	64.28
4	UBC 830	54	6	5	1	83.33
5	UBC 880	52	11	9	2	81.82
6	UBC 888	54	12	8	4	66.67
7	UBC 891	54	9	4	5	44.44
		Total	72	51	21	504.18
		Mean	10.3	7.3	3	72

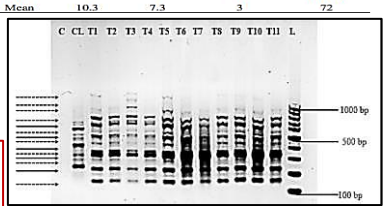

Figure 3 : *Amplification of genomic DNA from callus and leaf of E. elatior generated by primer UBC 811. Right lane corresponds to DNA ladder (L), Left lane C represents -ve control (no DNA added), CL represents +ve control leaf, and T1-T11 represent calli of E. elatior. Note: solid arrows point to the monomorphic bands, the dashed arrows point to the polymorphic bands*

CONCLUSION

As a conclusion, the highest induction of callus was achieved in treatment C16 (MS supplemented with 30 g/L glucose, 3 mg/L 2, 4-D + 1.5 mg/L BAP) with 50% of callus induction. Calli were proliferated before transferred onto shoot induction media. Treatment T11 (0.3 mg/L NAA +0.1 mg/L TDZ) shows the structure of root, probably from organogenic activity of calli. Calli from shoot induction treatments were genetically evaluated using ISSR marker. The percentage of polymorphism per primer ranged from 44.4% to 100% with an average of 72%. The results obtained in the present study showed the presence of some genetic variations at the DNA level during *in vitro* culture.

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