Chapter 4

Rhizome formation *in vitro*

4.1 Introduction

Species that normally produce such organs as bulbs, tubers, and corms can be induced to form these miniature propagates within *in vitro* cultures under appropriate environmental conditions. Plants that naturally produce tubers can be induced to produce miniature versions of the storage organs in a medium containing high sucrose concentrations (George, 1993).

C. alismatifolia Gagnep is vegetatively propagated by small pieces of rhizome. A large amount of this edible part is stored for seed purpose from one season to another. Beside its low multiplication rate (8-10 lateral buds from the rhizome of a single plant after 6 months), rhizome produced from field grown plants are affected by various pathogens, such as rhizome rot. It is therefore highly desirable to develop a method to produce disease multiplication rates. Tissue culture technique remains an indispensable tool for rapid multiplication and sustaining growth of slow propagating species. Propagation through axillary bud multiplication is an easy and safe method to obtain uniformity, and it also assures the consistent production of true-to-type plants within a relatively short time. Currently, interest in *in vitro* propagation is directed to rhizome or storage organ induction for efficient acclimatization and to minimize injury during transportation.

Microrhizomes are *in vitro* storage organs employed from micropropagation of turmeric and a variety of other geophytes. Microrhizomes develops engorged stem tissues in response to high concentrations of sucrose in medium (Adelberg and Cousins, 2007). They have got enough potential to be used by commercial growers and disease-free planting material, produced *in vitro* irrespective of seasonal fluctuations and are easily transferable and sown like seeds. *In vitro* induction of rhizome has been reported for ginger and *Curcuma longa*. Peter *et al*. (2002) reported

microrhizome of 0.05 to 15 g fresh weight per explants was induced in ginger tissue culture from 1 to 12 months on MS basal medium supplemented with higher levels of carbon source.

The concentration of sucrose is one of the important factors for rhizome formation *in vitro* of *Globba malaccensis* (Chidburee *et al*., 2003). Forsyth and Staden (1984) reported that 8% of sucrose concentration on MS medium induced rhizome formation in *Dioscorea bulbifera*. Garner and Blake (1989) improved tuberization in potatoes by increasing the sucrose levels from 4 to 8%. In *Alstroemeria* 'Zebra', the highest number of lateral rhizomes had been observed on a medium containing 6 or 8% sucrose (Gabryszewska-E, 1996). Rhizomes of calla were successfully formed on MS basal media containing 7% of sucrose (Ebrahim, 2003).The research aimed to study the effects of sucrose concentration to induce rhizome formation of *C. alismatifolia* Gagnep *in vitro*.

4.2 Materials and methods

4.2.1 Plant material

The mature young of single shoot explants were taken from *in vitro* maintained healthy plantlets on MS (Murashige and Skoog, 1962) basal medium. Single shoot, 3 cm long and 0.5 cm wide, having 3 fully developed leaves was excised after 4 weeks of proliferation (Fig. 4.1).

4.2.2 Effect of sucrose concentration

Shoots of *C. alismatifolia* Gagnep was initially grown on a proliferation medium consisting of modified strength MS salts supplemented with 8.79 µM BA and different sucrose levels, i.e.; 3, 4, 5 and 6% (20 ml of medium per culture vessel). The pH of all the media were adjusted to 5.8. The media were sterilized in an autoclave under 1.5 kg/cm^2 and 121^{o}C for 15 min.

4.2.3 Culture conditions

The explants were cultured for 6 months in growth chamber at 22 ± 2^{o}C under 16 hrs light/ 8 hr dark cycles. The light was provided by cool white fluorescent tubes about 30 μmol m^{-2}s^{-1} (two Philips TLD 36 W/95 fluorescent tubes).

Then changed the condition to induce rhizome formation had been changed for more 2 months (8 hrs light/ 16 hrs dark cycles and 18-20 ^{o}C temperature).

4.2.4 Measurements and Statistical Analysis of collected data

Twenty shoots (1 shoot/culture) were used as replication in each treatment. The number of shoots and rhizomes per explant, diameter of rhizome, number of contractile roots per rhizome, length of contractile roots, and fresh weight of rhizome were recorded after 8 weeks. Data were subjected to analysis of variance to test the individual effect of sucrose.

Figure 4.1 The young shoots of *C. alismatifolia* were cultured on MS medium with free plant growth regulator.

4.3 Results

4.3.1 Growth of rhizome

Initially, the plantlets of *C. alimatifolia* Gagnep were cultured on MS media supplemented with different sucrose levels for 6 months (Fig. 4.2a). The numbers of shoots per explant at 3, 4 and 5% sucrose were 17.67, 25.00 and 17.67 shoots per explants, respectively and they were greater than at 6% sucrose

(7.33 shoots per explant) (Table 4.1). Plantlets grew on MS medium supplemented with 6% sucrose was browning (Fig. 4.2b) as affected by high concentration of sucrose. Plantlets were induced to dormant by decreasing temperature at 6 months after culture and they were in dormancy at 8 months after culture (Fig. 4.2c).

The percentage of rhizome formation were ranged 14.54-28.08% which was not different among treatments (Table 4.1). At 4% sucrose concentration, the number of microrhizomes per explants was the highest at 6.67 rhizomes per explants and significantly higher than those of 5 and 6% sucrose treatments. However, diameter of rhizome was not significantly different among treatments. The use of 4% sucrose tended to increase in diameter of the microrhizomes produced (0.60 cm). The fresh weight of rhizomes was also similar in all treatments.

Figure 4.2 Culture of *C. alismatifolia* Gagnep in *In vitro*. (a) Shoot multiplication on MS medium containing 6, 5, 4 and 3% sucrose concentration (induction period: 6 months). (b) Browning of plantlets grew on MS medium containing 6% sucrose concentration (growing period: 6 months). (c) New rhizome and contractile roots cultured on MS medium containing different sucrose concentrations [3, 4, 5 and 6%] (induction period: 8 months).

Table 4.1 Effect of sucrose concentrations on *in vitro* shoot and rhizome inductions of *C. alismatifolia* Gagnep cultured on MS medium (induction period: 8 months).

Sucrose concentration (%)	Num. of shoots per explant [1/]	Percent of rhizome formation [2/]	No. of rhizomes per explant [1/]	Diameter of rhizome (cm) [2/]	Fresh weight of rhizome (g) [2/]
3	17.00 [a]	28.08	4.67 [ab]	0.35	0.15
4	25.00 [a]	28.03	6.67 [a]	0.60	0.21
5	17.67 [a]	19.31	3.33 [b]	0.47	0.15
6	7.33 [b]	14.54	1.00 [c]	0.43	0.13

[1/] Mean with in the same column followed by different letter were significantly different at $P<0.05$

[2/] Non-significantly different

4.3.2 Contractile root formation

Plantlets developed contractile roots in all treatments about 6 months after culture (Fig. 4.3a). Then, at 8 months after culture (Fig. 4.3b), the sucrose concentration significantly affected the number of contractile roots per rhizome and length of contractile roots (Table 4.2). At 4 and 6% sucrose concentrations in the medium, there were higher in the number of contractile roots per rhizome (4.00 and 4.00 contractile roots per rhizome, respectively) than the others. Three and five percent sucrose resulted in a decrease in number of contractile roots per rhizome (2.33 and 2.67 contractile roots per rhizome, respectively).

Length of contractile roots at 5 and 6% sucrose concentration treatments were reduced to 1.87 and 1.27 cm, respectively. At 3 and 4% of sucrose in medium there was an increase in the length of contractile roots (3.57 and 3.83 cm, respectively).

Table 4.2 Contractile roots of *C. alismatifolia* Gagnep cultured on MS medium at different sucrose concentrations (induction period: 8 months).

Sucrose concentration (%)	No. of contractile roots per rhizome [1/]	Length of contractile root (cm) [1/]
3	2.33 [b]	3.57 [a]
4	4.00 [a]	3.83 [a]
5	2.67 [b]	1.87 [b]
6	4.00 [a]	1.27 [b]

[1/] Mean within the same column followed by different letter were significantly different at $P<0.05$

Figure 4.3 Contractile roots of *C. alismatifolia* Gagnep cultured on MS medium containing 4% sucrose [(a) at 6 months and (b) 8 months].

4.4 Discussion

Base on this physiological aspect, addition of sucrose to the medium could result in excess of sugar content in their storage tissue because although the rate of sugar consumption is reduced by sucrose and absorbed and then accumulated in the tissue. Accordingly, the excess of sugar in the tissues would decrease its osmotic potential. Carbohydrates in the medium have besides their metabolic function and osmotic one

as well (Salisbury and Ross, 1992). Sugar was reported to have an osmotic role and also act as a source of energy and carbon in inducing shoot regeneration (Brown *et. al*., 1979). Therefore, in this experiment shoot regeneration in term of number of shoot per explants in excess sucrose concentration treatment (6%) was reduced to 7.33 shoots per explants compared with 2.5 shoots per explants at 4% sucrose.

The browning required the additional osmotic pressure of sucrose. Plant tissue produced toxin from collection sucrose. Torres (1957) reported that the browning was poisonous with the tissue and halts the growth and development of plantlets. It was the cause for the high tannins and hydroxyphenols. It seemed that high sucrose levels were not needed for the initiation of rhizome. Furthermore, an increase of sucrose to 6% induced browning of plantlets which were detrimental for growth of the shoots (Hazarika, 2003).

One possible presumption could be derived from physiological processes because growth and development of the bulblets were implicated with sugar metabolism and involved with energy source, such as starch reserved in their storage tissue (Salisbury and Ross, 1992).

Chirangini and Sharma (2005) reported that sucrose played an important role of carbohydrate source for rhizome formation and the optimum levels of sucrose affected to the development of rhizome under longer photoperiod in nature. The sucrose also plays an important role in developing rhizome of *Zingiber officinele* Rosc (Rout *et al*., 2001). Sucrose was required for the formation of lateral rhizomes, upright growing shoot and roots (Gabryszwqska, 1996). Dantu and Bhojuani (1995) reported that 96% of shoots cultures of *Gladiolus* 'Friendship' formed corms on liquid MS medium containing 6% sucrose. And in *C. longa* Linn, Sunitibala *et al*. (2001) reported that rhizome formation *in vitro* occurred in media containing 6 and 8% sucrose.

Although, sucrose was found to promote formation of various storage organs in most of the cases investigated so far. The enhanced rate of *in vitro* rhizome formation with increasing concentration of sucrose may be attributed to the presence of high carbon energy in sucrose since rhizomes mostly contain carbohydrates and sucrose (Nayak, 2000). However, it seems that contractile roots of *C. alismatifolia* were not modified cells to enlarge and store the carbohydrate source in this experiment.

4.5 Conclusion

The result of rhizome formation under field experiment indicated that the swollen of storage roots occurred during short sunshine duration in winter. This means that not only sucrose concentration but also other factors involved the translocation and accumulation of carbohydrate to the storage organ. Therefore, the optimum condition should be studied further in order to stimulate the storing of food reserve in storage roots.