

## EFFECT OF BAP AND SUCROSE ON THE DEVELOPMENT OF CORMEL IN MUKHI KACHU

M. K. R. BHUIYAN<sup>1</sup>, S. M. SHARIFUZZAMAN<sup>2</sup> AND M. J. HOSSAIN<sup>3</sup>

### Abstract

*In vitro* cormel development in Mukhi Kachu (*Colocasia esculenta*) Var. Bilashi was assessed in an experiment using three levels of BAP (0, 5 and 10 mg/l) and four levels of sucrose (0, 5, 10 and 15 %). Individual shoot excised from multiple shoot was used as explant in this experiment. *In vitro* cormel formation of *Colocasia* is an important means of organogenesis, which initiated earlier with 10% sucrose in 15% culture, whereas 15% sucrose produced cormels in 50% culture. While BAP at 10 mg/l formed cormels in 32.5% cultures but these two factors together formed cormels in 85% cultures, having 2.5 cormel per culture. The cormel weighed upto 1.7 g and contained 81.5% dry matter.

Keywords: Mukhi Kachu, BAP, Sucrose, Cormel development.

### Introduction

Mukhi Kachu (*Colocasia esculenta*) is known as taro, cocoyam, eddoe and dasheen in different places and used as an important vegetable in various parts of the tropics (Denham *et al.*, 2003). In Bangladesh, it comes to market as an important summer vegetable when most of the vegetable are not available. It is grown in high land and covering an area of 23,897 ha of land and production 236217 tones (Annon, 2012). Nutritionally, this crop is very rich in iron and yield potentially of this crop is 30-32 tons per hectare (Rashid, 1990).

The variety Bilashi produces corm and cormels which are the propagating materials but this cormels are also an important summer vegetables in Bangladesh. The major part of this crop is generally used as vegetable keeping a very small portion as seed. As cormels are used as planting materials in mukhi kachu and seed cormel supply is a limiting factor, propagation of cormels in *in vitro* can speed up the seed production program. *In vitro* cormels can be produced year round and can be used as a basic material for quality seed production in the country (Chandra *et al.*, 1988; Rahim and Alamgir, 1995).

*In vitro* tuberization of potato has been studied by many authors (Wang and Hu, 1982; Tover *et al.*, 1985; Pelacho and Mingo- Castel, 1991; Chandra *et al.*, 1992; Zakaria, 2003). But reports on taro are very scarce though these two crops are identical (Zhou *et al.*, 1999). The first report on taro micro-propagation was by Yamamoto and Matsumoto (1992), who induced *in vitro* cormels after adding 8

---

<sup>1</sup>Principle Scientific Officer, TCRC, Bangladesh Agricultural Research Institute (BARI), Gazipur, <sup>2</sup>Principle Scientific Officer, Floriculture Division, HRC, BARI, Gazipur,

<sup>3</sup>Director, TCRC, BARI, Gazipur, Bangladesh.

% sucrose in MS liquid medium. Many authors reported that Sucrose (Yamamoto and Matsumoto, 1992; Zhou *et al.*, 1999) and Benzyl adenine (Zhou *et al.*, 1999) are responsible for *in vitro* cormel production in taro. However, the work on *in vitro* cormel induction of taro is very scanty in abroad and there is no report in our country. Therefore, to develop cormels in *in vitro* plantlets, the present investigation has been under taken.

### **Materials and Method**

The experiment was conducted at the tissue culture laboratory of the Tuber Crops Research Centre (TCRC), BARI, Joydebpur, Gazipur. Well sprouted cormels of Mukhi Kachu variety ``Bilashi`` was used. The cormels were cut into small size with approximate 2.0 cm long sprout and were disinfected following the method as described by Hossain *et al.* (1998). This small size sprout was put in to test tube containing multiple shoot inducing MS media. Individual shoots were excised from multiple shoot and cultured in this experiment for cormel production.

Basic salts of Murashige and Skoog (Murashige and Skoog, 1962) culture media were used. In order to induce cormels in detached multiple shoots, three levels of BAP (0, 5 and 10 mg/l) and four levels of sucrose (0, 5, 10 and 15%) were used in this experiment. Individual shoots were excised from multiple shoot and multiplied. Shoots were inoculated into MS agar-solidified medium without growth regulators and grew for 25 DAC (Days after culture) before placing into corm induction medium. The cultures were maintained in a growth chamber at  $22 \pm 1^{\circ}\text{C}$  with a 16 h photoperiod, and a photosynthetic photon flux of 3000 lux was provided by white fluorescent lamps.

Shoots cultured in *in vitro* multiplication medium were cut off (3-5 cm high) above the roots and transplanted into culture vessels with MS (Murashige and Skoog, 1962) liquid medium, which was supplemented with sucrose and BAP according to the treatment. Cultures were maintained under light (16 h photoperiod). The number of DAC (days after culture) required for *in vitro* cormel induction was recorded as swelling of cormel was visible. The average number and weight was counted and recorded at harvest. The experiment was set in a Complete Randomized Design (CRD), replicated thrice. Each replication included three tubes. Data were analyzed following DMRT at 1% level of probability.

### **Results and Discussion**

#### **Effect of sucrose**

Results of cormel formation with sucrose is presented in Table 1. Cormel formation did not occurred up to 5 % sucrose and the highest percentage of

cormel (50.0) formed with 15 % sucrose. Cormel first appeared at 10 % sucrose after 12.5 DAC (days after culture) and it was 22.5 DAC for 15 % sucrose, which produced higher number of cormel per culture (1.5) than that with 10 % sucrose (0.3). The size of cormel was 0.4 cm in diameter, which on weight basis was 1.1 mg at 15 % sucrose. These values for 10 % sucrose were 0.2 cm and 0.5 mg, respectively. The DM % was highly varied over sucrose percentage; it was 22.5 % and 51.7 % for 10 and 15 % sucrose, respectively. In an experiment Zhou *et al.* (1999) found that 5-10% sucrose promoted corm formation. They reported that 15 % sucrose inhibited cormel formation. But in the present study 10-15 % sucrose promoted cormel formation. Zhou *et al.* (1999) used a diploid type variety which was quite different from that was used in the present study (a triploid type variety).

**Table 1. Main effect of sucrose on cormel induction and other parameters.**

| Sucrose (%) | Cormel formed/culture (%) | DAC to Cormel formation | Number of cormels / culture | Wt. of cormel (g) | Dia. of cormel (cm) | DM (%) of cormel |
|-------------|---------------------------|-------------------------|-----------------------------|-------------------|---------------------|------------------|
| 0           | 0.0 c                     | 0.0 c                   | 0.0 c                       | 0.0 c             | 0.0 c               | 0.0 c            |
| 5           | 0.0 c                     | 0.0 c                   | 0.0 c                       | 0.0 c             | 0.0 c               | 0.0 c            |
| 10          | 15.0 (3.9) b              | 12.5 b                  | 0.3 b                       | 0.5 b             | 0.2 b               | 22.5 (2.7) b     |
| 15          | 50.0 (7.1) a              | 22.5 a                  | 1.5 a                       | 1.1 a             | 0.4 a               | 51.7 (5.9) a     |

Figures in parenthesis indicate square root transformed data. In a column, means followed by common letters are not significantly different from each other at 1 % of level of probability by DMRT

**Table 2. Main effect of BAP on cormel induction and other parameters.**

| BAP (mg/l) | Cormel formed/culture (%) | DAC to cormel formation | Number of cormel/culture | Weight of cormel (g) | Diameter of cormel (cm) | DM (%) of cormel |
|------------|---------------------------|-------------------------|--------------------------|----------------------|-------------------------|------------------|
| 0          | 0.0 c                     | 0.0 c                   | 0.0 c                    | 0.0 c                | 0.0 c                   | 0.0 c            |
| 5          | 16.3 (4.0) b              | 8.8 b                   | 0.5 b                    | 0.4 b                | 0.1 b                   | 18.4 (2.1) b     |
| 10         | 32.5 (5.7) a              | 17.5 a                  | 0.9 a                    | 0.8 a                | 0.3 a                   | 37.3 (4.3) a     |

Figures in parenthesis indicate square root transformed data. In a column, means followed by common letters are not significantly different from each other at 1 % of level of probability by DMRT

Detail explanation of the role/ function of sucrose in *in vitro* cormel formation is not enough in the literature. The question may pose as to whether it performs an osmotic role or purely a nutritional one. It is thought that sucrose dissociates to allow a higher osmotic potential within the cells. Thus the role of sucrose in plant tissue culture media as an osmoticum as well as a carbohydrate source has been established. Cormel induction may depend on the osmotic stress of a high concentration of sucrose solution. However, developing cormels are sink for sucrose from the culture medium (Zakaria, 2003).

### Effect of BAP

The effect of BAP on cormel formation is shown in Table 2. No cormel was formed in the control. The highest (32.5%) cormel formed with BAP 10 mg/l. Whereas, it appeared first with 5 mg/l BAP after 8.8 DAC, whereas 10 mg/l BAP took 17.5 DAC. The number of cormel was 0.5 with 5 mg/l BAP, which increased to 0.9 with 10 mg/l BAP. The size of cormel was bigger for 10 mg/l BAP (0.3 cm in diameter) compared to 0.1 cm diameter for 5 mg/l. These values on weight basis were 0.8 and 0.4 mg, respectively. The DM % was higher for larger cormel (37.3) than smaller cormel (18.3). Cytokinins or BAP are believed to have strong promotive effects on cormel formation (Zakaria, 2003). The results are in agreement with the findings of many scientists (Priyakumari and Sheela, 2005 and Zhou *et al.*, 1999).

**Table 3. Combined effect of sucrose and BAP on cormel induction and other parameters**

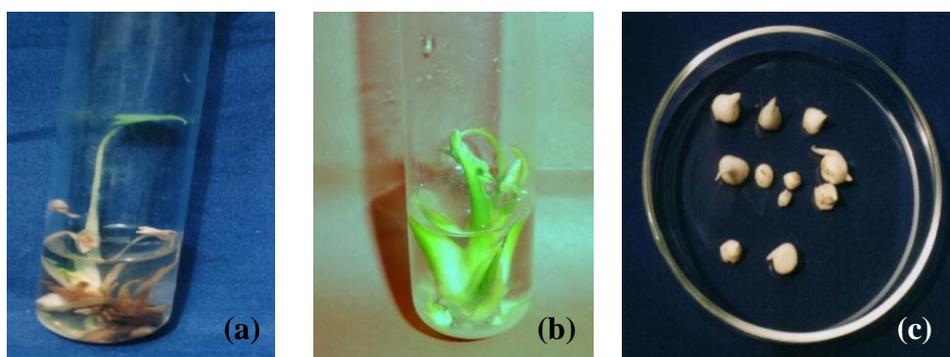
| Treatment      |               | Cormel formed/<br>culture (%) | DAC to<br>Cormel<br>formation | Number<br>of cormel<br>/culture | Wt. of<br>cormel<br>(g) | Dia. of<br>cormel<br>(cm) | DM (%) of<br>cormel |
|----------------|---------------|-------------------------------|-------------------------------|---------------------------------|-------------------------|---------------------------|---------------------|
| Sucrose<br>(%) | BAP<br>(mg/l) |                               |                               |                                 |                         |                           |                     |
| 0              | 0             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 5             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 10            | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
| 5              | 0             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 5             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 10            | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
| 10             | 0             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 5             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 10            | 45.0 (6.7) c                  | 37.5 a                        | 1.0 c                           | 1.4 c                   | 0.5 b                     | 67.5 (8.2) c        |
| 15             | 0             | 0.0 d                         | 0.0 c                         | 0.0 d                           | 0.0 d                   | 0.0 c                     | 0.0 d               |
|                | 5             | 65.0 (8.1) b                  | 35.0 ab                       | 2.0 b                           | 1.5 b                   | 0.6 a                     | 73.5 (8.6) b        |
|                | 10            | 85.0 (9.2) a                  | 32.5 b                        | 2.5 a                           | 1.7 a                   | 0.7 a                     | 81.5 (9.1) a        |

Figures in parenthesis indicate square root transformed data. In a column, means followed by common letters are not significantly different from each other at 1 % of level of probability by DMRT.

For several reasons, cytokinin has often been considered to be a major importance in cormel development process. Firstly, cytokinins are known to stimulate cell division (Skoog and Miller, 1957); secondly, there are indications that it inhibits cell elongation, while promoting cell expansion (Scott and Liverman, 1956). These phenomenon are required for cormel formation and development.

### Combined effect of BAP and sucrose

The combined result of BAP and sucrose for cormel formation is presented in Table 3. Cormel formation did not occur in most of the treatments except 10 mg/l BAP + 10% sucrose (45.0%), 5 mg/l BAP + 15% sucrose (65.0%) and 10 mg/l BAP + 15% sucrose (85.0%). The cormel formation was the earliest (32.5 DAC) in 10 mg/l BAP + 15% sucrose, which was followed by 5mg/l BAP + 15% sucrose (35.0 DAC). The maximum cormel was obtained from 10 mg/l BAP + 15% sucrose (2.5) (Fig. 1a) and the minimum was with 10 mg/l BAP + 10% sucrose (Fig. 1b). The size of cormel was the highest with 10 mg/l BAP + 15% sucrose (0.7 cm diameter), which on weight basis was 1.7 mg. The DM % was also higher for larger cormel (81.5). The detached cormels were shown in Fig. 1c. These results suggested that medium components are essential for cormel formation. In this experiment BAP and sucrose both promoted cormel formation, which is in accordance with previous work on potato (Ivan *et al.*, 1995; Khuri and Moorby, 1996) and on taro (Zhou *et al.*, 1999).



**Fig. 1. (a-c) : Cormel production in Mukhi Kachu (a) (sucrose 15% + BAP 10 mg/l) (b) (sucrose 10% + BAP 10 mg/l) (c) Detached cormels ready for planting in Mukhi Kachu.**

### References

- Anonymous. 2012. Year book Agricultural statistics of Bangladesh. Bangladesh Bureau of Statistics. P. 99.
- Chandra, R., J. H. Dodds and P. Tovar. 1988. *In vitro* tuberisation in potato. Newslet. Intl. Assoc. Plant Tissue Cult. **55**: 10-12.
- Chandra, R., G. R. Randhawa, D. R. Chaudhari and M. D. Upadhyya. 1992. Efficacy of triazole for *in vitro* micro-tuber production in potato. Potato Res. **35**: 339-341.
- Denham, T. P., S. G. Haberle, C. Lentfer, R. Fullagar, J. Field. M. Therin, N. Porch, and B. Winsborough. 2003. Taro cultivation. Origins of Agriculture at Kuk Swamp in the Highlands of New Guinea Science **301**: 189-193.

- Hossain, M. J., M. A. I. Khan and M. A. Hoque. 1998. Effect of IBA and NAA on rooting of potato stem cuttings. *J. Indian pot. Assoc.* **25**(1-2): 53-56.
- Ivan, G., M. Jiri, V. Josef, O. Milos, and A. V. O. Henri. 1995. The effect of an elevated cytokinin level using the *ipt* gene and N<sub>6</sub>-Benzyladenine on single node and intact potato plant tuberization *in vitro*. *J. Plant Growth Reg.* **14**: 143-150.
- Khuri, S and J. Moorby. 1996. Nodal segments or microtubers as explants for *in vitro* microtuber production of potato. *Plant Cell Tiss. Org. Cult.* **45**: 215-222.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-491.
- Pelacho, A. M. and A. M. Mingo-Castel. 1991. Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. *Plant Physiol.* **97**: 1253-1255.
- Priyakumari, I. and V. L. Sheela. 2005. Micropropagation of gladiolus cv. Peach Blossom through enhanced release of auxiliary buds. *J. Trop. Agric.* **43**(1-2): 4750.
- Rahim, M. A. and M. Alamgir. 1995. Effect of paclobutrazol and gibberellic acid on the growth of late planted Mukhikachu (*Colocasia esculenta*). *Progressive Agriculture* **6**(1): 39-46.
- Rashid, M. M. 1990. Varietal improvement of tuber crops in Bangladesh up to date progress and future possibilities. *In: Plant breeding in Bangladesh. Proceed of 1<sup>st</sup> National symposium held in June 5-7, 1989.* Pp. 253-261.
- Scott, P. A and J. L. Liverman. 1956. Promotion of leaf expansion by kinetin and benzyl aminopurine, *Plant Physiol.* **31**: 321-322.
- Skoog, F. and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *In: Symp. Soc. Expt. I. Bot.* **11**: 118-130.
- Tovar, P., L. Estrada, Schilde-Rentschler and J. H. Dodds. 1985. Induction of *in vitro* potato tubers. *CIP Circular* **13**: 1-4.
- Wang, P. J. and C. Y. Hu. 1982. *In vitro* mass tuberization and virus-free seed potato production in Taiwan. *American Potato J.* **59**: 33-37.
- Yamamoto, Y and O. Matsumoto, 1992. *In vitro* corm formation and growth habit of propagated seed corm in taro (*Colocasia esculenta* Schott.). *J. Japan. Soc. Hort. Sci.* **61**(1): 55-61.
- Zakaria, M. 2003. Induction and performance of potato microtuber. PhD Dissertation. Deptt. of Hort. BSMRAU. Salna. Gazipur. 188p.
- Zhou, Su. P., Y, K. He and S. J. Li. 1999. Induction and characterization of *in vitro* corms of diploid taro. *Plant Cell, Tissue and Org. Cult.* **57**: 173-178.