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Regeneration plantlets from somatic embryos of tea plant (*Camellia sinensis* L.)

Nguyen Van Thiep

Northern Moutainous Agriculture and Forestry Science Institute (NOMAFSI), Vietnam

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Somatic embryo of tea obtained from cotyledon of Shan Chat Tien vaiety gave a potential of germinating and developing mature tea plants. Using MS medium, supplementing with different concentrations of BAP and IBA in different culture stages created the new young teas. Somatic embryo was cut into small explants and then cultured on MS medium adding 8 mg/1BAP. As a results, 69.34% of explants produced new bud after 20 days. At the multiplying stage, nodal explants with one shoot were cultured in a medium consisted of 3 mg/l BAP and 0,3 mg/1IBA. Consequently, the height of young tea plants were 4.82 cm with 5-7 leaves and obtained 3.53 times higher for multiplying rate after 60 days. Stem explants, which had 3-4 leaves with 2.5-3 cm length were cultured in MS medium including 3 mg/1IBA. As a result, most of roots occurred and grown well after 3 weeks.

Key words: Tea, *Camellia sinensis*, Somatic embryo, cotyledon, MS medium.

Introduction

Tea has been playing an important role in midland and mountaous areas of Vietnam and it is the key economic industrial tree in some provinces in this region. Moreover, tea production is sound in terms of economy, social and enviroment. Therefore, areas, productivity and exportable tea quantity have been rapidly increasing in current years. However, yield and value of tea in Vietnam is low compared to those in the world. There are several reasons causing these limitations, and major cause is that Vietnam has not had good varieties to meet the demand of practical tea production. In addition, material sources for tea variety production in Vietnam are modest and multipling capacity of new tea varieties by cutting is limited and because of taking a certain time for mother tea plants to mature in order to cuttings. In order to enhance yield, productivity and quality of Vietnam tea, creating new variety is a vital way, together with cultivation techniques and processing technologies. Sellecting, importing and hybridize are main solutions in tea variety production. Adopting biotechnology in hybriding, multiplying is an effective way to

shorten time in producing new tea varieties. Tissue culture technologies can create perfect tea plants from the modest initial mother tea plants and quickly multiply them accoeding to several researchers (Aula and Dodd, 1998; Das *et al.,* 2005; Kato, 1985; and Zhou *et al*., 2005). This paper introduced the research results in multiplying somatic embryos, which can be applied in oder to enhance hybriding and multiplying new tea varieties.

Materials and methods

Somatic embryos are produced from cotyledon leaves of tea var Shan Chat Tien (*Camellia sinensis* var Shan) using MS medium and growth regulators. To research on bud creating medium from somatic embryos, the somatic embryos are formed from cotyledon leaves of tea seeds, which were selected from tea varieties of NOMAFSI. Somatic embryos were transferred into medium at pH 5.8 with following growth stimulus and treatments as follows:-treatment 1: MS + 2mg/l BAP + 30g sugar + 6g Agar, treatment 2: MS + 4mg/l BAP + 30g sugar + 6g Agar, treatment 3: MS + 6mg/l BAP + 30g sugar + 6g Agar, treatment 4: MS + 8mg/l BAP + 30g sugar + 6g Agar and treatment 5: MS + 10mg/l BAP + 30g sugar + 6g Agar. A ratio of bud break of somatic embryos and shoot growth capacity were observed.

To research multiplying tea shoots, when the height of shoots was 2.5-3 cm., they were cut and placed into medium containing IBA and BAP at pH5.8 in order to rapidly multiply the number of shoots and the treatments were as follows:- treatment 1: MS + 1mg/l BAP + 0,1mg/l IBA + 30g sugar + 6g Agar, treatment 2: MS + 2mg/l BAP + 0,2mg/l IBA + 30g sugar + 6g Agar, treatment 3: MS + 3mg/l BAP + 0,3mg/l IBA + 30g sugar + 6g Agar, treatment 4: MS + 3mg/l BAP + 0,4mg/l IBA + 30g sugar + 6g Agar and treatment 5: MS + 4mg/l BAP + 0,5mg/l IBA + 30g sugar + 6g Agar. The shoot growth capacity and multiply coefficient were observed.

To research of creating young tea roots, when the shoot length was 2.5-3 cm., they were cut and cultured in root creating medium at pH 5.8 with following treatments:- treatment 1: MS + 1mg/l IBA + 30g sugar + 6g Agar, treatment 2: MS + 2mg/l IBA + 30g sugar + 6g Agar, treatment 3: MS + 3mg/l IBA + 30g sugar + 6g Agar and treatment 4: MS + 4mg/l IBA + 30g sugar + 6g Agar. Numbers of roots were formed and time period for root growth was recorded.

All the experiments were maintained in 2000 lux of light intensity, light time was 12 hours and temperature was 25^0C.

Results and discussions

Formation of tea buds from somatic embryos

Tea seeds were chosen in order to create somatic embryos that are seeds of Shan Chat Tien Tea variety from NOMAFSI. Original somatic embryos (Fig.1a) were divided into smaller parts (Fig.1b) and transferred into creating bud medium. The results of bud break formation rate are given in Table 1.

Table 1. Impacts of BAP concentration on bud break from tea somatic embryos

Time	T1 (%)	T2 (%)	T3 (%)	T3 (%)	T5 (%)
10 days after culture	0.00	0.00	0.00	0.00	0.00
12 days after culture	0.54	1.76	2.64	5.08	2.12
15 days after culture	34.62	46.55	58.82	90.22	71.15
20 days after culture	38.84	50.27	64.18	96.34	78.67

LSD_{05}= 12.34

An explant of somatic embryos contained mass of many somatic embryos. After 12 days, somatic embryos mass started breaking buds. At the beginning, each somatic mass produced one bud, later on produced 1-2 buds. Bud break prolonged from 12^{th} day going forward and concentrated producing buds from 15 to 20 days after transferring. There were different rate of bud break at different BAP concentration. The increase of BAP concentration made the change of bud break rate. At 20^{th} day after culturing, 4 mg and 6mg/1BAP provided the similar rate of bud break. The highest rate of bud break was 3 mg/1BAP. In addition, most of somatic embryos masses gained 96.34% of the bud break. However, if increasing BAP concentration up to 10 mg/l, rate of bud break reached only 78.67%. As a result, Tapan et al (1986) and Zhou et al, 2005) stated on their works of induction of in vivo somatic embryos from tea and factors affecting the germination rate and form in culture of tea.

In conclusion, the best time for bud break rate from somatic embryos, which were made from Shan Chat Tien seeds was from 15 to 20 days after culturing and 3 mg/l BAP was the best medium.

Shoot multiply

After buds growing until 3-4 cm, they were cut into shorter parts of 0,5-1,0 cm. Each part, which had a dormant bud at the axil was placed into shoot multiplying medium after 30 days. The bud stumps were re-cultured in creating bud medium to produce more shoots. The aim of this experiment was to

identify bud multiplying coefficient and shoot growth capacity (Table 2). Result was similar to the report of Kato (1996).

Table 2. Impacts of growth stimulus concentration on tea bud growth after culturing 60 days

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**LSD_{05}**
Height (cm)	1.24	2.58	4.82	4.34	2.74	1.36
Number of leave	1.64	2.84	5.71	5.38	3.28	1.18

There were differences among different treatments on shoot growth of Shan Chat Tien Tea. There was a slow growth (average height of 1.24-2.58 cm and 1-3 leaves) at 1 mg/l and 2 mg/l of BAP and 0.1 mg and 0.2 mg/l of IBA concentration. Increasing BAP concentration up to 3mg/l helped shoots better growth (4. 34 cm height and 5.38 leaves). However, if increasing BAP and IBA up to 4 mg/l, bud growth kept unchanged and if rising up to 5 mg/l, the growth rate went down. Each stem part with one axil bud also cultured in order to identify multiplying coefficient. The experiment was tested at 3 replicates and 10 axil bud per glass pot (Table 3). Kato (1985) also reported on regeneration of plantlets from tea stem callus.

Table 3. Impacts of growth stimulus concentration on tea shoot multiplying coefficient after 60 days culturing

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**
Initial number	30	30	30	30	30
New creating number	52	64	106	95	81
Coefficient (time)	1.73	2.13	3.53	3.16	2.70

LSD_{05}= 0,42

After culturing, the trend of new shoots always come from the nearest surface of multiplying medium, the more new shoots produced the better tea multiply coefficient. After culturing 2 months, T1 and T2 (BAP concentration of 1 and 2 mg/l) gave the similar coefficient (from 1.73 to 2.13 times). The highest coefficients of shoots multiply were T3 and T4 (3.16 to 3.53 times). However, when increasing BAP concentration up to 5 mg/l and 0.5 mg/l, multiplying coefficient reduced to 2.7 times.

To conclude, the most suitable bud multiplying medium of Shan Chat Tien Tea variety was added 3 mg/l BAP and 0.3 mg/l IBA.

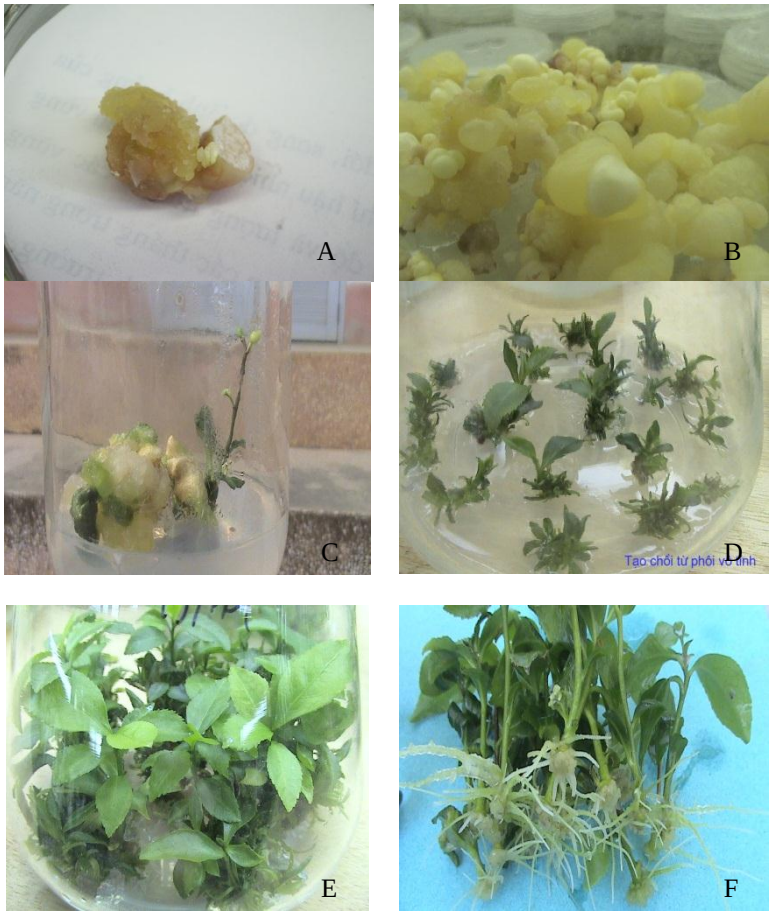

Fig. 1. Formation of new young tea from somatic embryos
A – Somatic embryos formed from cotyledon, B – Somatic embryos were cut into smaller parts
C – Bud break was developed from somatic embryos, D – shoots were separated to multiply,
E – Multiplying shoot stage and F – Tea roots and mature young tea plants were formed

Rooting

When the necessary number of young tea plants reached demand, 2.5 to 3 cm length of shoots was cut and transferred into MS medium adding IBA.(Table 4). Similar result was shown by Satoru *et al.* (1993) for the rooting of tea plants from in vitro culture for acclimation.

Table 4. Impacts of growth stimulus concentration on root creation of cutting (% root creation)

Time periods	T1	T2	T3	T4
After culture 10 days	0.00	0.00	0.00	0.00
After culture 13 days	0.00	1.38	4.22	0.00
After culture 16 days	3.84	31.82	50.37	3.27
After culture 19 days	16.02	60.71	71.06	16.66
After culture 22 days	21.11	68.27	82.54	19.38

LSD_{05}= 14.26

The roots occurred at the 13th day onward and more rapidly grow from 16 to 19 days after culturing. After occurring one week, tea root prolonged 1-3 cm more. T3 (3 mg/l IBA) had the highest root creation (82,54% after 22 days); the more or less IBA concentration created lower rate of tea root number (T1 and T4). Furthermore, IBA concentration also influenced on the tea root growth, Table 5 indicated the capacity of the tea root growth at different hormone concentration.

Table 5. Impacts of IBA concentration on root growth

Parameter	T1	T2	T3	T4	LSD_{05}
Numbers of roots	2.00	4.86	7.50	2.10	0.64
Root length (cm)	1.05	2.67	3.24	1.22	0.18

The highest numbers of roots and longest root length (7.5 roots per bud and 3.24 cm root length) were observed in T3. T1 and T4 are equivalent value and lowest gains. Therefore, 3 mg/1 IBA concentration was appropriated for the root creation and root growth in culture tissues of the Shan Chat Tien Tea variety. With this, Akula and Dodd (1998) stated that direct somatic embryogenesis in a selected tea clones was studied from nodal explants. Moreover, Das *et al.*(2005) studied on tea somaclones with high yield and quality.

Conclusion

Conclusions were based on above experimental results to regenerate tea var. Shan Chat Tien from its somatic embryos as following: MS medium supplementing with 8 mg/l BAP was the most appropriate to stimulus new bud formation from somatic embryos. In this medium, 96.34 % of shoots was formed from somatic embryos after 20 days. In the bud multiplying stage, after

2 months and gaining 4-6 cm height, 3 mg/l BAP and 0.3 mg/l IBA in MS medium was proved to be the most suitable invitro tea-multiplying medium, of which multiplying coefficient gained 3.53 times per transference. The tea roots started occurring after 13 days culturing and concentrated root formation from 16 to 22 days after culturing. MS medium adding 3 mg/l IBA was the most appropriate for root growth in the culture tissue. In this medium, 82.54% of shoots produced roots.

References

Akula, A. and Dodd, W.A. (1998). Direct somatic embryogenesis in a selected tea clone, "TRI-2025" *(Camellia sinensis (L.) O. kuntze* from nodal explants. Plant Cell Reports 17: 804-809.

Das suresh Chandra, Dutta Ranjan Kumar and Bordoloi Biswaiyoti (2005). Tea somaclones with high yield and quality potential. 2005 international symposium on innovation in tea science and sustainable development in tea dustry. Nov. 11-15 Hangzhou China.

Kato, M. (1985). Regeneration of plantlets from tea stem callus. Japan, J. Breedding. 35: 317-322.

Kato, M. (1996). Somatic embryogenesis from immature leaves of in vitro grown tea shoots. Plant cell report, Yamaguchi University, p. 920-923.

Kazumi Furukawa and Junichi Tanaka (2004). Makura-Ck2: A tea strain with a hight somatic embryogeneis. NIVTS, J. 6: p.109-115.

Satoru matsumoto, Masae kwada and Sadaaki Kondo (1993). Rooting of tea (Camellia sinensis) plants from in vitro culture for Acclimation. Japan, J. Breedding. 6: 1-10.

Shinsaku Nadamitsu and Yoshiaki Andoh (1986). Interspecific Hybrids between *Camellia vietnamnsis and C. chrysantha* by Cotylendon Culture. J. Breedding. 36: p. 309-313.

Tapan Kumar Mondal, Amita Bhattacharya, Madhu Sharma and Paramvir singh Ahuja (2001). Induction of in vivo somatic embryos from tea *(Camellia sinensis)* cotylendons. Current Science 81(3): p. 297-300.

Zhou Jian, Cheng hao and Wang liyuan (2005). Factors affacting the germination rate and the form in the culture of tea *(Camellia sinensis)* young embryo international symposium on innovation in tea science and sustainable development in tea dustry. Nov. 11-15 Hangzhou China, p. 594-601.

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