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The Study Effect of Cytokinin Hormone Types on Length Shoot *in vitro* Culture of Tea (*Camellia sinensis* L.)

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Astract: Tea (*Camellia sinensis* L.) is one beverage plant. Tea consumption estimate ninety thousand ton in Iran yearly. In order to study the effect of Adenine (An), Benzyl Adenine (BA), Kinetin (Kn) and 6-(γ,γ-dimethylallylamine)purine (2ip) hormones of *in vitro* culture of tea (*Camellia sinensis* L.), an experiment as RCBD with five replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. The culture bed was contained MS, sucrose (3%) and agar (75%). The results showed that cytokinin hormone types on length shoot *in vitro* culture of tea had a significant difference in 1% probability level. The highest length shoot *in vitro* culture of tea was obtained with application 3mg/L 2ip hormone (12.8 mm). The lowest length shoot *in vitro* culture of tea was obtained with application 3mg/L An hormone (4.14 mm). due to contamination of tea to nematode, mass production, produce for callus and extract of caffeine *in vitro* culture is signification important in tea.

Key words: Tea · *Vitro* culture · Cytokinin · Length shoot

INTRODUCTION

Vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells, a concept proposed by Haberlandt [1] and unequivocally demonstrated, for the first time, by Steward *et al*. [2]. Tissue culture is alternatively called cell, tissue and organ culture through *in vitro* condition [3]. It can be employed for large-scale propagation of disease free clones and gene pool conservation. Ornamental industry has applied immensely *in vitro* propagation approach for large-scale plant multiplication of elite superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide, especially in the developing countries due to cheap labour costs. However, micropropagation technology is more costly than conventional propagation methods and unit cost per plant becomes unaffordable compelling to adopt strategies to cut down the production cost for lowering the cost per plant [4].

Conventional tea breeding is well established, though time-consuming and labors intensive due to its perennial nature and long gestation period (4-5 years). Additionally, tea breeding has been slowed by lack of reliable selectionthe criteria. Vegetative propagation is standard, yet limited by slow multiplication rate, poor survivability of some clones and need for copious initial planting material [5]. Seed-borne plants are heterogeneous due to their highly allogamous nature; consequently, it is difficult to maintain their superior character. Research on tea micro-propagation has recently focused on exploring the potential of somatic embryogenesis as a more efficient means of plant manipulation and regeneration [5]. Somatic embryos have been used as a model system to understand the mechanisms regulating plant embryogenesis, being an alternative for the propagation of plants with high rates of multiplication, with relevance in tree improvement programmes [6]. To induce calli from tea explants and to cultivate the calli to produce plants, various kinds of media have been designed. Generally, it would be better to use only one or two kinds of basal media in combination of different kinds and concentrations of phytohormones [7]. The most suitable medium composition should be optimized afterwards in order to obtain higher level of products as well as higher growth rate. Plant hormones play essential roles in plant metabolism and can influence cell cycle proteins [8]. Tea (*Camellia sinensis* L.) is the oldest non-alcoholic caffeine-containing beverage crop in the world and health benefits attributed to tea consumption are well proven [9]. Tea is one of the most important plantation crops in the world. At present, the most parts of tea fields are planted

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with various clonal propagated cultivars. Fields performance of micro-propagated tea plants and the impact of cultural operations on growing tea plants have been reported previously [10]. For these reasons, tea plants were introduced into tissue culture for plant regeneration and genetic manipulation. Unlike other crops, reports are not available on the basic physiology of micro-propagated tea plants [10].

Due to contamination of tea to nematode, mass production, produce for callus and extract of caffeine *in vitro* culture is signification important in tea. The objective of the present research was to enhance the development of a culture method of tea (*Camellia sinensis* L.) with application cytokinin hormone types.

MATERIALS AND METHODS

In order to study the effect of Adenin (An), benzyladenine (BA), Kinetin (Kn) and 6-(γ,γ-dimethylallylamine) purine (2ip) hormones of *in vitro* culture of tea (*Camellia sinensis* L.), an experiment as RCBD with five replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. Tea apex meristems were obtained from 2-years-old rooted cuttings of tea plants grown in a green house, with a 16 h photoperiod and photosynthetic photon flux of 101.5 imol m-2 S-1 (400-700 nm) at the plant level. Leaf samples were washed with tap water and surface sterilized in a drop of liquid detergent for 1 min, followed by three rinses in sterile distilled water. Then, they were re-sterilized with 70% ethanol for 30s and with 20% sodium hypochlorite for 20 min, followed by three rinses in sterile distilled water, all under laminar flow. Discs of ca. 0.5 cm2 diameter were cut from the leaves and were cultured on MS [11] basal medium supplemented with (in mg/L) Indole-3-Butyric Acid (IBA), 3; Adenin (An), 3; 6-benzylaminopurine (BA), 3; Kinetin (Kn), 3; and 6-(γ,γ-dimethylallylamine) purine (2ip), 3 (Figure 1). PH was adjusted to 5.8 before adding 3% (w/v) sucrose and 75% (w/v) agar. Autoclaving was done for 20 min at 120°C and 150 KPa (Fazelienasab *et al*., 2004). The cultures were maintained with a 16 h photoperiod at 27±3°C.

Data were analyzed by using SAS software. The Duncan's multiple range tests was used to compare the means at%5 of significant.

RESULTS AND DISCUSSION

Results of variation analysis show that (Table 1), cytokinin hormone types on shoot length *in vitro* culture of tea had a significant difference in 1% probability level. The highest shoot length *in vitro* culture of tea was obtained with application 3mg/L 2ip hormone (12.8 mm) (Figure 2). The lowest shoot length *in vitro* culture of tea was obtained with application 3mg/L An hormone (4.14 mm) (Figure 2).

In plant tissue cultures, cytokinin is required for callus growth (an undifferentiated, tumor-like mass of cells) and ratio of cytokinin to auxin is important to determine the fate of the callus. Moreover, cytokinin is known to promote the light-induced formation of chlorophyll and conversion of etioplasts to chloroplasts [12]. This greening process was also observed in our

Table 1: Analysis of variance of cytokinin hormone types on shoot length *in vitro* culture of Tea

Source of variance	df	Length shoot
Replication	4	0.86
Cytokinin hormone types	3	68.55**
Error	12	0.037
C.V%		3

** and * respectively significant in 1% and 5% area

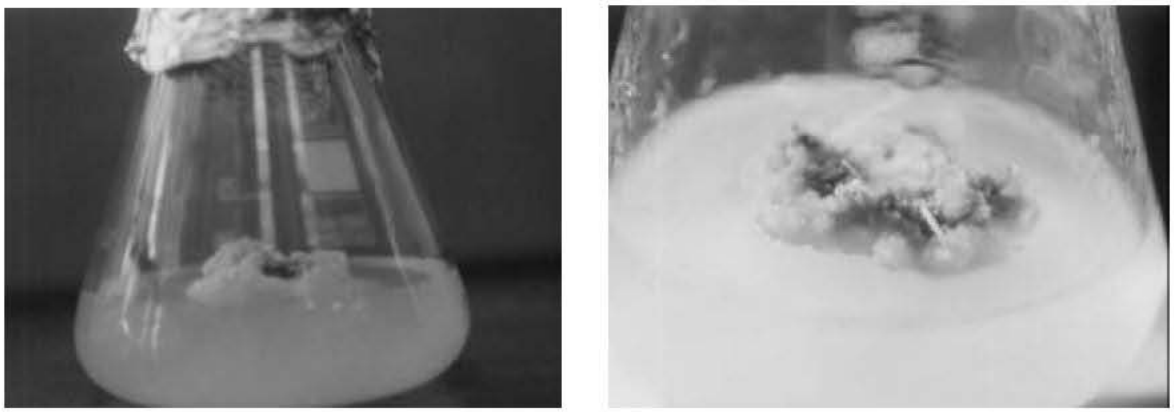

Fig. 1: Formation of shoot and root after *in vitro* culture on MS basal medium

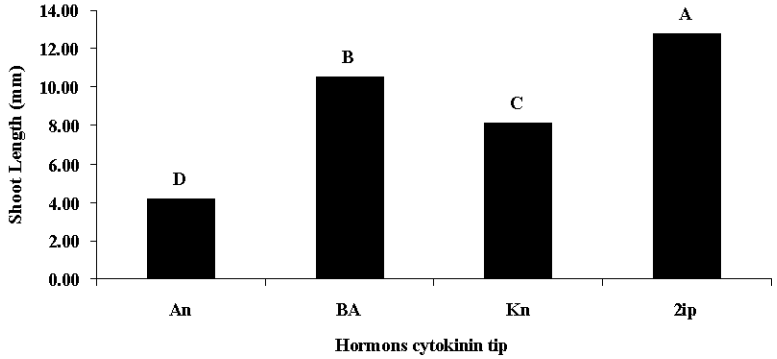

Fig. 2: The effect of cytokinin hormone types on shoot length *in vitro* culture of Tea

experiments. The effect of abscisic acid (ABA) and excess benzyladenine (BA) on the formation of shoot from tea (*Camellia sinensis* L.) leaf was investigated [13], As a result, we observed root formation in the transferred calli to B5 medium after one month. Subsequently, the calli were transferred to the aforesaid B5 medium supplemented with ABA (2 mg/L) and excess BA (400 mg/L) to form shoot. The calli turned green and showed differentiation of globular and heart embryos when transferred to the modified B5 medium, without formation of shoot. These findings showed that the applied concentration of ABA may cause inhibition of conversion of globular and heart embryos to shoot. The increased level of BA, however, was not able to ameliorate the effect of ABA. The effects of two types of auxin (IBA and NAA), an auxin pretreatment and the physical form of the culture medium on *in vitro* rooting of micro shoots of tea (*Camellia sinensis* L.) was studied [14], The incorporation of auxin in the rooting medium did not bring about the formation of roots but caused basal callus formation. Microshoots were successfully induced to root with a pretreatment of IBA (50 mg/litre dip for 3 h) followed by culture of shoots in auxin-free half-strength MS liquid medium with continuous agitation. Shake cultures accelerated the emergence of root and increased the number and the length of the roots compared to static cultures. An initial dark period of 7 or 14 days had no significant effect on root initiation, over cultures exposed to a continuous 12 h photoperiod. Aye *et al.* [15] indicated the invention provides regeneration method for tea plant from leaf explants via callus phase, where in explants were cultured on basal MS medium supplemented with 3,5,7 mg/L 2,4-D (2,4-dichloro phenoxy acetic acid).Callus induction was observed by 5mg/L 2,4-D for a period of 4-6 weeks. Shoot initiation and rhizogenesis from the leaf- derived callus of tea has been standardized using the various concentrations of 1,3,5,7 mg/L BAP only and combination with 1mg/L IBA. It is now accepted that the single concentration of 3mg/L BAP was the best for shoot initiation and rhizogenesis calli were obtained by combination of 1mg/L IBA and 3mg/L BAP.

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