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EVALUATION OF THE EFFECT OF MS MEDIUM LEVELS ON ROOTING IN MICRO CUTTINGS OF TEA (*Camellia sinensis* L.) UNDER IN-VITRO CULTURE CONDITION

Sirous Bidarigh[1] and Ebrahim Azarpour[2]
[1]Department of Agriculture, Lahijan Branch, Islamic Azad University, Lahijan, Iran
[2]Young Researchers Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran
E-Mail: Bidarig@yahoo.com

ABSTRACT

Tea [*Camellia sinensis* (L.)] is one of the most important beverage crops in the Iran. There has been a steady demand for production and supply of cuttings of different varieties for tea gardens in Iran. In order to study the effects of MS medium levels (full MS, half MS and redundancy MS) with 1mg/L indole-3-butyric acid (IBA) hormone on rooting in micro cuttings of tea, an experiment as RCBD with four replications was conducted during 2010 at Research Laboratory of Faculty of Agriculture, Lahijan University in Iran. The culture bed were contained MS levels (full MS, half MS and redundancy MS), 1mg/L IBA hormone, sucrose (3%) and agar (75%). The results show that MS medium levels with IBA hormone on root length and root number in micro cuttings of tea had a significant difference in 1 % probability level. The highest root length (17.22 mm) and root number (4.93) in micro cuttings of tea were obtained with application redundancy MS medium (25% macro element) and 1mg/L IBA. The lowest root length (3.17 mm) and root number (0.68) in micro cuttings of tea were obtained with application full MS medium (25% macro element) and 1mg/L IBA.

Keywords: tea, micro cutting, MS medium levels, root length, root number.

INTRODUCTION

A factor that must be considered when propagating a plant species in vitro is the type of medium to use. The medium is comprised of basal salts and essential nutrients that a plant requires for proper growth and development. In vitro culture techniques involving the use of high- and low-salt media, such as Murashige and Skoog (Murashige and Skoog, 1962; Daniel Lineberger, 2009) (MS).

In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plantcells, a concept proposed by Haberlandt (1902) and unequivocally demonstrated, for the first time, by Steward *et al*. (1985). Tissue culture alternatively called cell, tissue and organ culture through in vitro condition (Debergh and Read, 1991). 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 labor costs. However, micro propagation 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 (IAEA-TECDOC, 2004).

Single leaf-node cuttings conventionally propagate cultivated tea. This method of production becomes limiting when large numbers are required from new clones of which only very few stock plants are available. Moreover, the process in establishing tea plants from cutting is lengthy and labor-intensive, rendering it ineffective fore rapid dissemination of new clones for large-scale commercial plantings (Tahardi, 2003). Conventional tea breeding well established, though time-consuming and labors intensive due to its perennial nature and long gestation period (4-5 years). Additionally, tea breeding had been slowed by lack of reliable selection criteria. Vegetative propagation is standard, yet limited by slow multiplication rate, poor survivability of some clones, and need for copious initial planting material (Tahardi *et al*., 2003). 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 (Sghaier *et al*., 2009). Somatic embryos had 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 programmers (Misawa, 1994). 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 (Sánchez *et al*., 2005). 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 (Mondal, 2004). Tea (*Camellia sinensis* L.) is the oldest non-alcoholic caffeine-containing beverage crop in the world and health benefits attributed to tea consumption well proven (Marimuthu and Raj Kumar, 2001). Tea is one of the most important plantation crops in the world. At present, the most parts of tea fields was

planted 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 (Smart, L., 2008). 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 (Smart, L., 2008).

The objective of the present research was to enhance the development of a rooting in micro cuttings of tea (*Camellia sinensis* L.) with application MS medium levels and IBA hormone levels.

MATERIALS AND METHODS

In order to study the effects of MS medium levels (full MS, half MS and redundancy MS) with 1mg/L indole-3-butyric acid (IBA) hormone in vitro culture of tea (*Camellia sinensis* L.), an experiment as RCBD with four 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 µmol 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 (Murashige and Skoog, 1962) basal supplemented with 1mg/L Indole-3-Butyric Acid (IBA) (Figure-1). The macro element (N.P.K) levels on full, half and redundancy MS medium were included (M1=100%, M2=50% and M3=25%). 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 analyses were analyzed by using SAS software. The Duncan's multiple range tests was used to compare the means at 5% of significant.

RESULTS AND DISCUSSIONS

Results of variation analysis show that (Table-1), MS medium levels with IBA hormone on root length of tea had a significant difference in 1% probability level. The highest root length (15.06 mm) in micro cuttings of tea was obtained with application redundancy MS medium (25% macro element) and 1mg/L IBA, and the lowest root length (4.30 mm) was obtained full MS medium (100% macro element) and 1mg/L IBA (Figure-2). Results of variation analysis show that (Table-1), MS medium levels with IBA hormone on root length of tea had a significant difference in 1% probability level. The highest root number (5.38) in micro cuttings of tea was obtained with application redundancy MS medium (25% macro element) and 1mg/L IBA, and the lowest root number (0.54) was obtained with application full MS medium (100% macro element) and 1mg/L IBA (Figure-3). Result of analysis of liner (Figure-4) show that between root length and root number a positive and very significant correlation (R^2=0.93).

Micro propagation generally involves four distinct stages: initiation of cultures, shoot multiplication, rooting of in vitro grown shoots, and acclimatization. The first stage: culture initiation depends on explant type or the physiological stage of the donor plant at the time of excision. Explants from actively growing shoots were generally used for mass scale multiplication. The second stage: shoot multiplication is crucial and achieved by using Plant Growth Regulators i.e., auxin and cytokinin. The third stage: the elongated shoots, derived from the multiplication stage, are subsequently rooted either ex vitro or in vitro. In some cases, the highest root induction occurs from excised shoots in the liquid medium when compared with semi-solid medium. The fourth stage: acclimatization of in vitro grown plants is an important step in micro propagation (Rout *et al*., 2006; Briggs and McCulloch, 1964). Rooting the shoots produced in vitro or micro cuttings, has been achieved through in vitro and ex vitro or non-sterile, conditions (Lineberger, 1983). In some cases, micro cuttings root better in vitro environments. In vitro rooting was superior to ex vitro rooting for Prunus x 'Hally Jolivette'. Also, in some cases in vitro, it is beneficial to make changes to the medium. Li and Eaton (1984) reported that rooting 'Marechal Foch' grapevine in half-strength MS salts was superior to rooting in full strength MS salts. But in other cases, superior rooting can result under ex vitro conditions. Rooting of *Halesia carolina* L. non-sterile conditions was reported to be fart superior to rooting in sterile conditions (Gunasekare and Evans, 2000).

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. 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*. (2008) 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 had been standardized using the various concentrations of 1, 3, 5, 7 mg/L BAP only and

combination with 1mg/L IBA. It was 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. Martin *et al*. (2003) succeeded in direct shoot bud regeneration fromlamina explants of *Anthurium andraeanum* on MS medium fortified with 1.11 µMBA, 1.14 µM IAA and 0.46 µM Kn. Furthermore, the regenerated shoots were rooted on half-strength MS medium supplemented with 0.54 µM NAA and 0.93 µM Kn. The regenerated shoots were rooted on half-strength MS medium and successfully transferred to the greenhouse (Murch *et al*., 2003).

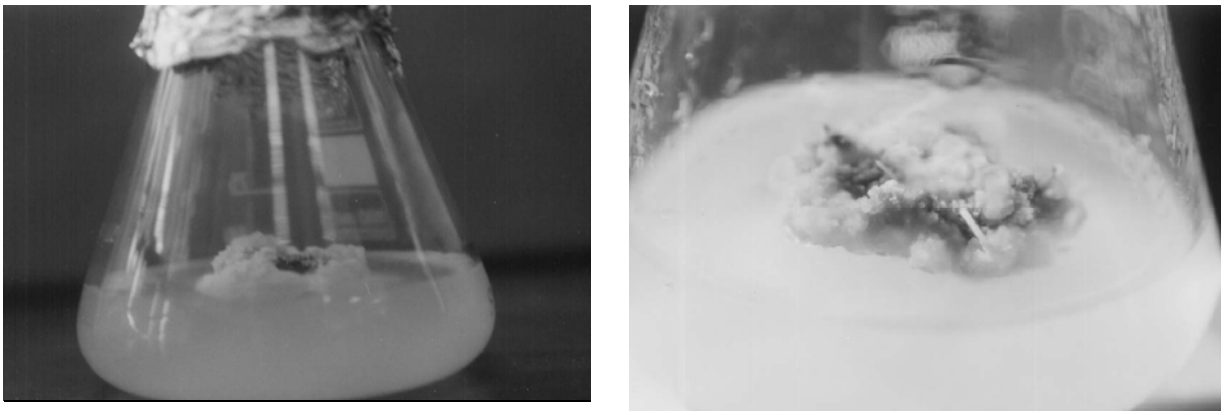

Figure 1. Formation of shoot and root after in vitro culture on MS basal medium.

Table-1.

Sours of variance	df	Root length	Root number
Replication	3	0.94	0.075
MS medium levels	4	162.08**	29.41**
Error	12	0.088	0.016
C.V %		3	4.15

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

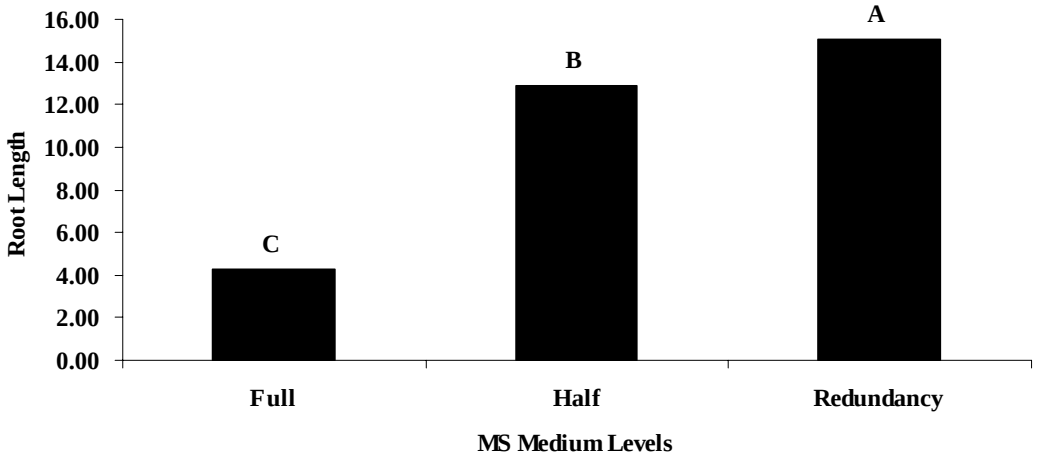

Figure-2. The effect of MS medium levels on root length in micro cuttings of Tea.

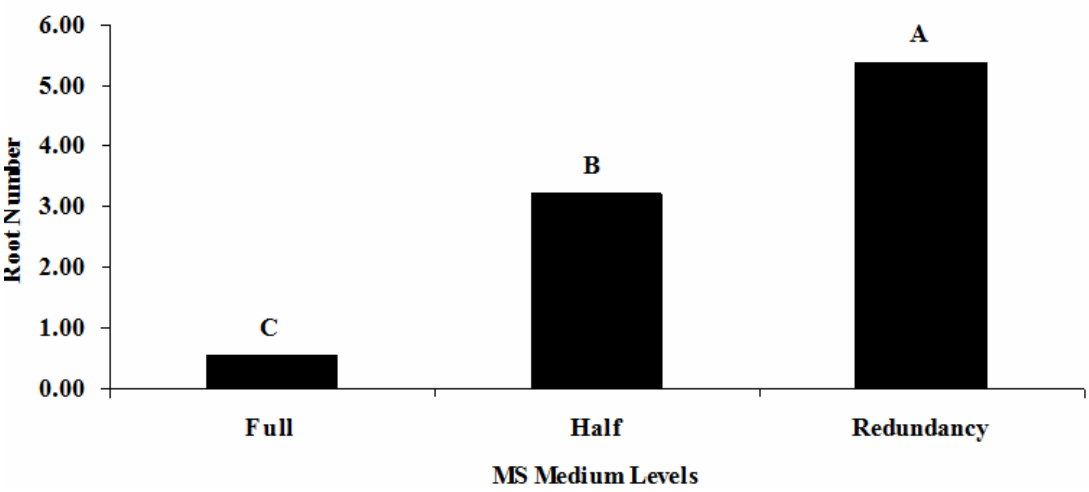

Figure-3. The effect of MS medium levels on root number in micro cuttings of Tea.

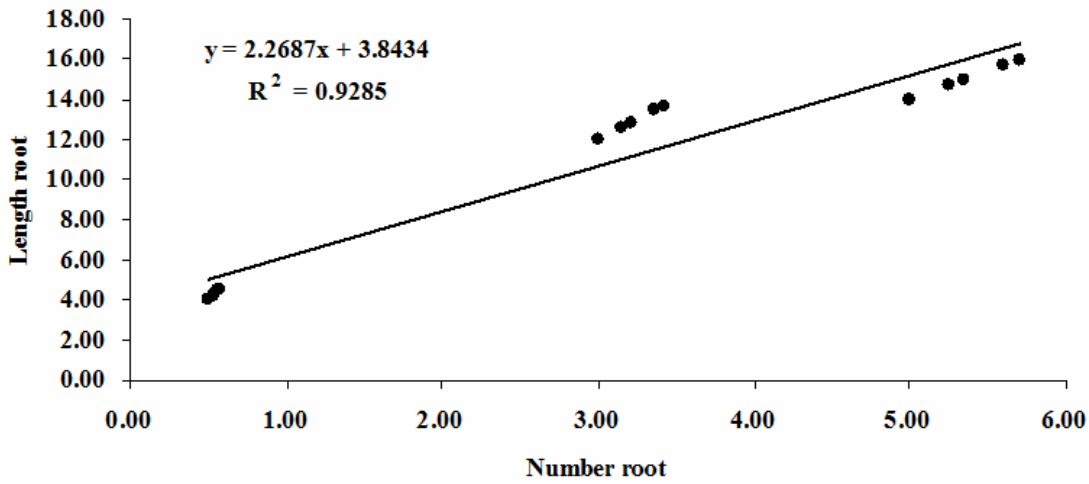

Figure-4. Correlation between root number and root length in micro cuttings of Tea.

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