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Research Paper

Determination of Potting Media for Effective Acclimatization in Micropropagated Plants of Tea Clone Iran 100

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Abstract: Establishment of *in vitro* plantlets in the greenhouse on various additives of soil mixtures is considered as one of the trickiest phases of micropropagation. In the present research, we report the results of an experiment aimed at optimizing acclimatization of tea clone Iran 100 plantlets (*Camellia sinensis* (L.) O. Kuntze) by using different types and ratios of potting media. Potting media like peat moss, vermiculite and perlite with different ratios were mixed together. This experiment was done in four repetitions using the randomized complete block design. The properties such as survival rate, number of leaves, plant height and root length were measured and recorded in the period of the experiment. The collected data was analyzed by SAS statistical software and the mean values were evaluated by the Tukey Test using the probability of 1 percent. The mixture containing peatmoss + vermiculite + perlite (2:1:1; v/v/v) resulted in increased percentage of plant survival, root length, plant height and number of leaves of tea clone Iran 100 and it is therefore, a recommended medium for growth of the cultivar during acclimatization.

Keywords: *Camellia sinensis*, tissue culture, acclimatized plantlets, peat moss, vermiculite and perlite.

Introduction

Iran is one of the top fifteen tea producing countries in the world. Iran contributes 1% to the total tea produced in the world while, China being the major tea producer contributes 33%. However, according to statistics, the trend shows a severe decrease in tea production in Iran (FAOSTAT, 2010). This trend has been attributed to various reasons; firstly, tea plant has an economic life span of 60-70 years. Most of the existing tea plantations in Iran are over 80-100 years old, hence resulting in low yield and poor quality. Secondly, a large number of existing populations are of seed origin and seed grown tea cultivars are mostly heterozygous. Thirdly, the total area under tea plantation is 34000 hectares in Iran (AzadiGonbad & FatemiChokami, 2009), and it is impossible to increase the area for tea plantation. In view of the above mentioned problems, the best way forward is to replace the old plants with new tea clones. In recent years, tea Clone 100 of Iran was shown to have higher yields and productivity than other clones; also it is highly preferable for black tea production in Iran. However, big scale planting is not possible with traditional propagation methods due to lack of insufficient planting materials as around 18,000 bushes are required per hectare. The increasing demand for tea has led to the use of biotechnological means of increasing productivity. Currently, micropropagation is used to produce identical pathogen-free plants of elite clones and is considered as an advanced system for agriculture, horticulture and forestry (George & Debergh, 2008). In micropropagation, a number of stages are followed. Acclimatization of *in vitro* plantlets is considered as one of the important stages of tissue culture. Usually rooted tea plantlets are transferred to a mixture of peat and soil in a 1:1 ratio, which is the standard procedure while using, misting or fogging units to create humidity (Agarwal, Singh, & Banerjee, 1992; Banerjee & Agarwal, 1990; Jain, Das, & Barman, 1993; Jha & Sen, 1992), but the use of vermiculite and soil in a equal ratio can also work very well (Kato, 1985; Tian-Ling, 1982). The intensity of nitrogen, organic anions, ash alkalinity, pH of soils and cations like Mg, Ca and K, result in determining the effect of unrefined material at soil acidity change (Noble, Zenneck, & Randall, 1996; Pocknee & Sumner, 1997; Tang & Yu, 1999; Yan & Schubert, 2000). Since several research have been conducted in order to determine the rooting of tea cutting, (Altinda & Balta, 2002; Bidarighfard, 1997; Chen, THSENG, & Ko, 1990; Fong, 1992; Waheed, Ahmad, Hamid, & Ahmad, 2010) but still limited information is obtained on hardening of tea clone Iran 100 plantlets. This study aims to utilize the available information in the literature to optimize a method in order to decrease mortalities of acclimatized tea clone Iran 100 plantlets by manipulation of potting media and the ratios.

Materials and Methods

Nodal segments measuring 1-1.5 cm in length was excised from shoot cuttings of tea (*Camellia sinensis* (L.) O. Kuntze) plants of clone Iran 100. These were then soaked in Tween 20 for 15 minutes followed by surface sterilization with 20% Clorox solution (0.53% sodium hypochlorite) for 15 minutes. All traces of Clorox solution were then washed off with sterile distilled water. Cultures were initiated on Murashige and Skoog (1962) medium supplemented with 1mg/l BAP and 3% sucrose (w/v) and solidified using agar (0.8%; w/v) for induction of shoots. After 30-35 days of inoculation the *in vitro* shoots were transferred to media containing 5 mg/l BAP in combination with 0.5 mg/l GA_3. Sub culturing was done at regular intervals of four weeks up to 16 weeks (four subcultures). For rooting, shoots (2-3 cm in height) were subjected to treatment with IBA solution 300 mg/l for 30 minutes and then transferred to half-strength basal MS medium without growth regulators. After 60 days the healthy plantlets 2-

2.5 cm height, with at least two leaves and well developed roots were dipped in 2% Benlate solution at the base for 1 min to protect the roots from fungus. The plantlets were then transferred to glass pots (5×12 cm) containing vermiculite and maintained in the culture room at 28 °C day/20 °C night, 16 h d-length and 70% relative humidity under low light (15-M photon.m^{-2}.s^{-1} over 16-hr). During this period the humidity was maintained by covering with a plastic cover. After 30 days, the glass pots were moved to normal greenhouse condition for further weaning. To acclimatize the plantlets, they were kept in glass pots for the duration of 10 days and then transplanted to plastic pot containing four different types of potting media, namely: peat moss + vermiculite + perlite (2:2:0 v/v/v), peatmoss + vermiculite + perlite (1:2:1 v/v/v), peatmoss + vermiculite + perlite (1:1:2 v/v/v) and peatmoss + vermiculite + perlite (2:1:1 v/v/v). By the eighth week the effect of potting media on the survival rate, the mean number of leaves produced and the root length and plant height were attained. This experiment was carried out in RCBD (Randomized completely block design) with 4 replications.

Results and Discussion

Plantlets of tea clone Iran 100 were transferred directly from *in vitro* system to glass pots containing vermiculite for acclimatization. By the third day of acclimatization, the plantlets showed wilting symptoms, the stems were bent, and the leaves turned yellowish. After two weeks, the plantlets recovered slowly and started to thrive. By week 4, the plantlets were transferred to 50% shade house for adaptation to *ex vitro* situation. In this stage, new leaves emerged and the plantlets recovered fully, and their normal growth was resumed.

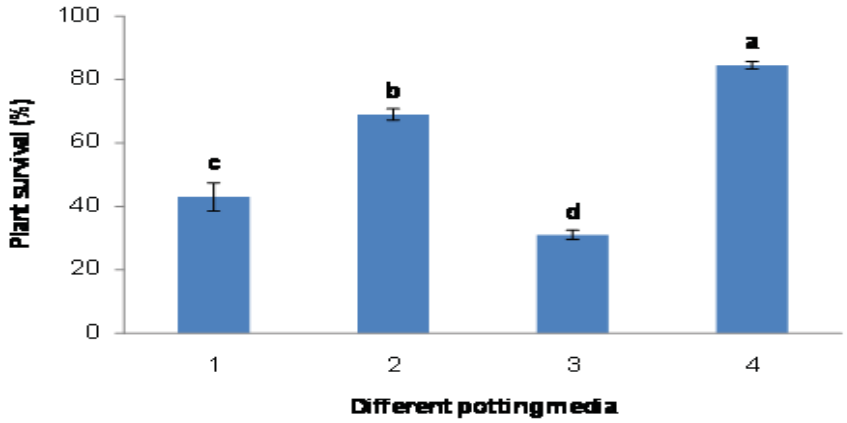

Figure 1. The effect of different potting media on survival percentage attained at week 8 of acclimatization 1) (Pm.V.P., 2:2:0 v/v/v); 2) (Pm.V.P., 1:2:1 v/v/v); 3) (Pm.V.P., 1:1:2 v/v/v) 4) (Pm.V.P., 2:1:1 v/v/v).Symbol: Pm (peatmoss), Ver (vermiculite), P (perlite).

Finally, the plantlets were transplanted to plastic pots containing four types of potting medium namely: peatmoss + vermiculite + perlite (2:2:0 v/v/v), peatmoss + vermiculite + perlite (1:2:1 v/v/v), peatmoss + vermiculite + perlite (1:1:2 v/v/v) and peatmoss + vermiculite + perlite (2:1:1 v/v/v). By the eighth week the effect of potting media on the survival rate, the mean number of leaves produced and the plants height, became apparent among the different media.

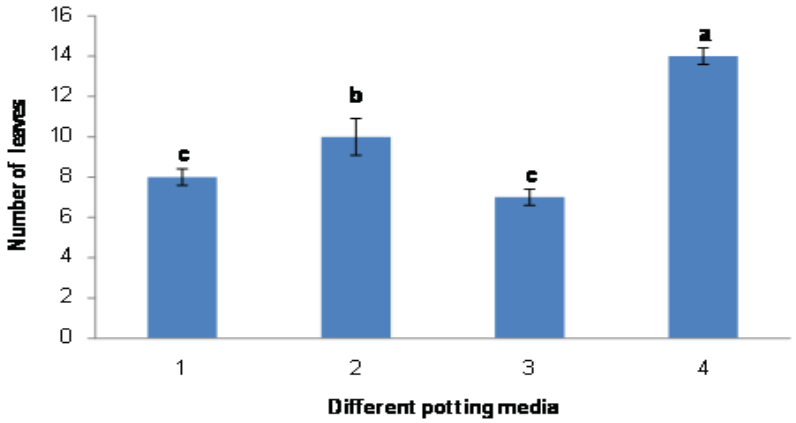

Figure 2. The effect of different potting media on number of leaves produced at week 8 of acclimatization 1) (Pm.V.P., 2:2:0 v/v/v); 2) (Pm.V.P., 1:2:1 v/v/v); 3) (Pm.V.P., 1:1:2 v/v/v) 4) (Pm.V.P., 2:1:1 v/v/v).Symbol: Pm (peatmoss), Ver (vermiculite), P (perlite)

Table 1 shows highly significant differences ($p \leq 0.01$) among the four potting media on the rate of survival of plantlets during acclimatization. The highest survival (84.5%) was obtained in medium consisting of peatmoss+ vermiculite+ perlite (2:1:1 v/v/v) followed by 69% and 43% in peatmoss+ vermiculite+ perlite (1:2:1 v/v/v) and, peatmoss+ vermiculite+ perlite (2:2:0 v/v/v)

media respectively by the eighth week of acclimatization (Figure 1). The results indicated that the lowest survival percentage (31%) was obtained in the medium containing peatmoss + vermiculite + perlite (1:1:2 v/v/v).

As shown in Table 2, all treatments showed highly significant differences ($p \leq 0.01$) in the number of leaves produced. The medium containing peatmoss+ vermiculite+ perlite (2:1:1 v/v/v) produced the highest number of leaves per plant with 14 leaves followed by 10 in peatmoss+ vermiculite+ perlite (1:2:1 v/v/v) (Figure 2). However, there was no significant difference in the number of leaves produced per plantlet between peatmoss+ vermiculite+ perlite (2:2:0 v/v/v) and peatmoss+ vermiculite+ perlite (1:1:2 v/v/v) with 8 and 7 leaves, respectively.

Table 1. Results of ANOVA on the percentage of plant survival after eight weeks of acclimatization

Source	Sum of Squares	df	Mean Square	F	P value
rep	106.250	3	35.417	1.527	0.273
treatment	7088.750	3	2362.917	101.874**	0.000
Error	208.750	9	23.194		
Total	59160.000	16			
CV	18.46%	**R square**	0.953		

Table 2. Results of ANOVA on the number of leaf per plantlet after eight weeks of acclimatization

Source	Sum of Squares	df	Mean Square	F	P value
rep	1.500	3	0.500	0.310	0.818
treatment	115.000	3	38.333	23.793**	0.000
Error	14.500	9	1.611		
Total	1652.000	16			
CV	23.01%	**R square**	0.889		

The results revealed that different potting medium had significant effect on plant height attained after eight weeks of acclimatization in the greenhouse (Table 3). The highest mean plant height of 8.1 cm was attained in potting medium containing peatmoss+ vermiculite+ perlite (2:1:1 v/v/v) followed by 5.9 and 4 in peatmoss+ vermiculite+ perlite (1:2:1 v/v/v) and peatmoss+ vermiculite+ perlite (1:1:2 v/v/v) respectively (Figure 3). The results also showed the lowest mean height 3.02 cm obtained in the medium containing peatmoss+ vermiculite+ perlite (1:2:1 v/v/v).

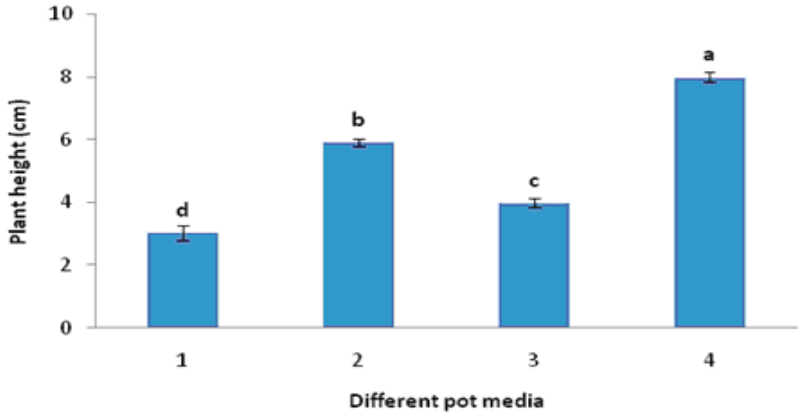

Figure 3. The effect of different potting media on plant height attained at week 8 of acclimatization 1) (Pm.V.P., 2:2:0 v/v/v); 2) (Pm.V.P., 1:2:1 v/v/v); 3) (Pm.V.P., 1:1:2 v/v/v) 4) (Pm.V.P., 2:1:1 v/v/v).Symbol: Pm (peatmoss), Ver (vermiculite), P (perlite)

Table 3. Results of ANOVA on height of plant after eight weeks of acclimatization

Source	Sum of Squares	df	Mean Square	F	P value
rep	0.383	3	0.128	1.133	0.387
treatment	58.123	3	19.374	172.215**	0.000
Error	1.013	9	0.113		
Total	498.420	16			
CV	16.4%	**R square**	0.972		

Highly significant differences ($p \leq 0.01$) were observed among the different potting media in terms of root length (cm) attained after eight weeks of acclimatization in the greenhouse (Table 4). The highest mean root length (5 cm) was obtained in potting media consisting of peatmoss+ vermiculite+ perlite (2:1:1 v/v/v). The results showed that the lowest mean root length was attained in potting media containing peatmoss+ vermiculite+ perlite (2:2:0 v/v/v) and peatmoss+ vermiculite+ perlite (1:1:2 v/v/v) producing mean length of 2.97 cm and 3.0 cm respectively. However, there was no significant difference between these two potting media in terms of root length (cm) obtained after eight week of acclimatization (Figure 4).

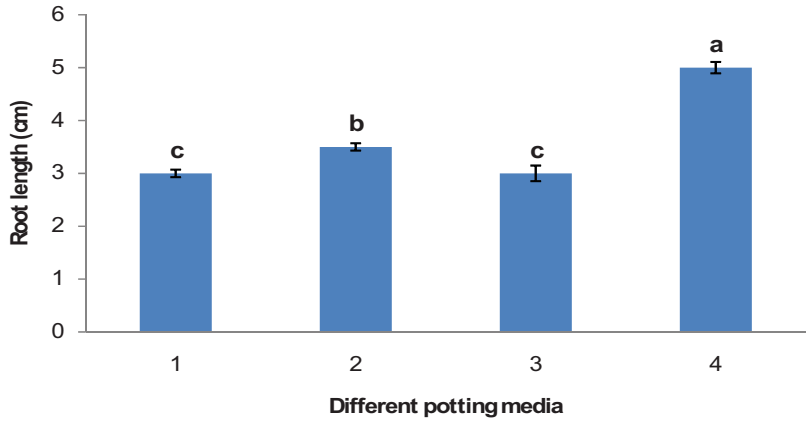

Figure 4. The effect of different potting media on mean root length attained at week 8 of acclimatization 1) (Pm.V.P., 2:2:0 v/v/v); 2) (Pm.V.P., 1:2:1 v/v/v); 3) (Pm.V.P., 1:1:2 v/v/v) 4) (Pm.V.P., 2:1:1 v/v/v).Symbol: Pm (peatmoss), Ver (vermiculite), P (perlite)

Table 4. Results of ANOVA on the length of root after eight weeks of acclimatization

Source	Sum of Squares	df	Mean Square	F	P value
rep	0.145	3	0.048	1.160	0.377
treatment	10.750	3	3.583	86.000**	0.000
Error	0.375	9	0.042		
Total	221.520	16			
CV	15.63%		**R square**	0.967	

Rooted plants were successfully acclimatized in different potting media and grew naturally in the greenhouse. Referring to Figure 5 the maximum survivalpercentage, number of leaves, root length and height were obtained on plants grown in peatmoss+ vermiculite+ perlite mixture (2:2:0). This response maybe due to the ability of the mixture to provide enough moisture and aeration to the plants, thereby resulting in good root growth. Perlite is an important component in the potting mixture when mixed with peatmoss.

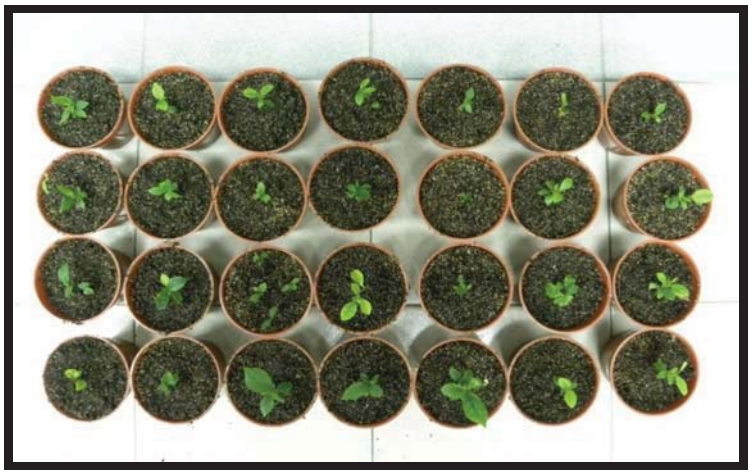

Figure 5. The acclimatized plants in potting media in peatmoss+ vermiculite+ perlite mixture (2:1:1; v/v/v)

The addition of perlite to peatmoss increases the amount of air (oxygen) held in the peatmoss, as well as the amount of water retained by the peatmoss. This obviously improves the growing conditions for plants (Miller & Donahue, 1990). Vermiculite also possesses cation exchange properties, thus it can hold and made available ammonium, potassium, calcium and magnesium to the

growing plants. Vermiculite, when combined with peatmoss promotes faster root growth and gives quick anchorage to young roots (Hartmann, Kester, Davies, Robert, & Geneve, 2007).

The well-rooted plantlets performed best in terms of quick acclimatization when these were transferred to medium consisting peatmoss + vermiculite + perlite (2:1:1; v/v/v) with 84.5% survival. The root length, plant height and number of leaves were maximum (5, 8.1 cm and 14, respectively) in comparison to other substrates tested. Therefore, the mixture containing peatmoss + vermiculite + perlite (2:1:1 v/v/v) is recommended for growth of tea clone Iran 100 plants during acclimatization.

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References

Agarwal, B., Singh, U., & Banerjee, M. (1992). *in vitro* clonal propagation of tea (*Camellia sinensis* (L.) O. Kuntze). *Plant Cell, Tissue and Organ Culture, 30*(1), 1-5.

Altinda, E., & Balta, F. (2002). Comparison of rooting capabilities of Turkish tea clones. *Turkish Journal of Agriculture and Forestr, 26*, 195-201.

AzadiGonbad, R., & FatemiChokami, A. (2009). Effects of plant growth regulators on rooting of tea cuttings (*Camellia sinensis*) clone 100 Iran. *Asian Journal of Chemistry, 21*(4), 3298-3300.

Banerjee, M., & Agarwal, B. (1990). *in vitro* rooting of tea, *Camellia sinensis* (L.) O. Kuntze. *Indian Journal of Experimental Biology, 28*, 936-939.

Bidarighfard, S. (1997). *Effect of IBA, zinc sulfate, wounding and the type of rooting media on tea cutting.* Islamic Azad University, Tehran.

Chen, J. E. E. S., THSENG, F. M., & Ko, W. (1990). Improvement of survival and subsequent growth of tea cuttings. *HortScience, 25*(3), 305-306.

FAOSTAT (2010). Retrieved 20 January 2012: http://faostat.fao.org/DesktopDefault.aspx?PageID=567&lang=en#ancor

Fong, C. H. (1992). The effect of organic fertilizer and growth substances on rooting of tea cuttings. *Hort. Abst., 62-6*, 5316.

George, E. F., & Debergh, P. C. (2008). Micropropagation: Uses and Methods. In E. F. George, M. A. Hall & G. D. Klerk (Eds.), *Plant propagation by tissue culture* (Vol. 1 pp. 29-64). Dordrecht, The Netherlands: Springer.

Hartmann, H., Kester, D., Davies, F., Robert, L., & Geneve, F. L. (2007). Plant Propagation: Principles and Practices: Prentice-Hall International, Inc., London.

Jain, S. M., Das, S. C., & Barman, T. S. (1993). *Enhancement of root induction from in vitro regenerated shoots of tea (Camellia sinensis L.).* Paper presented at the Proc. Ind. Natl Sci. Acad. B.

Jha, T., & Sen, S. K. (1992). Micropropagation of an elite Darjeeling tea clone. *Plant Cell Reports, 11*(2), 101-104.

Kato, M. (1985). Regeneration of plantlets from tea stem callus. *Japanese Journal of Breeding, 35*, 317-322.

Miller, R. W., & Donahue, R. L. (1990). *Soils: An introduction to soils and plant growth*: Prentice Hall.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Pantarum, 15*(3), 473-497.

Noble, A., Zenneck, I., & Randall, P. (1996). Leaf litter ash alkalinity and neutralisation of soil acidity. *Plant and Soil, 179*(2), 293-302.

Pocknee, S., & Sumner, M. E. (1997). Cation and nitrogen contents of organic matter determine its soil liming potential. *Soil Science Society of America Journal, 61*(1), 86-92.

Tang, C., & Yu, Q. (1999). Impact of chemical composition of legume residues and initial soil pH on pH change of a soil after residue incorporation. *Plant and Soil, 215*(1), 29-38.

Tian-Ling, L. (1982). Regeneration of plantlets in cultures of immature cotyledons and young embryos of *Camellia oleifera* Abel. *Acta Biologiae Experimentalis Sinica, 4*, 393-403.

Waheed, A., Ahmad, H., Hamid, F., & Ahmad, N. (2010). Co-relation of IBA indole-butyric acid with different media used for early rooting of tea cuttings. *Science Technology and Development, 29*(1), 15-20.

Yan, F., & Schubert, S. (2000). Soil pH changes after application of plant shoot materials of faba bean and wheat. *Plant and Soil, 220*(1), 279-287.