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Acclimatization and performance of micropropagated clonal rootstocks and scion cultivars of apple under different growing structures

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Abstract

***In-vitro* multiplied plantlets of apple cvs. Scarlet Gala and Vance Delicious, and clonal rootstocks M-7 and Merton-793 at three leaves stage were studied for acclimatization and performance in the Department of Horticulture, CSKHPKV, Palampur in 2011. The plantlets were first kept under hi-tech polyhouse condition for hardening and acclimatization. Three months later the cultivar Scarlet Gala and clonal rootstock (M-7) were further transplanted under ordinary plolyhouse, shade-net house and cultivars Scarlet Gala, Vance Delicious and rootstocks M-7 and Merton- 793 were planted in nursery bed under hi-tech polyhouse. The observations on vegetative growth, leaf area and chlorophyll content were recorded. Among various structures, the growth of clonal rootstock (M-7) and scion variety (Scarlet Gala) was more under ordinary polyhouse, followed by shade-net house and minimum was under hi-tech polyhouse. Similarly, leaf area, number of leaves per plantlets and chlorophyll content were also found maximum in plantlets under polyhouse followed by shade-net and minimum under hi-tech polyhouse condition.**

Key words: Clonal rootstock, Scarlet Gala, polyhouse, Vance Delicious, hi-tech polyhouse.

Introduction

In apple, all kinds of clonal rootstocks with varying growth habits from very vigorous to ultra dwarf are available in India, but their availability to the farmers are limited, secondly if available there are chances of virus infestation at initial stage of nursery production resulting spread of unhealthy and spurious planting materials among the growers. As, at initial stage of orchard establishment the basic objective remains the farmers should get true-to-type and healthy planting materials. Once unhealthy planting materials are distributed later on this mistake can't be corrected therefore, plants must be free from any diseases including viruses and true-to-type. Although, the eradication of viruses is very difficult in plants once they get infested but removal of specific viruses by meristem-tip culture has not only reduced chances of viruses but has lead to a dramatic yield increase and rejuvenation of plant varieties that are multiplied through *in-vitro* (Murashige, 1980). This is a unique contribution of meristem-culture not achievable by any other techniques. Therefore, this technique is the only option to reduce the chances of virus infestation at initial stage of plant multiplication. Although, plants propagated through this method didn't fully eradicate viruses but the chances of virus infestation were reduced (Pierik, 1987).

Conventionally, clonal rootstocks are multiplied by layering method (mound and trench), being seasonal, irregular and slow this method is not appropriate to meet the ever growing demand of apple plants. In contrast, micropropagation is a quick and efficient method of propagation for production of large quantity of propagules. There are several reports of *in-vitro* multiplication of clonal rootstocks of apple successfully (Snir and Erez, 1980; Patal-Ochatt *et al.*, 1989 and Webster and Jones, 1989).

Further, the success of micropropagation depends upon the ability to transfer plantlets to potting medium and to acclimatize them successfully to free living conditions. Unlike, various herbaceous plants many fruit trees grown *in-vitro* have been difficult to acclimatize due to various factors and substantial number of such plants donot survive when transferred from *in-vitro* condition to either polyhouse or open field conditions. Since they exhibits typical anatomical and physiological characteristics necessitates that they should be acclimatized gradually to the polyhouse or open field condition. But there are reports of successfully acclimatization of fruit trees propagated through tissue culture method by altering the growing condition such as high humidity, (Machnik and Orlikowska, 1981), misting (Hutchinson 1984) and low moisture (Brainerd and Fuchigami, 1981). Similarly, Sutter and Hutzell (1984) used humidity tents and antitranspirants to increase the survival percentage of micropropagated plants under low relative humidity. Further, the successful *ex-vitro* acclimatization of micropropagated plants determines the quality of the end product (Conner and Thomas, 1982) and successful results can be achieved by careful environmental control during acclimatization. The other factors apart from environment are potting medium, fertilization and control of diseases etc.

Therefore, this study was carried out in the Department of Horticulture, CSKHPKV, Palampur HP, India, during the year 2011 in collaboration with TERI, New Delhi to find out effect of different environmental conditions on growth of micro-propagated clonal rootstocks and scion cultivars of apple.

Materials and Methods

Plant material and hardening method

In-vitro multiplied self rooted apple plantlets were brought to the Department of Horticulture, CSKHPKV, Palampur from TERI, New Delhi in April, 2011. These plantlets were at three leaves stage in plug trays with media mixture; cocopeat+ perlite + vermiculite. Plantlets were kept under hi-tech polyhouse in controlled conditions (RH; 70±5%, temperature; 25±2°C in natural photoperiodic condition without additional photoperiod) as such for one month. The plantlets were irrigated by intermittent misting twice in a day, after one month the plants were further transplanted in polythene tubes containing growing media mixture of sand, soil and vermi-compost in the ratio of 1:1:1 for another three months, during this period the temperature and RH was kept as such. After three months (August) the survival rate was recorded and observed 24-31 %, and the well hardened plantlets were further transplanted under different growing structures in well prepared nursery beds as mentioned in Table 1.

The observations on various growth parameters

Table 1. Treatment combinations

Cultivar/rootstock	Growing structure
T_1: M-7	: Shade net-house 50 % UV stabilized green colour
T_2: Scarlet Gala	: do
T_3: M-7	: Ordinary polyhouse
T_4: Scarlet Gala	: do
T_5: Scarlet Gala	: Hi-tech polyhouse
T_6: Vance Delicious	: do
T_7: Merton-793	: do
T_8: M-7	: do

and leaf characteristics (number, area, fresh and dry weight and total chlorophyll content) were taken from ten randomly selected plants from each replication. To estimate leaf chlorophyll a sample of ten representative leaves per plant was detached in the morning hours (Halfacre *et al*. 1968), immediately collected in ice box and stored in refrigerator below 0 ^{0}C to avoid degradation of chlorophyll pigments. Leaves were then washed and chopped in to fine pieces under subdued light and 100 mg of chopped material was placed in vial containing 7 ml of dimethyl sulphoxide (DMSO). The contents of the vials were incubated at 65 ^{0}C temperature for half an hour and then extract was transferred to graduated test tube and final volume was made to 10 ml with DMSO as suggested by Hiscex and Isralistan (1979). Then optical density was recorded at 645 and 663 nm wavelengths against a DMSO blank and total chlorophyll content was calculated and expressed in mg/g. The data obtained were then analyzed by using Statistical Software CPCS1 and processed by one-way ANOVA at 0.05 % level of significance.

Results and Discussion

Vegetative growth

During secondary hardening process under hi-tech polyhouse condition using sand, soil and vermi-compost (1:1:1) as growing media, the overall survival rate was observed 24-31 per cent. But when the plantlets which were further transplanted under different growing structures viz; polyhouse, shade-net and hi-tech polyhouse, survived well and percentage was 100 (data not presented). The different structures exerted significant influence on vegetative growth of plantlets as presented in Table 2. The scion cultivar Scarlet Gala (58.33 cm) and clonal rootstock M-7 (51.33 cm) under ordinary polyhouse condition registered maximum growth. Whereas, the plantlets irrespective of cultivars or rootstocks, under hi-tech polyhouse condition attained minimum height at the end of growing season. Although, the hi-tech polyhouse was equipped with misting/fogging and exhaust fans to reduce the temperature within structure but overall performance of the tissue cultured plants was found lesser as compared to other structures. This might be the fact that with frequent misting and fogging resulted the water logging condition around the plants which might have hampered the overall performance of plants. The same results were also reported by Meera and Sathyanarayana (2010) during acclimatization process of micropropagated stevia. In which they observed better survival in greenhouse (27.89%) compared with mist house (23.11%).

Similarly, other growth parameters like; shoot diameter and internode length were also significantly influenced by different growing structures and were recorded maximum in plantlets which were transplanted under ordinary polyhouse, followed by shade-net and minimum under hi-tech polyhouse. After *ex- vitro* transfer, the plantlets get easily impaired by sudden changes in environmental conditions, and need a period of acclimatization to correct the abnormalities. Environmental changes can further influence morphological and physiological changes in plants that allow them to adapt novel climatic conditions. The various physiological, morphological and physical factors are involved for acclimatization of tissue cultured plantlets. Osorio *et al.* (2013) reported 88 % survival of micro-propagated *Tuberaria major* plants under growth chamber and 84 per cent in the greenhouse. Lavanya *et al*. (2009) suggested that light is the most important factor during acclimatization process but Pospisilova *et al.* (1999) reported humidity as one of the most important factor. According to Bhatt and Dhar (2000) carbohydrate concentration was the most important during plant acclimatization process.

Leaf characteristics (number of leaves, fresh & dry weight of leaf and chlorophyll content)

The plantlets which had vigorous growth produced maximum number of leaves/plantlet (Table 3), as compared to those which registered lesser

Table 2. Effect of different growing structures on vegetative growth of *in-vitro* grown clonal rootstocks and scion cultivars of apple

Vegetative growth→ Treatments↓	Plantlet height (cm)	Plantlet diameter (cm)	Internode length (cm)
	Under shade-net		
T_1: M-7	43.33	0.144	1.56
T_2: Scarlet Gala	50.33	0.178	1.62
	Under polyhouse		
T_3: M-7	51.33	0.165	1.76
T_4: Scarlet Gala	58.33	0.191	1.82
	Under Hi-tech polyhouse		
T_5: Scarlet Gala	39.33	0.150	1.31
T_6: Vance Delicious	11.33	0.079	0.92
T_7: Merton-793	26.00	0.097	1.10
T_8: M-7	18.00	0.097	1.03
CD (P=0.05)	7.36	0.038	0.23

Table 3. Effect of different growing environment on leaf characteristics of *in-vitro* grown apple rootstocks and scion cultivars

Leaf characteristics→ Treatments↓	No. of leaves/plant	Leaf area (cm^2)	Leaf weight (g)		Total chlorophyll content of leaf
			Fresh	Dry	
	Under shade-net				
T_1: M-7	23.67	24.81	0.33	0.09	1.80
T_2: Scarlet Gala	31.33	24.13	0.36	0.10	1.85
	Under polyhouse				
T_3: M-7	31.67	28.88	0.57	0.17	2.21
T_4: Scarlet Gala	33.00	33.30	0.62	0.19	2.28
	Under Hi-tech polyhouse				
T_5: Scarlet Gala	30.00	21.55	0.32	0.10	1.80
T_6: Vance Delicious	10.67	14.55	0.23	0.06	1.69
T_7: Merton-793	23.67	20.80	0.28	0.08	1.76
T_8: M-7	16.33	16.75	0.24	0.09	1.79
CD (P=0.05)	6.00	5.69	0.11	0.02	0.22

growth. The scion cultivar Scarlet Gala, irrespective of growing structures (polyhouse, shade-net and under hi-tech polyhouse condition) produced more number of leaves per plantlet. The maximum number of leaves per plantlet was observed under polyhouse condition (33.0 leaves/plantlet). Similarly, clonal rootstock M-7 under polyhouse condition also produced more number of leaves but was statistically par with scion cultivar Scarlet Gala under same growing structure. In general, all plantlets irrespective of scion cultivars or clonal rootstocks under hi-tech polyhouse condition produced lesser number of leaves (Vance Delicious 10.67 leaves / plantlet). Similarly, the corresponding leaf area, fresh and dry weight of the leaf was also more in same plantlets under same condition (Table 3).

Irrespective of the cultivar and rootstock, the leaf chlorophyll content of plantlets under ordinary polyhouse condition was more as compared to other growing structures (Table 3). The leaf chlorophyll content and leaf weight (fresh and dry) increment is the result of photosynthesis and accumulation of carbohydrate, as in this study the hi-tech polyhouse and shade-net structures were equipped with 50% UV stabilized green colour net, which might have prevented the sunlight to enter inside, ultimately the lesser synthesis of chlorophyll content thus low carbohydrates in leaf. Osorio *et al.* (2013) also observed higher chlorophyll contents under greenhouse and growth chamber condition while acclimatizing *in-vitro* grown *Tuberaria major* plants. The amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content. Thus the chlorophyll content can directly determine the photosynthetic potential (Curran *et al.*, 1990 and Fiella *et al*., 1995). Further, leaf chlorophyll content is closely related to plant stress as well as senescence (Merzlyak *et al.*, 1999).

Although, considerable efforts have been made to acclimatize *in-vitro* grown plantlets to field condition/under protected structures but the process of acclimatization remains inconclusive due to various factors. In this study, it was observed that even hi-tech structure equipped with environment controlling equipments to lower down temperature and to maintain relative humidity could not improve performance of plantlets as compared to ordinary polyhouse condition, where plants have shown better adaptability and better growth as compared to other structures.

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