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Efficacy of *In vitro* Propagation and Crown Sizes on the Performance of Strawberry (*Fragaria×ananassa* Duch) *cv*. Festival under Field Condition

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ABSTRACT

***In vitro* propagated and conventional runner propagated plantlets of strawberry (*Fragaria×ananassa* Duch) cv. 'Festival' were transferred to the field and the size of crown for plants derived from both sources of planting material were compared. Four sizes of the crown, i.e., < 0.5, 0.5-0.8, 0.8-1, and > 1 cm were taken from both sources of planting material. The results obtained flashed that vegetative and reproductive responses of *in vitro* propagated plants in the field were superior over the runner propagated plants. The upshots among the various crown sizes of the planting material revealed that the plants of crown size > 1 cm performed better in all the vegetative and reproductive parameters like number of crowns per plant, number of leaves, plant height, number of runners, plant spread, terminal leaf area, number of days to flower induction, number of flowers, number of fruits, average fruit weight and fruit size. Thus, *in vitro* propagated derived plants can be advocated for large scale commercial cultivation. The crown size > 1 cm can be set as a selection criterion for planting materials.**

Keywords: Direct regenerated shoot, Field performance, *in vitro* propagated plants, Runner derived plants, Strawberry.

INTRODUCTION

The cultivated strawberry is a hybrid of two American species, *Fragaria chiloensis* and *F. virginiana*, developed in France in the seventeenth Century. Though a basically temperate fruit crop, strawberry has adapted to different climatic conditions *viz*., moderate, Mediterranean, subtropical, and even to high altitudes of tropical climate. *In vitro* regeneration techniques are indispensible tools for mass propagation, germplasm preservation and genetic engineering of horticultural crops. Conventional method of strawberry propagation is mainly achieved through runners. However, obtaining healthy stocks through this method is challenging. Henceforth, *in vitro* techniques have been amplified to be an efficient method of mass propagation (Boxus, 1974; Boxus *et al.,* 1977). Micropropagation of strawberry has been used in horticultural production for more than 25 years (Boxus, 1974). Several improvements of the technology have been recommended by authors working with strawberry (Damiano, 1980; Drew *et al.,* 1986; Theiler Hedtrich and Wolfensberger, 1987; Boxus, 1974; Jemmali *et al.*, 1995). Besides, the possibility of obtaining disease free plants through *in vitro* meristem culture is also a boon as demonstrated by Morel and Martin (1952), Adams (1972), Nishi and Oosawa (1973), Mullin *et al.* (1974) and Scott and Zanzi (1981). Consequently, it is important to increase the national production of plantlets to meet the grower's needs. It was estimated that several millions of plants

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can be produced within a year from a few mother plants by tissue culture technique (Boxus, 1983). Moreover, the storage of tissue cultured propagules entails less space than traditional runner plants and *in vitro* storage can be initiated at any time during the production cycle (Swartz *et al.*, 1981).

For commercial utilization of tissue culture, extensive field evaluation of the *in vitro* derived plants is necessary. Increased branching and vigorous vegetative growth are often noted in plants produced through *in vitro* culture. Tissue culture derived strawberry plants grow more vigorously, producing more crowns and runners with increased petiole length, yield per area, and number of inflorescences per crown than conventionally propagated plants (Swartz *et al.*, 1981; Cameron *et al.*, 1989; Lopez-Aranda *et al.*, 1994). Studying the validation of these findings is interesting as this might prompt the replacement of runners with tissue culture plantlets in the near future for commercial cultivation, especially in the developing countries. The plant size is imperative since flower number increases with the increased crown diameter of daughter plant (Jemmali and Boxus, 1993), and the crown diameter is correlated not only to the number of flowers and size but to the inflorescence instigation as well (Mason, 1987). Chercuitte *et al.* (1991) denoted that the optimum crown size has to be determined for each individual cultivar. Several researchers (Bish *et al.*, 2002; Giménez *et al.*, 2009; Takeda and Hokanson, 2003) suggested larger size of the crowns for greater performance in the field. These reports signify that size of the crown can be considered as a selection criterion for planting materials. Studies, however, have been conducted only with runner propagated planting materials. Exploring the effect of crown sizes on the performance of plants derived from both runner and *in vitro* propagation would be interesting as to whether the effect of crown sizes are applicable or not for both the source of planting materials for better returns. Reports on the relation of crown diameter with plant growth, development, and fruit yield are meager. Therefore, in the present study, the field performance of *in vitro* and runner propagated plants with various crown sizes have been assessed for their various responses on the vegetative and reproductive parameters wherein the salient findings of the study are discussed in the light of contemporary published data.

MATERIALS AND METHODS

Propagation and Acclimatization of Plantlets

In vitro cultures were initiated from field grown plants at Indian Institute of Horticultural Research, Hessarghatta, Bangalore, India. The method of decontamination suggested by Biswas *et al.* (2007) for runner tips was ineffective for the explants collected from the open field in the present study. Hence the decontamination treatment procedure was modified. Tender runners (7-10 cm) were severed, leaves and roots trimmed off, and then nodal segments (1-2 cm) obtained were pretreated with a mixture of 1% Bavistin, 0.1% antibiotic formulation (streptomycin+tetracycline) and 0.5% CTAB (Cetyl Trimethyl Ammonium Bromide) for 90 minutes. The explants were washed 3 times in sterile distilled water, followed by disinfection with 75% ethanol containing 2 drops of Tween-20. This was followed by washing of explants with sterile distilled water. The pre-treated explants were treated again with Mercuric chloride (0.1%) for 3 minutes followed by washings in sterile distilled water for six times. After washing, the leaf sheaths were removed and the exposed ends were trimmed off, and the excised nodal explants (1-1.5 cm) were again transferred to a bottle containing sterile water. The excised explants were further retreated with Mercuric chloride (0.1%) for 10 seconds, and subsequently washed 5 times with sterile distilled water. The explants were then inoculated in culture tubes containing 10 mL of the prepared

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culture medium. Direct shoot regeneration was induced on MS (Murashige and Skoog, 1962) medium containing 1 mg L^{-1} TDZ (Thidiazuron). The shoots obtained from the cultured explants were separated and sub-cultured after 3 weeks of culture. Medium supplemented with 1 mgL IBA (Indole-3-Butyric Acid) was used for root proliferation of microcuttings. After subsequent rooting for 1 month, plantlets were then subjected to hardening media composed of a mixture of sterilized sand, soil and cocopeat (1:1:2). Hardening media were filled to 1/3rd of the plastic cups punched with holes. Wetting of the soil mixture with water to field capacity was accomplished and the plantlets were transplanted into plastic cups containing media overlaid with an inverted punched holes plastic cup, then plantlets were subsequently placed under cool fluorescent light in the culture room. After 1 week, the inverted plastic cup covers were taken off for further hardening, and one month thereafter, the hardened plantlets were shifted to glasshouse conditions. Plantlets of 2 months old were ready for transplanting to the field.

Field Transfer of Plantlets and Evaluation

Runners and acclimatized micropropagated plantlets with 5-7 cm height were planted in the prepared field during September 2012. Four sizes of the crown, i.e., < 0.5, 0.5-0.8, 0.8-1 and >1 cm were taken from two sources of planting material *viz.,* (1) crown derived from runners, and (2) crown derived from *in vitro* plantlets. Their performance was evaluated in the field. For each of the total 8 treatment combinations, 4 plants were planted in an experiment using a 2 factors factorial Randomized Block Design (RBD) with 4 replications of 32 plants each. The plants spacing was kept at 35×60 cm. An average temperature of 29.4 (maximum) and 17.9°C (minimum), with relative humidity of 77.6% (at 07.30 hours.) and 51.2% (14.00 hours.) was prevalent during the period of investigation.

Meticulous observations on vegetative, floral, and fruit characters were recorded at various stages of growth. The vegetative parameters included number of crowns per plant, plant height, plant spread, number of leaves per plant, number of runners per plant, and terminal leaf area (cm^2). Floral and fruit variables included number of days to flower bud initiation, number of flowers per plant, number of fruits per plant, weight of fruits (g), fruit size (mm^2) and TSS (°B). The average of all the observations recorded was worked out. Primary berries obtained were used for the measurement of percent Total Soluble Solids (TSS).

Statistical Analysis

Analysis of variance for various observations was carried out using 4×2 Factorial RBD with 4 replications using SPSS statistics 17.0. Test for significance ($P \leq 0.05$) was conducted among source of planting materials, crown sizes, and their interactions.

RESULTS

The vegetative parameters of *in vitro* direct regenerated shoot derived plants and runner derived plants with various crown sizes were assessed as displayed in Figures 1, 2, and 3. Higher height and spread was obtained in plants derived from *In vitro* Direct Regenerated shoot (IDR) compared to Runner derived plants (R) at 30 and 75 DAP (Days After Planting) (Figure 1). The plants derived from IDR also exhibited higher number of leaves per plant and number of runners per plant (Figure 2), number of crown per plant, and terminal leaf area (Figure 3) compared to R plants at 30 and 75 DAP. These mentioned parameters were also found to be higher in the plants of

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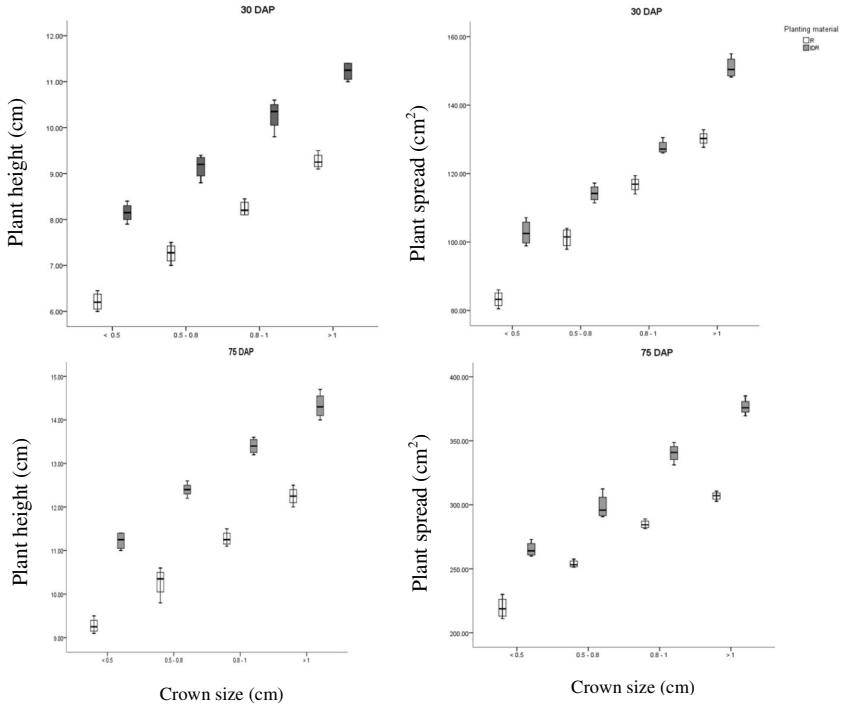

Figure 1. Plant height (cm) and spread (cm^2) of strawberry *cv.* 'Festival' as a function of crown size, under field condition. Bars represent the significant difference ($P \leq 0.05$) for mean values comparison. R: Runner derived, IDR: *In vitro* Direct shoot Regeneration.

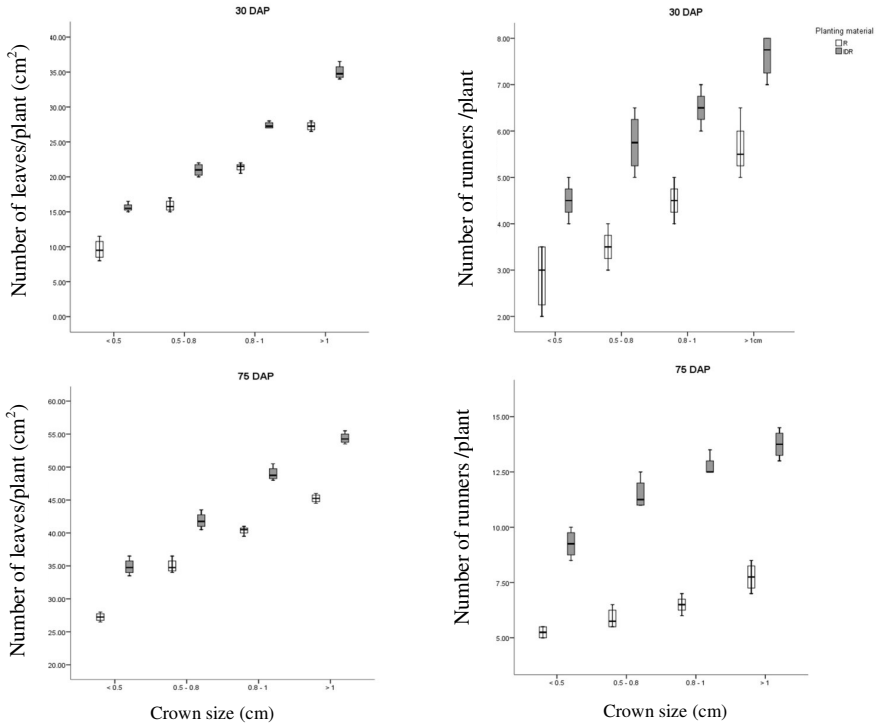

Figure 2. Number of leaves and number of runners per plant of strawberry *cv*. 'Festival' as a function of crown size, under field condition. Bars represent the significant difference ($P \leq 0.05$) for mean values comparison. R: Runner derived, IDR: *In vitro* Direct shoot Regeneration.

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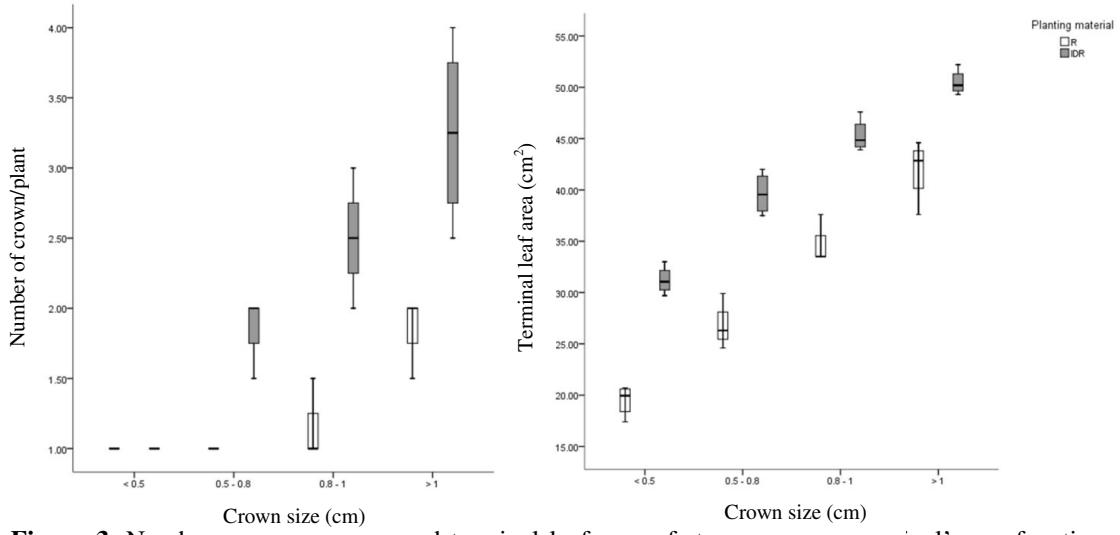

Figure 3. Number of crowns/plant and terminal leaf area of strawberry *cv.* 'Festival' as a function of crown size, under field condition. Bars represent the significant difference (P≤ 0.05) for mean values comparison. R: Runner derived, IDR: *In vitro* Direct shoot Regeneration.

crown size >1 cm, while the lowest was found in crown size < 0.5 cm of the planting materials irrespective of the sources at 30 and 75 DAP. There was a hike in the performance of strawberry irrespective of the sources of planting materials in all the vegetative parameters as the size of the crown increased, and when plantlets derived from IDR were used as planting materials, field performance of the plants was supreme.

The IDR shoot derived plants and R plants with various crown sizes were also evaluated for flowering and fruiting parameters as displayed in Table 1. The lowest number of days to first flower appearance and higher number of flowers per plant, number of fruits per plant, weight per fruit and bigger size of fruit were observed in IDR shoot derived plants of *cv.* 'Festival' with crown size >1 cm of the planting materials as compared to R plants with various other smaller sizes of the crown. With an increase in crown size of the planting materials, irrespective of the sources, the performance of strawberry in various reproductive parameters also increases. The interaction effects revealed that the number of days taken to first flower appearance, higher number of fruits per plant, higher weight per fruit and bigger size of fruit was noticed in IDR > 1 cm. Surprisingly, no variation in TSS of fruits were observed from both source of planting material, i.e. IDR and R plants. Regarding various crown sizes, maximum TSS was obtained in crown size >1 cm, whereas, crown size < 0.5 cm of the planting materials exhibited minimum TSS. Interactions between source of planting materials and sizes of crowns showed no significant effect on TSS of the fruits.

DISCUSSION

The chronicled data indicated that *in vitro* propagated plants performed better in the field compared to runner derived plants. This is because of increased vigor and axillary bud activity, possibly related to the forced proliferation *in vitro* through hormonally induced crown branching (Swartz *et al.*, 1981). The increased runner production of *in vitro* propagated plants is also believed to be due to enhanced axillary bud activity caused by the carry over effect of cytokinin in the shoot proliferation medium as reported by Waithaka *et al.* (1980). Similarly, the increase

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Table 1. Reproductive responses and TSS of strawberry *cv.* 'Festival' to source of planting materials with various crown sizes in field conditions.

Factors	Days to first flower induction	Number of flowers per plant	Number of fruits per plant	Average fruit weight (g)	Fruit size (L×B) (mm²)	TSS (°B)
Planting materials						
R [a]	38.81 ± 0.57	16.56 ± 0.82	8.91 ± 0.56	8.88 ± 0.40	892.28 ± 22.27	10.72 ± 0.21
IDR [b]	34.03 ± 0.85	21.75 ± 0.75	12.38 ± 0.50	11.23 ± 0.42	1167.25 ± 32.55	10.81 ± 0.26
CD (P= 0.05)	0.47	0.34	0.39	0.23	19.34	NS
Crown size						
< 0.5 cm	39.19 ± 0.70	11.81 ± 0.75	7.19 ± 0.33	6.26 ± 0.29	686.56 ± 18.41	8.83 ± 0.27
0.5 to 0.8 cm	37.31 ± 0.44	16.81 ± 0.75	8.81 ± 0.56	8.38 ± 0.42	810.81 ± 17.79	9.83 ± 0.16
0.8 to 1 cm	35.44 ± 0.78	21.88 ± 0.85	11.88 ± 0.67	11.36 ± 0.49	1109.88 ± 25.29	11.96 ± 0.25
> 1cm	33.75 ± 0.92	26.13 ± 0.78	14.69 ± 0.56	14.23 ± 0.44	1511.81 ± 48.16	12.44 ± 0.25
CD (P= 0.05)	0.66	0.48	0.56	0.32	27.36	0.17
Interaction effect						
R< 0.5	41.13 ± 0.75	9.25 ± 0.65	5.88 ± 0.25	5.00 ± 0.21	654.13 ± 24.41	8.78 ± 0.25
R 0.5 to 0.8	39.50 ± 0.41	14.38 ± 0.85	7.38 ± 0.48	7.50 ± 0.34	722.75 ± 10.00	9.80 ± 0.14
R 0.8 to 1	37.88 ± 0.48	19.13 ± 0.85	9.38 ± 0.85	9.94 ± 0.65	883.75 ± 13.56	11.95 ± 0.21
R> 1	36.75 ± 0.65	23.50 ± 0.91	13.00 ± 0.65	13.08 ± 0.40	1336.00 ± 41.12	12.35 ± 0.24
IDR< 0.5	37.25 ± 0.65	14.38 ± 0.85	8.50 ± 0.41	7.53 ± 0.38	719.00 ± 12.40	8.88 ± 0.29
IDR 0.5 to 0.8	35.13 ± 0.48	19.25 ± 0.65	10.25 ± 0.65	9.25 ± 0.51	898.88 ± 25.58	9.85 ± 0.17
IDR 0.8 to 1	33.00 ± 1.08	24.63 ± 0.85	14.38 ± 0.48	12.78 ± 0.32	1308.50 ± 37.03	11.98 ± 0.30
IDR> 1	30.75 ± 1.19	28.75 ± 0.65	16.38 ± 0.48	15.39 ± 0.48	1715.13 ± 55.19	12.53 ± 0.26
CD (P= 0.05)	0.94	N.S	0.79	0.45	38.69	NS

[a] Runner derived, [b] *In vitro* Direct shoot Regeneration.

in plant height of *in vitro* propagated plants might be due to physiological influence of growth hormones in the culture medium or a physiological response to higher leaf densities. The results obtained are consistent with the findings of Zebrowska *et al.* (2003), Swartz *et al.* (1981), Cameron *et al.* (1989) and Lopez-Aranda *et al.* (1994) who reported that micropropagated plants produced more leaves and runners in comparison to vegetatively propagated plants through runners. Similar results have been reported in meristem culture propagated plants of 3 other genotypes by Swartz *et al.* (1981). Gantait *et al.* (2010) also reported that the *in vitro* generated plants develop higher number of leaves enabling them to intercept incoming radiation for photosynthesis earlier than conventionally propagated plants. The present study also complies with the report of Gustavsson and Stanys (2000) wherein they observed a better performance of *in vitro* derived lingonberry plants than the conventionally propagated plants. Early flowering in *in vitro* propagated plants of strawberry is in agreement with results reported in some other genotypes (Cameron and Hancock, 1986; Swartz *et al.*, 1981). Early flowering in *in vitro* propagated plants might have been caused by the action of IBA in the rooting medium which adds up with the endogenous auxins responsible for flower induction as reported in strawberry by Moore and Hough (1962). Similarly, Zebrowska *et al.* (2003), Swartz *et al.* (1981), Cameron *et al.* (1989), and Lopez-Aranda *et al.* (1994) reported that *in vitro* propagated plants produced more number of flowers than runner propagated plants. The increased number of fruits per plant of tissue cultured plants was mainly due to an increased number of flowers per plant. Increased fruit production was not accompanied by reduced fruit weight. Similar results have also been reported by Nehra *et al.* (1994). However, this is in contrary to the previous reports of Cameron *et al.* (1985) and Swartz *et al.* (1981) where increased flower and fruit production were found to be associated with a reduction in mean fruit weight. These differences might have been due to overcrowding of tissue culture plants in matted rows in previous studies.

Plants with crown size larger than 1 cm resulted in maximum performance in both vegetative and reproductive characters in the field. The findings are consistent with the reports of other workers, in which larger crowns resulted in higher vigor and faster initial growth, earlier and higher fruit yield (Bish *et al.*, 2002; Giménez *et al.*, 2009), inflorescence initiation and number of flowers (Mason, 1987; Jemmali and Boxus, 1993). Even strawberry number and average fruit weight seem to be positively correlated to crown size (Le Mière *et al.*, 1998). Due to stress in the days after planting, the new plant roots are not immediately able to absorb water and nutrients (Gautier *et al.*, 2001), moreover strawberry is considered having a slow root growth (Hennion and Veschambre, 1997). Consequently, plants with bigger crown contained more number of roots, thereby absorbing water and nutrients soon after planting, resulting in faster initial growth and early and higher total fruit yield, as also reported by Hochmuth *et al.* (2001), Durner *et al.* (2002) and Takeda and Hokanson (2003). Thus, the crown size >1 cm can be set as a selection criterion for planting materials.

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کارایی تکثیر درون شیشه ای و اندازه پاهنگ (طوقه) بر عملکرد رویشی توت فرنگی (*Fragaria×ananassa* Duch) کولتیوار Festival در شرایط مزرعه ای

س. دینگنگان، م. ماهادواما، و ب. ن. سرینیواسا مورتی

چکیده

گیاهچه های توت فرنگی کولتیوار Festivalکه به روش درون شیشه ای یا با استفاده از ساقه رونده تکثیر و افزایش یافته بودند به مزرعه منتقل شدند و اندازه پاهنگ (طوقه) بوته های به دست آمده از هر دو منبع مواد گیاهی با هم مورد مقایسه قرار گرفتند. چهار اندازه طوقه شامل۰/۵ cm >، ۰/۸-۰/۵ سانتی متر،۱- ۰/۸ سانتی متر و 1 cm< از هر دو منبع مواد گیاهی تهیه شد. نتایج به دست آمده روشن ساخت که در شرایط مزرعه، واکنش های رشد سبزینه ای و رشد زایشی بوته های به دست آمده از تکثیر درون شیشه ای درمقایسه با بوته های تکثیر شده از ساقه رونده برتری داشت. نتایج به دست آمده

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از میان پاهنگ های بوته های آزمایش شده نشان داد که در همه پارامترهای رویشی وزایشی مانند تعداد پاهنگ در بوته، تعداد برگ ها، ارتفاع بوته، تعداد ساقه های رونده، سطح گسترش بوته، مساحت برگ انتهایی، تعداد روز تا گل انگیزی، تعداد گل ها، تعداد میوه، میانگین وزن و اندازه میوه ،عملکرد بوته های دارای پاهنگ های بزرگتر از ۱ سانتی متر بهتر بود. از این قرار، برای تولید تجارتی در مقیاس کلان می توان کاشت بوته های گیاهان تکثیر شده درون شیشه را توصیه کرد. نیز، پاهنگ بزرگتر از ۱ سانتی متر را می شود به عنوان ضابطه انتخاب مواد گیاهی در نظر گرفت.

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