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Multiplication of strawberry stock plants at different planting times

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ABSTRACT

The objective of this research was to determine the effectiveness of the multiplication of micropropagated strawberry stock plants as a method for producing runner tips to be used in the production of plug transplants. Micropropagated stock plants of cultivars INIA-Arazá, INIA-Guenoa and INIA-Yvapitá were planted at October 1st, 2008. Runner tips emitted by the micropropagated stock plants were harvested and planted as multiplied stock plants at 37; 40; 46; 51; 58; 64; 67; 75 and 87 days after planting the micropropagated stock ones. Runner tips emitted by all plants between October, 2008 and April, 2009, were harvested and counted, the number of root nodules counted and the crown diameter measured. The number of runner tips decreased linearly on all stock plants by delaying planting time, while crown diameter and number of root nodules were not affected. Higher number of runner tips was produced by micropropagated stock plants. The cultivar INIA-Arazá was the most prolific. Multiplying stock plants may be a new propagation method than can be used for reducing the number of micropropagated stock plants required for the production of strawberry commercial plug transplants.

Keywords: *Fragaria x ananassa*, plant propagation, runner tips, plug plants.

RESUMO

Multiplicação de plantas matrizes de morangueiro em diferentes épocas de plantio

O objetivo desta pesquisa foi determinar a eficácia da multiplicação de plantas matrizes micropropagadas como um método para a produção de pontas de estolões para serem utilizadas na produção de mudas em bandejas. As plantas matrizes micropropagadas das cultivares INIA-Arazá, INIA-Guenoa e INIA-Yvapitá foram plantadas em 1° de outubro de 2008. As pontas de estolões emitidas pelas plantas matrizes micropropagadas foram colhidas e plantadas como plantas matrizes multiplicadas aos 37; 40; 46; 51; 58; 64; 67; 75 e 87 dias após o plantio das plantas micropropagadas. As pontas de estolões emitidas por todas as matrizes entre outubro, 2008 e abril, 2009, foram colhidas e contadas, o número de primórdios radiculares foi contado e o diâmetro de coroa medido. O número de pontas de estolões diminuiu linearmente com a época de plantio enquanto o diâmetro da coroa e o número de nódulos radiculares não foram afetados. O número mais elevado de pontas foi obtido com as plantas matrizes micropropagadas. A cultivar INIA-Arazá foi a mais prolífica. A multiplicação de plantas matrizes pode ser um novo método de propagação a ser empregado para reduzir o número de plantas matrizes micropropagadas na produção de mudas de morangueiro em bandejas.

Palavras-chave: *Fragaria x ananassa*, propagação de plantas, pontas de estolões, mudas em bandejas.

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The use of transplants of high physiological and sanitary quality is a critical step in the strawberry crop production. In Southern Brazil, transplants are mainly of bare root type and they are produced in soils contaminated by diseases, leading to low fruit yield. As a consequence, growers import every year from Argentina and Chile a great proportion of transplants they need to establish their crops (Oliveira *et al*., 2005).

For strawberry commercial fruit production has been a trend in recent years and in many countries to use plug transplants instead of bare root transplants (Durner *et al*., 2002; ARMEFLHOR, 2006). Plug transplants are produced from runner tips rooted in trays filled with substrate. Main advantages are the ease of transplanting, reduction in pesticide requirements to control soil borne diseases, lower water requirements and improved plant survival (Durner *et al*., 2002; Giménez *et al*., 2008). In the United States and Canada, to produce runner tips for further production of plug transplants, micropropagated plants are transplanted as stock plants in double-rows on fumigated raised beds covered with polyethylene mulch (Durner *et al*., 2002). In Europe, plastic bags or small pots are used (ARMEFLHOR, 2006). Runner tips are emitted from early spring to late summer, when growers need transplants to establish their crops. Therefore, runner tips emitted before the right time for plug transplant production had to be picked out and discarded, increasing labor costs. Another consequence is that a high number of micropropagated plants had to be planted at spring to reach a high production of plug transplants in a short time period in late summer.

For production of strawberry plug transplants in Brazil, acclimatized *in*

vitro stock plants are planted at early spring in soilless growing systems (Giménez *et al*., 2008). About one month later the emission of runners begins and proceeds until late summer. In Southern Brazil, growers plant their crops for fruit production in March, April and May. The production of commercial plug transplants by nurseries begins in late January and has to be planned in such a way to attain the grower's planting timing.

Strawberry runner tips to be used for plug transplant production has been considered as bearing two trifoliate leaves, with the oldest one measuring between 6.5 and 10.0 cm and a crown diameter between 2.0 to 5.0 mm, with visible root nodules, preferably 0.5 to 1.0 cm long (Hennion *et al*., 1997; Lieten, 2000; Durner *et al*., 2002). Tips harvested with such characteristics stay for about 30-35 days in trays with substrate for rooting and starting growing new leaves. From October until December, runner tips emitted by stock plants are periodically cut off and discarded, because it is not the right time for production of commercial transplants. During this time, intensive labor is required to plant and nutrient solution management. Production costs are increased by additional labor, fertilizers and substrate in relation to the traditional production method of bare root transplants.

A way to increase the number of stock plants may be planting runner tips from October to December, to generate new stock plants. By this way, the number of stock plants available for plug transplant production in January may be increased. This practice may also be a way to improve the efficiency of this propagation method and reducing production costs. However, in late spring, emission of runners by multiplied stock plants is stimulated by temperature and photoperiod. In such conditions, a competition for assimilates between growth of runners and the stock plant could take place, leading to reduction in number, growth and development of runner tips. Data to clarify such questions were not found in the literature.

The objective of this research was to determine the effectiveness of the multiplication of micropropagated strawberry stock plants as a method for producing runner tips to be used in the production of plug transplants.

MATERIAL AND METHODS

The experiment was carried out at the Universidade Federal de Santa Maria (UFSM) (29º42'S; 53º42'W; 95 m height), between October 1st, 2008, and April 15th, 2009, inside a polyethylene greenhouse. During this time period, maximum, minimum and monthly average air temperatures were 34.2ºC, 17.4ºC and 25.8ºC, respectively.

Acclimatized strawberry stock plants were obtained from the laboratory of UFSM. Micropropagated stock plants from the laboratory were acclimatized in the greenhouse (Bisognin, 2007) and planted at October 1st, 2008. On 37; 40; 46; 51; 58; 64; 67; 75 and 87 days after planting, runner tips emitted by micropropagated stock plants bearing as least one visible root nodule were harvested, counted and its crown diameter was measured. Tips were rooted in trays under mist for 12 days, in a similar way as done for plug transplant production (Durner *et al*., 2002), acclimatized during four days and planted in bags. Ten planting times were obtained, the first one by micropropagated stock plants and the other nine by multiplied stock plants.

Planting was done in 2.3 L polyethylene bags, filled with commercial substrate for leaf vegetables and placed on top of a polyethylene film-covered-bed, at 0.80 m height from soil surface. A density of 12 plants m^{-2} was used, with a distance of 0.27 m between rows and 0.30 m between bags. Water and nutrients were supplied by fertigation, with a 30% coefficient of drainage, using a nutrient solution containing, in mmol L^{-1}: 10.60 NO_3^-; 0.43 NH_4^+; 2.00 $H_2PO_4^-$; 6.15 K^+; 3.00 Ca^{2+}; 1.00 Mg^{2+} and 1.00 SO_4^{2}, and in mg L^{-1}: 0.03 Mo; 0.42 B; 0.06 Cu; 0.50 Mn; 0.22 Zn and 1.00 Fe. The pH and electrical conductivity were maintained between 5.5 and 6.5 and 1.4 and 1.5 dS m^{-1}, respectively (Andriolo, 2007). Plants were daily fertigated three times during 15 minutes, at 9:00 am, 12:00 noon and 4:00 pm from October 1st and November 20th. From November 20th to April 15th, additional five minutes daily fertigations were done at 10:30 am, 1:30 pm, 3:00 pm, 5:00 pm and 7:00 pm.

The short day strawberry cultivars INIA-Arazá, INIA-Guenoa and INIA-Yvapitá were used. "INIA-Arazá" has a very early harvest season under plastic tunnels and produces a high number of runners in the nursery. "INIA-Guenoa" is an erect plant with good architecture, equilibrated in vigor and production. "INIA-Yvapitá" is a genotype only adapted to open field crop. This cultivar has a strong and vigorous plant and produces a good number of runners in the nursery (Vicente *et al*., 2009).

Treatments were three cultivars and ten planting times, in a split plot block randomized experimental design, with seven replications of one plant, cultivars in plots and planting times in subplots.

All runner tips emitted in the experimental period were harvested, counted, the crown diameter measured (mm) and the number of visible root nodules was recorded. Counted data were transformed by the equation $(x+0.5)^{½}$. All data were submitted to analysis of variance and variables differing by the F test at $p>0.05$ were compared by the Scott Knott test or polynomial regression.

RESULTS AND DISCUSSION

Number of runner tips decreased linearly along the ten planting times, in spite of genotypic differences among cultivars (Figure 1). Higher numbers were produced by micropropagated plants of the three cultivars. The decreasing rate was stronger on "INIA-Arazá" and similar on "INIA-Guenoa" and "INIA-Yvapitá". The decrease in the production of runner tips by delaying planting dates of multiplied stock plants was a consequence of the number of days for plant growth and development from planting to the end of the experiment. At this time, the amount of runner tips produced along the ten planting times was 257, 122 and 215 by "INIA-Arazá", "INIA-Guenoa" and

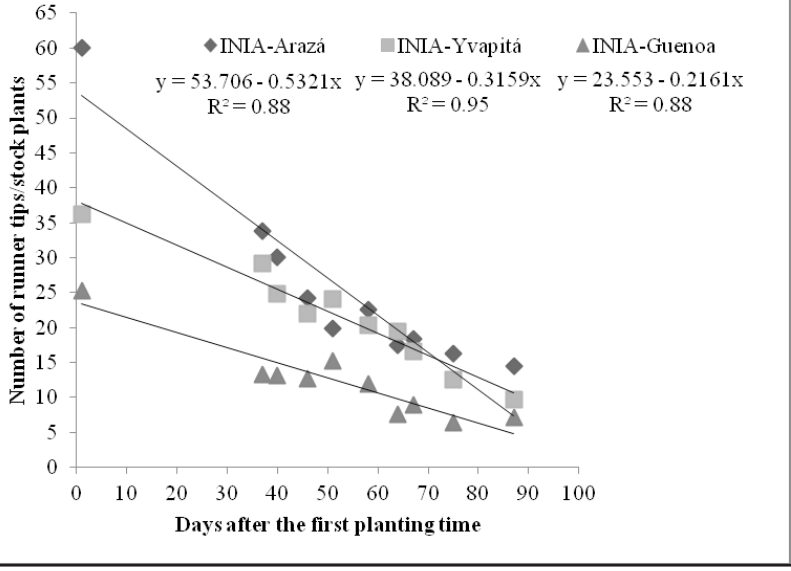

Figure 1. Average number of runner tips per stock plant produced by micropropagated (first serial planting time) and multiplied strawberry (2nd-10th serial planting times) stock plants [número médio de pontas de estolão por planta matriz produzidas por plantas matrizes de morangueiro micropropagadas (primeira época de plantio) e multiplicadas (2ª-10ª época de plantio)]. Santa Maria, UFSM, 2009.

Table 1. Crown diameter of runner tips produced by micropropagated (first serial planting time) and multiplied strawberry (2nd-10th serial planting times) stock plants (diâmetro de coroa de pontas de estolões produzidas por plantas matrizes de morangueiro micropropagadas (primeira época de plantio) e multiplicadas (2ª-10ª) épocas de plantio). Santa Maria, UFSM, 2009.

Serial planting time	Days after the first planting time	Cultivars		
		INIA-Arazá	INIA-Guenoa	INIA-Yvapitá
1	0	5.69 nsc	7.00 nsb	8.22 nsa
2	37	5.56 c	7.00 b	8.19 a
3	40	5.57 c	6.92 b	8.04 a
4	46	5.40 b	7.22 a	7.94 a
5	51	4.97 c	7.35 b	8.33 a
6	58	5.79 c	7.40 b	8.25 a
7	64	5.46 c	7.19 b	8.36 a
8	67	6.10 b	7.74 a	8.00 a
9	75	5.82 c	7.14 b	8.01 a
10	87	6.07 b	7.55 a	7.68 a
Means		5.65	7.25	8.10
CV (%) planting time			10.16	
CV (%) cultivars			7.49	

Means followed by same letters in the line do not differ by the Scott Knott test at 5% probability (médias seguidas da mesma letra na linha não diferem significativamente pelo teste Scott Knott a 5% de probabilidade); nsnon significant (não significativo).

"INIA-Yvapitá" plants, respectively.

Crown diameters of runner tips produced along the ten planting times differed among cultivars as a consequence of genotypic differences (Table 1). Higher mean values were recorded on cultivar INIA-Yvapitá and lower ones on cultivar INIA-Arazá. No significant differences were recorded in the crown diameter of runner tips harvested on multiplied plants at different planting times.

The number of root nodules of runner tips also differed among cultivars (Table 2). The higher number of root nodules was recorded in the cultivar INIA-Arazá, which had the lower crown diameter. Similar numbers were observed in cultivars INIA-Guenoa and INIA-Yvapitá. Such differences in number of root nodules of strawberry runner tips have been considered as a genotypic characteristic (Hokanson, 2004). Thus, differing the planting time by multiplying stock plants did not affect the crown diameter and number of root nodules of runner tips and the survival rate was 100% on all cultivars (data not shown).

By the usual method of strawberry plug plant production, only runner tips of micropropagated stock plants would be produced. Of those, 71% on average were available for plug transplant production at the right time for sale. Among the remainder runner tips not used for plug transplant production, 25.4% were planted as multiplied stock plants and produced runner tips for plug transplant production at the right time. The total number of runner tips produced by micropropagated stock plants during the experimental period was 300 tips m^{-2} in "INIA-Guenoa", 432 tips m^{-2} in "INIA-Yvapitá" and 720 tips m^{-2} in "INIA-Arazá". These numbers lie within the range reported by ARMEFLHOR (2006), of about 400 tips m^{-2}, on commercial cultivars. In a per plant basis, numbers were 60; 25 and 36 tips for clones "INIA-Arazá", "INIA-Guenoa" and "INIA-Yvapitá", respectively (Figure 1). They were higher than the 10 and 22 tips $plant^{-1}$ reported by Hokanson (2004) in cultivars Northeaster and Lateaster, the 19 tips $plant^{-1}$ by Treder (2007) in "Elsanta" and the 12-20 tips $plant^{-1}$ by Lieten (2000) in "Elsanta".

Among the ten planting times, a small number of runner tips was not used for production of plug transplants in plants from micropropagated and multiplicated stock plants at 37, 40 and 46 days after the first planting time. Tips were not used because it was too late to more multiplications of stock plants

Table 2. Number of root nodules of runner tips produced by micropropagated (first serial planting time) and multiplied strawberry (2^{nd}-10^{th} serial planting times) stock plants [número de primórdios radiculares de pontas de estolões produzidas por plantas matrizes de morangueiro micropropagadas (primeira época de plantio) e multiplicadas (2^{a}-10^{a} época de plantio)]. Santa Maria, UFSM, 2009.

Serial planting time	Days after the first planting time	Cultivars		
		INIA-Arazá	INIA-Guenoa	INIA-Yvapitá
1	0	7.20 ^{ns}a	3.83^{ns}b	3.99 ^{ns}b
2	37	7.35 a	4.12 b	4.66 b
3	40	7.04 a	4.83 b	3.78 b
4	46	7.30 a	4.03 b	3.71 b
5	51	7.10 a	3.94 b	4.13 b
6	58	8.07 a	3.56 b	4.26 b
7	64	7.13 a	4.01 b	4.66 b
8	67	7.35 a	3.77 b	4.77 b
9	75	8.01 a	4.32 b	4.81 b
10	87	8.34 a	4.03 b	4.67 b
Means		7.49	4.04	4.34
CV (%) planting time			26.21	
CV (%) cultivars			15.19	

Means followed by same letters in the line do not differ by the Scott Knott test at 5% probability (médias seguidas de mesma letra na linha não diferem entre si pelo teste de Scott Knott a 5% de probabilidade). ^{ns} non significant (não significativo). Data were transformed by the equation $(x+0.5)^{½}$ [os dados foram transformados pela equação $(x+0.5)^{½}$].

and too early for production of plug transplants for sale. Considering all ten planting times and cultivars, 95.4% of runner tips were used for plug transplant production. The number of runner tips produced by micropropagated plants was 1,164 tips m^{-2} in "INIA-Guenoa", 2,148 tips m^{-2} in "INIA-Yvapitá" and 2,364 tips m^{-2} in "INIA-Arazá". These numbers are higher than that reported by ARMEFLHOR (2006) on commercial cultivars, of about 400 tips m^{-2} for production of transplants for sale. Using this method of multiplying stock plants, the number of runner tips for plug transplant production was increased 3.3; 3.9 and 5.0 times in "INIA-Arazá", "INIA-Guenoa" and "INIA-Yvapitá", respectively.

In the commercial production of strawberry transplants, the planting time of stock plants have to be planned in such a way to maximize the production of runner tips at the right time to sale plug transplants. This may be done by early planting a small number of micropropagated stock plants, followed by its multiplication, as done in the present experiment. Another way is planting a great number of microprogated stock plants later in spring. The choice depends on availability of stock plants from laboratories and local production costs for crop management. In both cases, genotypic differences should be taken into account. The prolificacy of the cultivar being used should be considered to estimate the number of stock plants necessary to attain commercial goals of plug transplant production. The growing system is another factor to be considered. In hydroponic facilities, plant densities from 6 to 12 stock plants m^{-2} have been reported in the literature (Treder *et al*., 2007; ARMEFLHOR, 2006). In the present experiment using individual polyethylene bags the 12 stock plants m^{-2} was reached, maximizing the surface use efficiency of the greenhouse.

Multiplying stock plants may be a new propagation method that can be used in the production of strawberry commercial plug transplants. Its main advantage is the reduction in the number of micropropagated stock plants required for runner tip production. To attain a production of 100,000 ones by the traditional method, 1,667 micropropagated plants would be necessary using the most prolific cultivar INIA-Arazá, which has an average rate of 60 runner tips per micropropagated stock plant (Figure 1). By the present method, one micropropagated plant might originate 14 new stock plants in the further nine multiplications. Each one of these 14 new multiplied stock plants might produce 22 runner tips, at a rate of about 368 runner tips per micropropagated stock plant. With only 291 micropropagated stock plants the same 100,000 commercial plug transplants might be produced, which represent a reduction of about 83% on the required number of micropropagated stock plants. In conclusion, multiplying stock plants is a method than can be used for reducing the number of micropropagated stock plants required for the production of strawberry commercial plug transplants.

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