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Influence of shoots type and light intensity on carnation seedlings production

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Abstract

The aim of this study was to determine the influence of shoots type and light intensity on plantlets rooting and duration of seedling preparation for transplantation of a red carnation cultivar (*Dianthus caryophyllus* L.). The study was conducted during two consecutive years, 2017-2019, at the Flowers Greenhouse under the ownership of Mehmet Loci in Fushë Kruja, at the central part of Albania. Two different types of shoots, semi-hardwood shoots and green shoots, as well as two different light intensities, natural light intensity (with an mean of 14367 lux) and reduced light intensity (with an mean of 1574 lux), with 5 replication with 104 shoots, 3.5 and 4 cm in length, for each replication, were used in both years. Green and semi-hardwood shoots were placed for rooting in small styrofoam boxes (0.010 liters) filled with perlite-peat mixture (60:40), under controlled environmental conditions (18±2^{o}C and 70±5% RH). Observed results showed that both factors, shoots type and light intensity, had significantly influenced the rooting ability and the duration period of transplantation of seedlings. Rooting percentage of plantlets for semi-hardwood shoots was 63% and 45%, while for green shoots, rooting percentage of plantlets was 86% and 68% at natural light intensity (with an mean of 14367 lux) and reduced light intensity (with an mean of 1574 lux), respectively. Duration of the period planting to root initiation was 19 and 21 days for green shoots and 13 and 16 days for for semi-hardwood shoots, at 14367 lux and 1574 lux light intensity, respectively. Plantlets were ready for the permanent transplantation after 39-42 days for green shoots and after 28-31 days for semi-hardwood shoots, at 14367 lux and 1574 lux light intensity, respectively. The use of semi-hardwood shoots has significantly accelerated rooting and transplantation duration by 11 days compared to green shoots, for the same light intensity level. Results showed that there exists a strong negative correlation between the light intensity, rooting initiation, and transplantation period ($r = -0.93576278$ and $r = -0.916217561$, respectively), while, between rooting initiation duration, transplantation period, and rooting percentage exists a strong positive correlation ($r = +0.983803957$ and $r = +0.965430639$), which means that the acceleration of rooting initiation significantly affect the acceleration of transplantation and the number of seedlings. Relationship between shoots type, light intensity, and rooting percentage was significant and statistically confirmed by ANOVA ($p \leq 0.05$).

Keywords: carnation, *Dianthus caryophyllus* L., green shoots, light intensity, plantlet, rooting percentage, semi-hardwood shoots.

Introduction

Ornamental plant production is an important part in the global market for agricultural products, and, among them, cut flowers are the 337th most traded product and the 1122nd most complex product according to the Product Complexity Index (PCI) (OEC, 2019). Carnations are one of the most popular commodities (Acosta *et al.*, 2009), with of the longest-lasting shelf life up to 21 days, having a wonderful clove-like fragrance (Iannotti, 2019). Carnations are perennial herbaceous plants, native to Eurasia or the Mediterranean region, commonly referred by their scientific name "*Dianthus*", which have been extensively cultivated for the last 2,000 years (CBI, 2017). In 2015, exports of carnations from UK in/out of the Netherlands, Germany, Poland and France valued of €87 million (CBI, 2017). The natural flowers of *Dianthus caryophyllus* L are of bright pinkish-purple coloration, but, today, due to extensive breeding, many colour varieties exist, including red, white, yellow and green. Light red carnations convey admiration, whereas the dark red version expresses deeper sentiments of love and affection. White carnations are associated with purity and luck, and pink carnations are a sign of gratitude. Phytochemical analysis of *Dianthus caryophyllus* L showed that it contained triterpenes, alkaloids, coumaruns, cyanogenic glycoside, cyanidin, pelargonidin, the yellow isosalipurposide, essential oil, volatile oil, and many other chemical contents. Pharmacological studies revealed that the plant possessed anticancer, antiviral, antibacterial, antifungal, insecticidal, repellent, antioxidant, reno-protective, anaesthetic and analgesic effects, reduce the stress, depression, fatigue, inflammation and insomnia, and improves the cardiac rhythm and blood pressure (Al-Snafi, 2017).

Efficient propagation of uniform starting material is a critical requirement for mass production of most ornamental plants, including carnation. The rooting of stem shoots with 4 to 6 pairs of mature leaves, is the most common way of propagating carnation plants and many commercial companies offer shoots of selected registered varieties just rooted or ready for rooting (Acosta *et al.*, 2009). Vegetative propagation is based on the totipotency phenomenon of plant cells, which is the ability of a single cell (i/e, *the parenchymal cells*) of the plant to divide and to transform into meristematic cells and produce all of the differentiated cells in an organism. Totipotency is the phenomenon that each plant cell contains the genetic material needed to produce a new plant identical to the parent plant (Susaj, 2011; Susaj *et al.*, 2011/a). Not all plant cells are totipotent, but under appropriate conditions, certain cells may become totipotent. A cell (and only a single cell) can be considered as totipotent if it is able to develop autonomously into a whole plant via embryogenesis (Susaj, 2011).

The rooting process is a complex sequence, where, initially some cells take on a special status (stem cells) to divide around an organized center, allowing the appearance of a meristem, and later on, cell growth will lead to a structured root appearing. In addition to other signals, auxin is involved in the rooting process (Acosta *et al.*, 2009). Adventitious roots formation in stem shoots depends on a large set of exogenous (water, light intensity, temperature, air humidity, etc.) and endogenous factors (water content, hormone levels, carbohydrate content, genotype, etc.). (Hartman *et al.*, 2002; Gjeloshi *et al.*, 2014; Justamante *et al.*, 2019). Adventitious roots are plant roots that form from any non-root tissue and are produced both during normal development (crown roots on cereals and nodal roots on strawberry and in response to stress conditions (Steffens and Rasmussen, 2015). Villanova *et al.* (2017) and Susaj *et al.*, 2011/a have reported that the initiation of adventitious roots in the stem cutting base depended on its endogenous auxin and cytokinin levels at harvest time, while the physiological status of the mother plant at harvest time and of the stem cutting influence their subsequent growth and development during the rooting process. According to Stockdale (2019), the shoots require high lighting and cool temperatures (<18^{o}C) for optimum growth. The shoots should be well rooted and ready for transplantation in approximately 30 days.

Materials and Methods

Experimental site and design

The study for the influence of shoots type and light intensity on plantlets rooting and duration of seedling preparation for transplantation of carnation seedlings was conducted during the period November-January (2017-2018 and 2018-2019), at the Flowers Greenhouse under the ownership of Mehmet Loci in Fushë Kruja, at the central part of Albania. There are studied the influence of two factors: Factor A: shoots type (age) and Factor B: light intensity, on plantlets rooting and duration period of transplantation of carnation seedlings.

Planting material

Planting shoots were collected from selected carnation mother plants, grown at the main nursery. Two types of standardized height and weight shoots lateral shoots, 3.5 cm green shoots and 4 cm semi-hardwood shoots, were used.

Light intensity

There were used two levels of light intensity: natural light intensity (with a mean of 14367 lux) and reduced light intensity (with a mean of 1574 lux). The last light intensity was secured constructing a small plastic greenhouse (36 m^3 volume - 6 m x 3 m x 2 m) inside the large glass greenhouse.

Planting conditions

Lateral shoots (green and semi-hardwood shoots) were placed for rooting directly in placed for rooting in small styrofoam boxes (13 x 8 sockets with a volume of 0.010 liters each socket) filled with perlite-peat mixture (60:40), on November 15, each year. Styrofoam boxes with plantlets were kept for rooting and growth in the greenhouse with controlled conditions (18 ± 2°C temperature, 9 hr photoperiod, natural light (14367 lux) and reduced light (1574 lux) 70 ± 5% relative humidity).

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In total, there were monitored 10400 plantlets (5200 plantlets for each shoots type: 520 plantlets for each variant in 5 repetitions, from which 2600 planted shoots were displayed to natural light intensity and 2600 plantlets were displayed to reduced light intensity) (Table 1).

Table 1: Experimental design

		Factor B: Mean light intensity (lux)	
	Variants	**B1 (14367 lux)**	**B2 (1574 lux)**
Factor A: Shoots type	**A1: green shoots**	A1B1	A1B2
		A1B1	A1B2
		A1B1	A1B2
		A1B1	A1B2
		A1B1	A1B2
	A2: semi-hardwood shoots	A2B1	A2B2
		A2B1	A2B2
		A2B1	A2B2
		A2B1	A2B2
		A2B1	A2B2

Observations, measurements and assessments

Planted shoots for rooting were treated using common practices for plant propagation up to the transplantation period. Light intensity was measured every day, using a "Standard ST-1301" instrument, outside the greenhouse at the open air, inside the large glass greenhouse, and inside the small greenhouse, and were calculated the 15 days' mean light intensity, for each year, and mean light intensity for both years of the study.

Every 3 days, there was observed roots initiation and was calculated the period of adventive root formation. At the transplantation time, was assessed the rooting percentage, and was determined the rooting duration period, as well. At the planting time and at the transplantation time was measured the shoots length, using normal instruments.

Statistical Analysis

Observed results were subject to correlation analysis and ANOVA Test ($p \leq 0.05$) aiming the raise of the accuracy of the relationship and differences between two types of shoots (Factor A) and two light intensities (Factor B) on the duration of rooting initiation, rooting period, and rooting percentage of carnation shoots as is described by Lekaj *et al*. (2014).

Results

Light intensity (lux) outside the Flowers Greenhouse and at the shoots rooting environments

Every day, for a period of 62 days, was measured the light intensity, according to the previously described methods, using a "Standard ST-1301" instrument. During the periods 15th November 2017 – 15th January 2018 and 15th November 2018 –

15th January 2019, light intensity was measured outside the greenhouse at the open air, inside the large glass greenhouse, and inside the small greenhouse, and were calculated the 15 days' mean light intensity, for each year, and mean light intensity for both years of the study. Observed results showed that the two years mean light intensity, measured outside the greenhouse, at the open air, was 37098.40 lux, and varied from 35100 lux to 38200 lux. The two years mean light intensity measured inside the large greenhouse was 14367 lux, and varied from 13750 lux to 15200 lux, while the two years mean light intensity measured inside the small greenhouse was 1574 lux, and varied from 13750 lux to 15200 lux (Table 2). Light intensity at the shoots rooting environments was 38.7% and 4.25% of the outside light intensity, inside the large greenhouse and inside the small greenhouse, respectively (Table 2). Light intensity inside the small greenhouse was about 10.95% of the large flowers greenhouse.

Table 2: Light intensity (lux) at the experimental site outside and at the shoots rooting environments (two years mean data)

Measurement period	**Days**	**Light intensity outside the large greenhouse**		**Light intensity inside the large greenhouse**		**Light intensity inside the small greenhouse**	
		Daily mean (lux)	**Total (lux)**	**Daily mean (lux)**	**Total (lux)**	**Daily mean (lux)**	**Total (lux)**
15/11–30/11	16	38200	611200	15200	243200	1850	29600
01/12 – 15/12	15	37600	564000	14700	220500	1560	23400
16/12 - 31/12	16	37 400	598400	13800	220800	1520	24320
01/01 - 15/01	15	35100	526500	13750	206250	1350	20250
Sum	62	-	2300100	-	890750	-	97570
Mean	**-**	**37098.4**		**14367**		**1574**	

Influence of shoots type and light intensity on rooting initiation and plantlets transplantation periods

Observed results showed that both factors, lateral shoots type (Factor A) and light intensity (Factor B), have had significant influence on rooting initiation and plantlets transplantation periods.

Under 14367 lux light intensity, the mean rooting initiation period was 19 days for lateral green shoots and 13 days for semi-hardwood shoots, while, under 1574 lux light intensity, the mean rooting initiation period was 21 days for lateral green shoots and 16 days for semi-hardwood shoots (Table 3).

Influence of shoots type and light intensity on rooting ability and seedlings production

Observed results showed that shoots type and light intensity had significantly influenced the rooting ability, and seedlings production quantity and quality. There were observed and measured 10400 shoots, in total, 520 planted shoots for each variant in five repetitions, every year.

Table 3: Influence of shoots type and light intensity on rooting initiation and plantlets transplantation periods (two years mean data)

Factor A: Shoots type	Factor B: Light intensity (lux)	Rooting initiation		Transplantation	
		Date	Duration (days)	Date	Duration (days)
A1: green shoots	14367 lux	04/12	19	24/12	39
	1574 lux	06/12	21	27/12	42
A2: semi-hardwood shoots	14367 lux	28 /11	13	13/12	28
	1574 lux	01/12	16	16/12	31

Two years mean data of seedling production from green shoots was 1638 seedlings or 63% (under 14367 lux light intensity), and 1170 seedlings or 45% (under 1574 lux light intensity), while seedling production from semi-hardwood shoots was 2236 seedlings or 86% (under 14367 lux light intensity), and 1768 seedlings or 68% (under 1574 lux light intensity) (Table 4).

Table 4: Seedling production of carnation according to the shoots type and light intensity (two years mean data)

	Variants	Factor B: Mean light intensity (lux)							
		B1 (14367 lux)				B2 (1574 lux)			
		Variant / repetition	Planted shoots	Seedlings number	% vs total	Variant / repetition	Planted shoots	Seedlings number	% vs total
Factor A: Shoots type	A1: green shoots	A1B1	520	308	59	A1B2	520	280	54
		A1B1	520	338	65	A1B2	520	238	46
		A1B1	520	332	64	A1B2	520	226	43
		A1B1	520	344	66	A1B2	520	208	40
		A1B1	520	316	61	A1B2	520	218	42
		Total	**2600**	**1638**	**63**	**Total**	**2600**	**1170**	**45**
	A2: semi-hardwood shoots	A2B1	520	412	79	A2B2	520	376	72
		A2B1	520	468	90	A2B2	520	348	67
		A2B1	520	432	83	A2B2	520	312	60
		A2B1	520	485	93	A2B2	520	406	78
		A2B1	520	439	84	A2B2	520	326	63
		Total	**2600**	**2236**	**86**	**Total**	**2600**	**1768**	**68**

The best results were observed using semi-hardwood shoots and normal (difuse) light intensity at an average of 14367 lux. Reduction of light intesity to 1574 lux had significantly reduced the number and the quality of seedling production of carnation. It was recommended that the best way for production of carnation seedlings is using of semi-hardwood shoots as a very successful vegetative propogation method.

Discussion

There were observed significant differences between shoots type under the same light intensity. In both cases of the light intensity, the mean rooting initiation period for semi-hardwood shoots was shorter than the mean rooting initiation period for green shoots. Results are similar to Susaj *et al.* (2011/b), who report that different cutting types have significant influence on rooting ability of *Rosmarinus officinalis* L cuttings and *Thuya occidentalis* var Emeraud cuttings, and that rooting initiation period for hardwood and semi-hardwood cuttings was shorter than green cuttings. Villanova *et al.* (2017), Hartman *et al.* (2002), Susaj *et al.* (2011/a; 2011/b) and several other authors report that high carbohidrate content influences rooting ability, accelerates adventivious root formation and rooting initiation period for 2 or 3 days, for green shoots and semi-hardwood shoots, respectively, delayed rooting initiation period of both shoot types under low light intensity. At the same time, rooting percentage and transplanted plantlets number were significantly lower under low light intensity. These results confirmed that low light intensity inhibit root formation and delay the seedling preparation period, as it was previously concluded by Hartman *et al.* (2002).
Plantlets transplantation should be done out when they are 11-14 cm in length and have 5-6 healthy mature leaves (Stockdale, 2019). Plantlets deriving from green shoots were ready for transplantation after 39 days (under 14367 lux light intensity) to 42 days (under 1574 lux light intensity), while semi-hardwood shoots were ready for transplantation after 28 days (under 14367 lux light intensity) to 31 days (under 1574 lux light intensity. Use of semi-hardwood shoots has accelerated seedling production period by about 11 days, compare to green shoots of carnation. Stockdale (2019) and other authors recommend that the transplantation period of for rooted carnation shoots is approximately 30 days. Shoots type has influenced the plantlets features such as length and leaves number. Observations and measurements showed that, after 39-42 days, plantlets deriving from green shoots were 11-12 cm in length and had 5-6 leaves, while plantlets deriving from semi-hardwood shoots were 14-16 cm in length and had 7-8 leaves. Higher carbohydrate content is one of the factors of the acceleration of root induction and shoot growth (Susaj *et al.*, 2011/a; Hartman *et al.* 2002). Results showed that low light intensity under small greenhouse delayed the plantlets transplantation by 2-3 days.

Correlation analysis for the relationship between phenological and fruit quality indicators

Correlation analysis shows that there exists a strong negative correlation between the light intensity, rooting initiation, and transplantation period ($r = -\ 0.93576278$ and $r = -\ 0.916217561$, respectively). This result statistically proves that reduction of light intensity in the rooting environment influence on the rooting initiation and transplantation period of the carnation plantlets, while between rooting initiation duration, plantlets transplantation period, and rooting percentage exists a strong positive correlation ($r = +\ 0.983803957$ and $r = +\ 0.965430639$), which means that the acceleration of rooting initiation significantly influenced the acceleration of plantlets transplantation and the raise of seedlings production (Table 5).

Tabela 5. Correlation between the light intensity in the rooting environment, rooting initiation duration, plantlets transplantation period, and seedling production of carnation

	Light intensity in the rooting environment	Rooting initiation duration	Plantlets transplantation period	Seedlings production (%)
Light intensity in the rooting environment	1			
Rooting initiation duration	-0.93576278	1		
Plantlets transplantation period	-0.916217561	+0.983803957	1	
Seedlings production (%)	+0.965430639	-0.968636739	-0.921917765	1

Observed results showed that there exists a significant relationship between light intensity on the rooting environment and shoots type with rooting initiation, plantlets transplantation, and seedling production percentage. Differences between shoots type and light intensity for the abovementioned features were significant and statistically confirmed by ANOVA ($p \leq 0.05$), as performed by Lekaj *et al.* (2014), where for the light intensity, F = 9.305 > F crit = 4.413 and P-value = 0.00688 < α= 0.05, while for shoot types F = 23.551 > F crit = 4.351 and P-value= 9.643E-05 < α= 0.05 (Table 6).

Table 6. Results of ANOVA for the relationship between light intensity and shoots type with rooting initiation, plantlets transplantation, and seedling production

Source of Variation	SS	MS	F	P-value	F crit
Shoots type (Rows)	77291.636	77291.636	23.551	9.643E-05	4.351
Light intensity (Columns)	43804.8	43804.8	9.305	0.00688	4.413

Conclusions

Two shoots type (green and semi-hardwood), tested under two levels of light intensity (14367 lux and 1574 lux), showed to have significant differences related to the rooting initiation period, transplantation period, rooting ability, and seedlings quality. Duration of root initiation, transplantation period, rooting ability, and seedlings quality depended upon the shoots type. Root initiation

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period ranged from 13 days to 16 days for semi-hardwood shoots, to 19 days to 21 days for green shoots. Transplantation period ranged from 28 days to 31 days for semi-hardwood shoots, to 39 days to 42 days for green shoots. Results showed that light intensity significantly influenced root initiation period and transplantation period duration. Both shoots types rooted 2-3 days earlier under high light intensity (14367 lux), compare to low light intensity (1574 lux).
Rooting percentage and seedling quality determine the effectiveness of propagation and the final product destination, ornamental value of carnation plants and flowers. Observed results showed that shoots type and light intensity had significantly influenced the seedling production of carnation. Seedling production from green shoots ranged from 45% to 63%, while for semi-hardwood shoots ranged from 68% to 86%, under 1574 lux and 14367 lux light intensity, respectively. Reduction of light intesity to 1574 lux had significantly reduced the number and the quality of seedling production of carnation.
Between the light intensity, rooting initiation and transplantation period exists a strong relationship confirmed by correlation analysis and the absolute value of the correlation coefficient ($r > 0.7$). Between light intensity in the rooting environment and rooting initiation, and transplantation period exists a strong negative correlation, while between rooting initiation duration, plantlets transplantation period, and rooting percentage exists a strong positive correlation. Therefore, and according to the results obtained in this study, the highest propagation results were obtained using of semi-hardwood shoots is recommended as a very effective way on propagation of carnation (*Dianthus caryophyllus* L.).

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