Evaluation of new promising hybrids of Greek sage (*Salvia* sp.) species in relation to their growth

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Abstract

Research took place to investigate the growth of six new interspecific hybrids obtained by interspecific crossings of four wild sage species found in Greece, i.e., *Salvia officinalis*, *S. ringens*, *S. pomifera* ssp. *pomifera* and *S. tomentosa*, with the aim of introducing them to the horticultural industry, particularly as ornamentals for xeriscaping. Two hybrids of each crossing *S. officinalis* × *S. tomentosa*, *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* × *S. ringens* were evaluated as regards their growth in a greenhouse culture. Rooted cuttings, one month old, were transplanted in two different peat-based substrates, i.e., peat mixed with wood fibre (PWF, pH 5.0-5.5) and PWF: perlite (1/1 v/v), in plastic pots. They were fertilized either weekly or monthly with a water-soluble fertilizer (10N-3P-11K). The water had a pH of 5.9 and an EC of 2.3 dS m^{-1}. Plant growth was evaluated on a monthly basis for three months, recording the number of lateral shoots formed, and the length and node number of three randomly selected lateral shoots. All hybrids presented vigour growth without any effect of substrate type and fertilization frequency. The two *S. officinalis* × *S. tomentosa* hybrids tested had similar plant growth between them, while they developed longer with more nodes lateral shoots compared to all other hybrids. One out of the two *S. officinalis* × *S. ringens* hybrids tested showed more compact growth due to the shorter and slightly more lateral shoots developed compared to all the other hybrids.

Keywords: fertilization, interspecific hybrid, native xerophytic ornamentals, plant breeding, peat-wood fibre substrate, xeriscaping

INTRODUCTION

Adaptation of native plants to the local environmental factors exhibits high degree of genetic diversity, which is necessary for selection and breeding of new cultivars (Bernáth, 2002). Wild species are a large gene pool for genetic improvement of various species. Many studies of breeding and introduction of new ornamental or medicinal and aromatic species have been published the recent years (Datta, 2021). Hybridization and improvement of specific species is the main target of research studies and horticulture industry (de Dreux, 2001). Classical methods like pollination have been used leading to success of interspecific hybridization by specific cross combinations and successful fertilization (Van Tuyl and Lim, 2003). Interspecific hybridization is of high importance in ornamental breeding and hybrids coming from interspecific hybridization can both enclose hybrid vigour and combine traits from different species (Volker and Orme, 1988).

Interspecific hybridization could be used in the genetic improvement of native *Salvia* species to enhance useful characteristics. Under this aspect *S. officinalis*, *S. ringens*, S. *pomifera* ssp. *pomifera* and *S. tomentosa* could be interesting ornamental plants or horticultural crops for semi-arid areas combining different ornamental, morphological and biometric characteristics. Xeriscaping needs the exploitation of new species and hybrids maintaining a traditional look and using species selected for their water efficiency (Welsh, 2000; Ozyavuz and Ozyavuz, 2012). *Salvia* species of the Mediterranean basin being drought tolerant are ideal for xeriscaping, requiring a few cultivation practices. Rich flowering and colour diversity

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of flowers of different species enhance ornamental value being of high importance for bees too.

Fertilizers are important for plant growth providing the necessary nutrients for optimum plant development. Inorganic fertilizers are easy to use in commercial farming being quickly absorbed and utilized by crops (Masarirambi et al., 2010). Information on the fertilization needs of Mediterranean sage species could only be found for *S. officinalis*, which is a commercial crop, with an emphasis on mycorrhiza symbiosis (Geneva et al., 2010; Giannoulis et al., 2021).

The decomposition of peat leads to carbon dioxide emissions and various raw materials are used in horticultural growing media to reduce the use of peat targeting to reduce the proportion of peat in growing media (Laun et al., 2021). The use of peat-reduced substrate containing wood fibre has been successfully used in horticulture (Gruda et al., 2001; Laun et al., 2021).

Six interspecific hybrids between *S. officinalis*, *S. pomifera, S. ringens* and S. *tomentosa* have been developed and selected for their ornamental characteristics among others in a research program (SALVIA-BREED-GR). The *S. officinalis* × *S. pomifera* hybrids develop a few long lateral shoots with intermediate characteristics of the parents in terms of plant height, leaf colour and shape, as well as in leaf aroma. The *S. officinalis* × *S. ringens* hybrids have unique, segmented, particularly decorative, and slightly aromatic leaves. They develop long flowering stems (40 cm) as *S. ringens*, with more flowers (light violet-blue) than those of *S. ringens*. They are taller than *S. ringens* with many lateral shoots. The *S. officinalis* × *S. tomentosa* hybrids are compact plants with numerous lateral shoots and grey-green leaves smaller than those of *S. officinalis.* They form many flowering stems, with light pink flowers looking more like those of *S. officinalis.* The leaf aroma is lighter than *S. officinalis*.

In the present study, the vegetative growth of the above six interspecific sage hybrids was evaluated under the effect of two peat -wood fibre-based substrates and two fertilization frequencies, aiming to establish greenhouse protocols for fast and successful growth in order to be introduced in the horticultural industry serving the target of improvement and promotion of Greek sage species for ornamental use.

MATERIALS AND METHODS

Six interspecific sage hybrids, i.e.: M-2019-0013-01 and M-2019-0013-03 (*S. officinalis* × *S. tomentosa*), M-2019-0011-04 and M-2019-0011-05 (*S. officinalis* × *S. pomifera* ssp. *pomifera)* and M-2019-0009-02 and M-2019-0009-05 (*S. officinalis* × *S. ringens)* (Table 1) were evaluated for their growth. One-month-old, rooted stem cuttings (Figure 1) excised from two-year-old hybrid plants, after pinching their shoot tips, were transplanted singly, in plastic pots (2 L), on two different media, i.e., either peat containing wood fibre (30% wood fibre) (PWF, Forest Gold, Pindstrup, Denmark, pH 5.0-5.5) or PWF:perlite (1:1, v/v). Two different fertilization treatments were applied, i.e., fertilization either weekly or monthly with a water-soluble fertilizer (10N-3P-11K), the water having a pH of 5.9 and an EC of 2.3 dS m^{-1}. The experiment took place in a glasshouse of the company Kalantzis Plants, at Marathon Attiki, Greece, (38°09'34.7"N, 23°56'02.4"E), from end of March to end of June of 2021 (three months). The minimum and maximum temperature of 18.0 and 28.0°C, respectively, were maintained in the greenhouse, average temperature being 21.0°C. Plant growth was evaluated after three months from transplanting, recording the number of lateral shoots formed on each plant, and the length and node number of three randomly selected lateral shoots $plant^{-1}$. The plants did not flower during the period of three months.

Factorial experiments were designed, one for each hybrid, the main experimental factors being substrate type and fertilization frequency. Fifteen plants per treatment were used, the significance of the results was tested by three-, two- or one-way analysis of variance (ANOVA) and treatment means were compared by Student's *t* test at $p \leq 0.05$.

Table 1. Parental species, crossbreeding, names and codes of the six *Salvia* hybrids tested.

Female parent	Male parent	Cross	Hybrid name	Hybrid code
S. officinalis	*S. pomifera*	*S. officinalis* × *S. pomifera*	M-2019-0011-04	1104
			M-2019-0011-05	1105
S. officinalis	*S. ringens*	*S. officinalis* × *S. ringens*	M-2019-0009-02	0902
			M-2019-0009-05	0905
S. officinalis	*S. tomentosa*	*S. officinalis* × *S. tomentosa*	M-2019-0013-01	1301
			M-2019-0013-03	1303

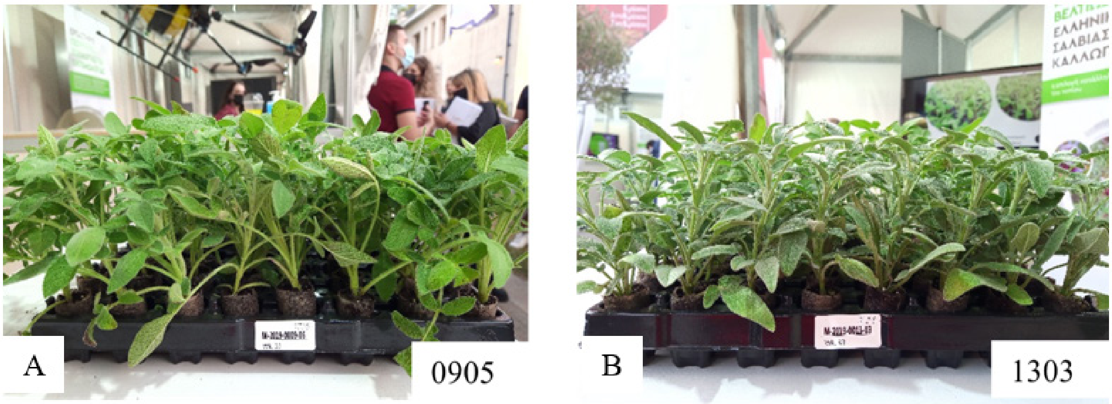

Figure 1. Rooted cuttings of *S. officinalis* × *S. ringens* (0905) and *S. officinalis* × *S. tomentosa* (1303).

RESULTS AND DISCUSSION

All three-way interactions among hybrids, fertilization and substrate were significant. Two-way analysis revealed that there was no significant effect of fertilization schedule or substrate in most of the traits, except for the substrate on node number of 1303 and shoot length of 1301 and 1105, as well as the fertilization schedule on lateral number of 0905. All hybrids had vigour growth, in terms of shoot length, node number per shoot and lateral shoot number plant^{-1} in all treatments (Tables 2-4).

In a previous study (Karimi et al., 2021) increasing the application rate of N fertilizer declined nitrogen use efficiency for *S. officinalis*. Similar reports can be found as regards N use efficiency of another xerophytic species, *Origanum vulgare* (Yazdani Biouki et al., 2014; Xie et al., 2019) and vector analysis was suggested to assess the role of substrate elements (Grigatti et al., 2007). During the last decades a global warning to reduce the use of peat has been established. The high cost of peat being at the same time a non-renewable resource and the environmental constraints led to the use of reduced peat-based media or peat-free substrates (Benito et al., 2005). Modern floriculture industry widely uses peat-based media mixed with wood fibre (Frangi et al., 2008; De Lucia et al., 2013). In the present study the peat-wood fibre medium (PWF) mixed with perlite (1/1 v/v), proved an efficient substrate for the vigour of all six hybrids, resulting to reduced use of peat, compared to the PWF routinely used in the commercial production of ornamentals including sage species.

The *S. officinalis* × *S. tomentosa* hybrids (1301 and 1303) showed higher shoot elongation in general compared to the other hybrids (Figure 2a, b). One (0902) out of the two *S. officinalis* × *S. ringens* hybrids had more compact growth due to shorter and more lateral shoots compared to all other hybrids, being similar to parent plants *S. ringens.* The 0905 hybrid, the second one from the same parental species, formed less lateral shoots than 0902, being closer to the other four hybrids (Figure 2c).

Table 2. The effect of the main experimental factors, i.e., substrate type (PWF or 1 PWF:1 perlite) and fertilization frequency (weekly or monthly) on shoot length (cm), node number shoot^{-1} and lateral shoot number plant^{-1} of the *S. officinalis* × *S. tomentosa* hybrids, after three-month culture.

Main factor	1301			1303		
	Shoot length	Node number	Lateral number	Shoot length	Node number	Lateral number
PWF	58.3 a	14.1 a	3.4 a	44.3 b	13.4 b	3.5 a
1 PWF:1 P	50.5 b	13.3 a	3.4 a	56.5 a	16.4 a	3.4 a
Weekly	55.5 a	14.1 a	3.6 a	44.3 b	15.0 a	3.4 a
Monthly	53.1 a	13.2 a	3.2 a	55.4 a	14.7 a	3.5 a
$F_{substrate}$	*	NS	NS	-	*	NS
$F_{fertilization}$	NS	NS	NS	-	NS	NS
$F_{substrate} \times F_{fertilization}$	NS	NS	NS	*	NS	NS

Mean comparison in columns within each main factor with Student's t test at P<0.05; means followed by the same letter are not significantly different at p≤0.05.
*Significant at p≤0.05; NS: non-significant.
PWF: peat mixed with wood fibre; P: perlite; substrate ratios by volume.

Table 3. The effect of the main experimental factors, i.e., substrate type (PWF or 1 PWF:1 perlite) and fertilization frequency (weekly or monthly) on shoot length (cm), node number and lateral number increase of the *S. officinalis* × *S. pomifera* ssp. *pomifera* hybrids shown after three-month culture.

Main factor	1104			1105		
	Shoot length	Node number	Lateral number	Shoot length	Node number	Lateral number
PWF	39.4 a	9.2 a	3.4 a	34.0 b	10.1 a	3.3 a
1 PWF:1 P	28.9 b	7.3 a	3.2 a	40.6 a	11.1 a	3.6 a
Weekly	29.5 b	7.1 b	3.3 a	37.4 a	10.5 a	3.5 a
Monthly	39.8 a	9.6 a	3.4 a	38.0 a	10.9 a	3.3 a
$F_{substrate}$	-	-	-	*	NS	NS
$F_{fertilization}$	-	-	-	NS	NS	NS
$F_{substrate} \times F_{fertilization}$	*	*	*	NS	NS	NS

Mean comparison in columns within each main factor with Student's t test at p≤0.05; means followed by the same letter are not significantly different at p≤0.05.
*Significant at p≤0.05; NS: non-significant.
PWF: peat mixed with wood fibre; P: perlite; substrate ratios by volume.

Table 4. The effect of the main experimental factors, i.e., substrate type (PWF or 1 PWF:1 perlite) and fertilization frequency (weekly or monthly) on shoot length (cm) node number and lateral number increase of the *S. officinalis* × *S. ringens* hybrids shown, after three-month culture.

Main factor	0902			0905		
	Shoot length	Node number	Lateral number	Shoot length	Node number	Lateral number
PWF	20.9 b	3.6 a	4.5 a	39.9 a	3.8 a	8.0 a
1 PWF:1 P	28.0 a	4.8 a	4.4 a	38.9 a	3.8 a	7.1 a
Weekly	25.3 a	3.5 a	4.4 a	42.3 a	3.9 a	8.5 a
Monthly	23.4 a	4.8 a	4.3 a	36.8 a	3.7 a	6.7 b
$F_{substrate}$	-	NS	NS	NS	NS	NS
$F_{fertilization}$	-	NS	NS	NS	NS	*
$F_{substrate} \times F_{fertilization}$	*	NS	NS	NS	NS	NS

Mean comparison in columns within each main factor with Student's t test at p≤0.05; means followed by the same letter are not significantly different at p≤0.05.
*Significant at p≤0.05; NS: non-significant.
PWF: peat mixed with wood fibre; P: perlite; substrate ratios by volume.

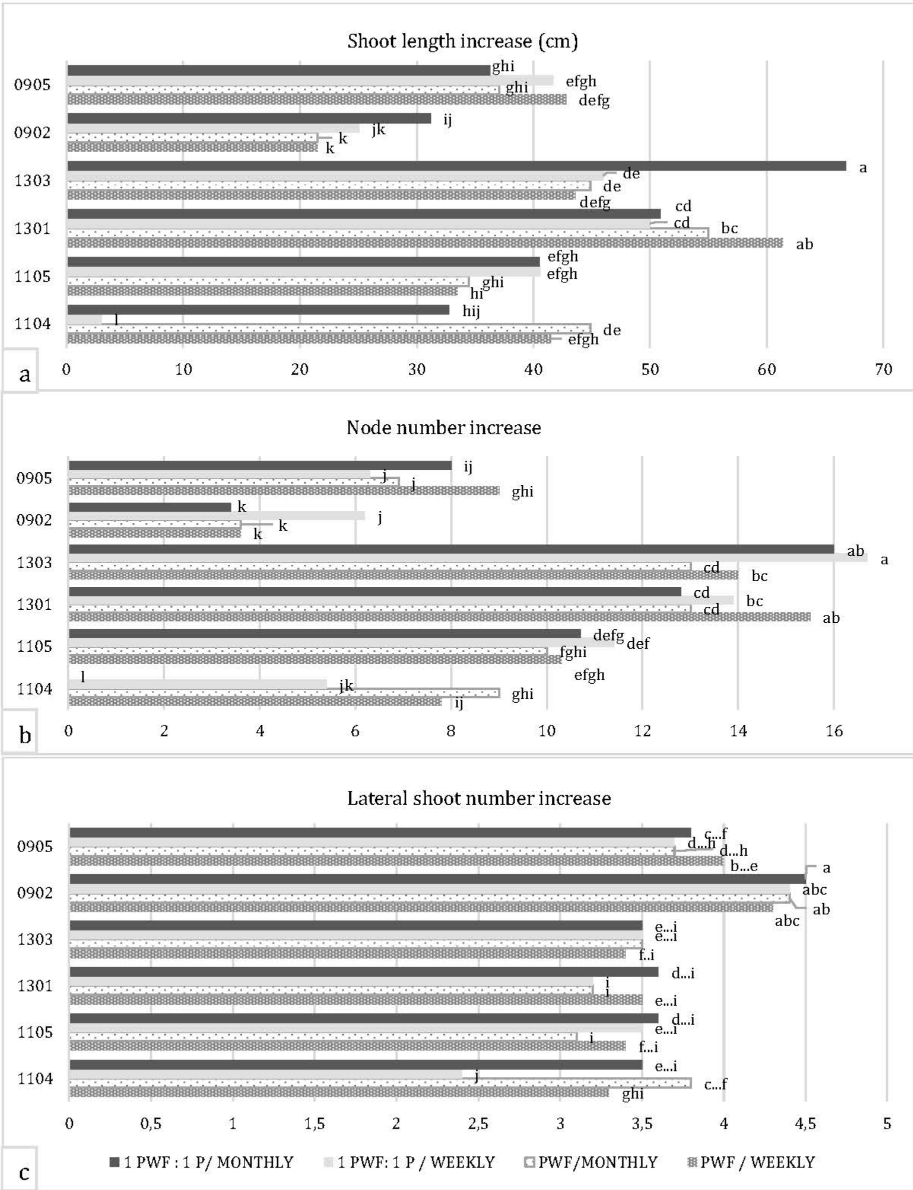

Figure 2. The effect of experimental treatments on the increase of shoot length (cm) (a), node number (b) and lateral shoot number (c) of the six sage hybrids, after three-month growing. Mean comparison p≤0.05; means followed by the same letter are not significantly different at p≤0.05. PWF: peat mixed with wood fibre; P: perlite; substrate ratios by volume.

In conclusion, in the present study vigour shoot growth of all six interspecific sage hybrids was achieved using peat-wood fibre and perlite medium (1/1 v/v) and low fertilization frequency, leading to sustainable production serving the need to reduce peat and fertilizer use in the floricultural practice. Moreover, one out of the two *S. officinalis* × *S. ringens* hybrids tested had more compact growth due to shorter and slightly more laterals compared to all other hybrids; the second hybrid from the same parental species developed more lateral shoots as well, traits that are desirable in the ornamental plants industry.

ACKNOWLEDGEMENTS

This research has been co-financed by the European Regional Development Fund of the

European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH - CREATE - INNOVATE (Project code: T1EDK-04923, Project: SALVIA-BREED-GR).

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Evaluation of new promising hybrids of Greek sage (*Salvia* sp.) species in relation to their growth

Salvia-Breed-GR

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European Union
European Regional Development Fund

HELLENIC REPUBLIC
MINISTRY OF ECONOMY & DEVELOPMENT
SPECIAL SECRETARY FOR ERDF & CF
MANAGING AUTHORITY OF EPAnEK

EPAnEK 2014-2020
OPERATIONAL PROGRAMME
COMPETITIVENESS ENTREPRENEURSHIP INNOVATION

ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη
Partnership Agreement 2014 - 2020

Co-financed by Greece and the European Union

Introduction

- Wild species are a large gene pool for genetic improvement of various species.
- Hybridization and improvement of specific species is main target of research studies and horticulture industry.
- Xeriscaping needs the exploitation of new species and hybrids maintaining a traditional look and using species selected for water efficiency.
- Salvia species of Mediterranean basin being drought tolerant are ideal for xeriscaping, as they require few cultivation practices.

As part of a research program (SALVIA-BREED-GR), aiming to the improvement and promotion of Greek sage species for ornamental use, in the present study, the effect of two different peat-based substrates and fertilization dose on the growth of rooted-stem cuttings of six different interspecific hybrids of Salvia was investigated, aiming to obtain mother plants that could be used as plant-clones for horticultural use.

Materials and Methods

- Glasshouse
- March to June of 2021 (three months)
- Rooted cuttings (one-month old)

Effect of two factors

Two substrate types

peat-based media mixed with wood fibre (PWF, Forest Gold, Pindstrup, Denmark)

Two schedules of fertilization

water-soluble fertilizer, 10N-3P-11K

Two levels for each factor

- PWF
- PWF : perlite (1/1 v/v), (plastic pots, 2 L)

- once
- four times per month

Plant growth evaluation:

- Every 30 days for 3 months
- number of lateral shoots
- length
- node number of three lateral shoots

Female parent	Male parent	Hybrid	Hybrid name	Hybrid Code
S. officinalis	*S. pomifera*	*S. officinalis* × *S. pomifera*	M-2019-0011-04	1104
			M-2019-0011-05	1105
S. officinalis	*S. tomentosa*	*S. officinalis* × *S. tomentosa*	M-2019-0013-01	1301
			M-2019-0013-03	1303
S. officinalis	*S. ringens*	*S. officinalis* × *S. ringens*	M-2019-0009-02	0902
			M-2019-0009-05	0905

Statistical Analysis

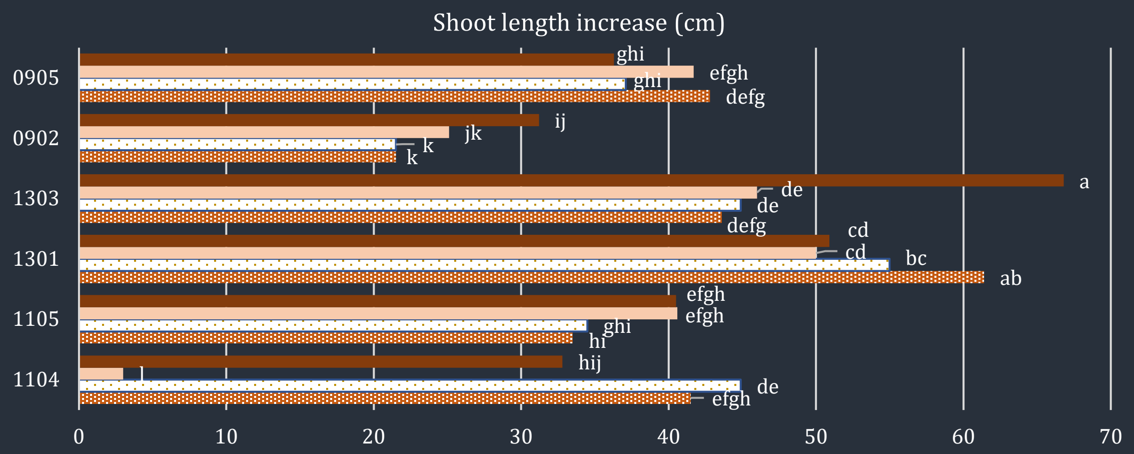
- Completely randomized design
- 15 plants/treatment
- one- and two-way analysis of variance (ANOVA)
- Student's t test at $p \leq 0.05$

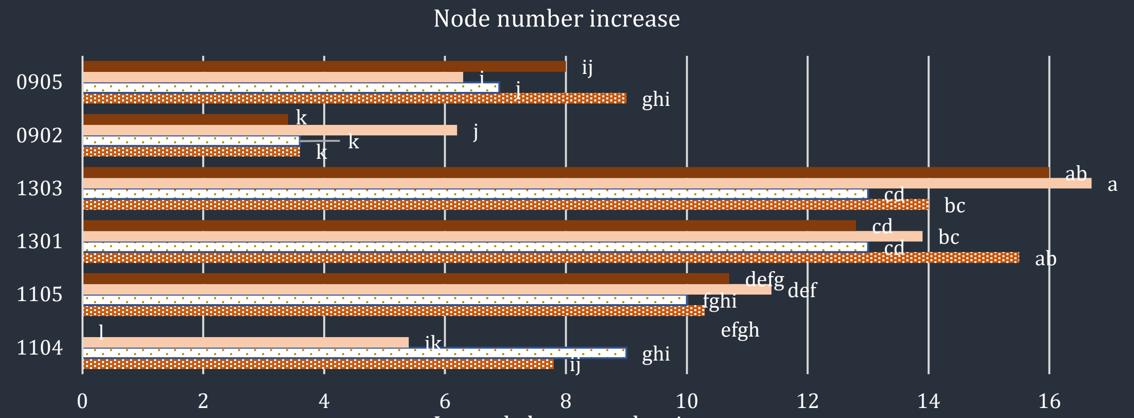
Results and Discussion

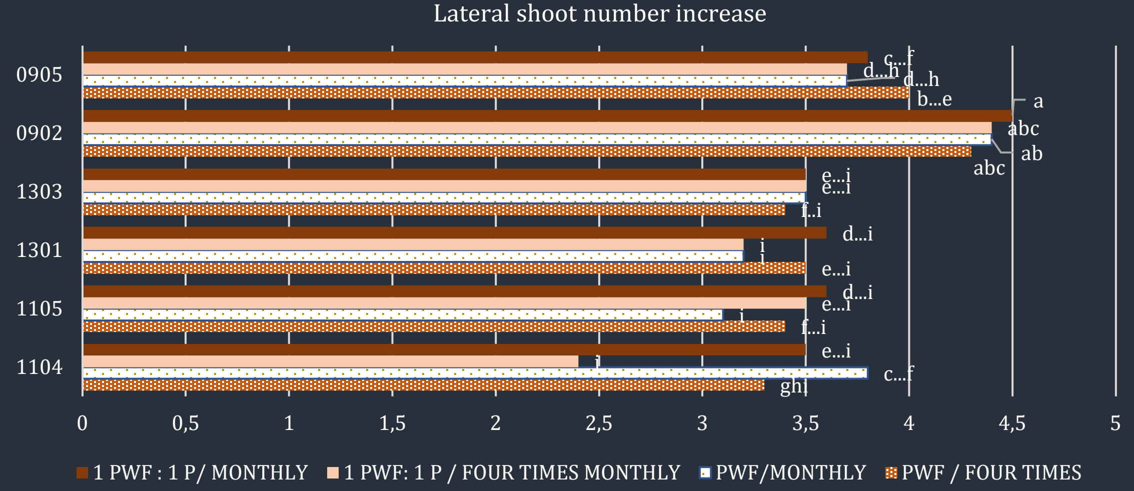
Female parent	Male parent	Hybrid	Hybrid name	Hybrid Code
S. officinalis	*S. pomifera*	*S. officinalis* × *S. pomifera*	M-2019-0011-04	1104
			M-2019-0011-05	1105
S. officinalis	*S. tomentosa*	*S. officinalis* × *S. tomentosa*	M-2019-0013-01	1301
			M-2019-0013-03	1303
S. officinalis	*S. ringens*	*S. officinalis* × *S. ringens*	M-2019-0009-02	0902
			M-2019-0009-05	0905

Figure 1. Rooted cuttings of six different hybrids, one-month old, in PWF : perlite (1/1 v/v); 0902, 0905 (*S. officinalis* × *S. ringens*), 1104, 1105 (*S. officinalis* × *S. pomifera*), 1301 and 1303 (*S. officinalis* × *S. tomentosa*).

Figure 2. *S. officinalis* × *S. pomifera* (1104) hybrid, after 60 days' culture.







Conclusions

- One out of the two *S. officinalis* × *S. ringens* hybrids tested had more compact growth due to shorter and slightly more laterals compared to all other hybrids
- All hybrids had vigorous growth in both substrate types and irrigation schedules tested

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