Intergeneric Grafting of Ornamental Incense Cedar: First Results

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Abstract— *Horticultural grafting is important in propagating conifers, mainly because many species are hard to root, especially when using cuttings from mature trees. Incense cedar (Calocedrus decurrens (Torr.) Florins) was recently introduced in Albania as an ornamental tree in public and private urban green spaces and is now much in demand. Intergeneric grafts are rarely used in conifers, and there is little information regarding incense cedar grafting onto rootstocks from different genera. This work aimed to evaluate the effects of intergeneric grafting on the quality of ornamental incense cedar, which is little known in Albania. Scions were prepared by taking 8-10 cm apical shoot from young C. decurrens ‘Aureovariegata’ plants. Rootstocks were prepared from one-year-old Mediterranean cypress (Cupressus sempervirens L.) seedlings. First results show that the height of grafted plants tended to increase slowly from 30 to 90 days after grafting (DAG), with a minimum increase of 4,9 %; this is because the plant grafted takes several days to join scion and rootstock. The Relative Growth Rate (RGR) of the scion was generally higher than that of the rootstock, which even showed negative values 60 DAG; this trend grew after 90 DAG. At the end of our experimental work, we obtained 410 plants, representing an 82% graft success rate available for planting in different Albanian soils.*

Keywords*— *Calocedrus decurrens (Torr.) Florins; conifer; RGR; vegetative propagation.

I. INTRODUCTION

Vegetative propagation is an important tool in both ornamental and forest tree improvement [1]. Grafting, an ancient agricultural and vegetative propagation technique, generally involves connecting two plant segments, a portion of shoot (‘scion’) and a root piece (‘rootstock’) [2]. It is most commonly employed in woody perennial crops to indirectly manipulate scion phenotype. A wide range of classical grafting techniques can be found in Garner [3]. Among these, bark grafting is relatively easy and very successful; it can be performed in the spring, only when the bark slips or separates easily from the wood. Taxonomic proximity is a general requirement for successful graft-take and long-term survival of the composite plant [4]. Rootstocks are usually from the same species as the scion, although some interspecific graft combinations have been successful. Grafting is widely used to propagate conifers [5] because many species are hard to root, especially when using cuttings from mature trees [6]. In general, only few species can be grafted: i.e. dwarf conifers, which cannot be successfully cloned using cuttings [7]. For conifers, grafters have historically used the following combinations: *Picea abies* for all spruces, and *Pinus sylvestris* for all two-needled and some three-needled pines [8]. Vuksani et al. [9] showed that the grafting compatibility of Arizona cypress plants and Mediterranean cypress rootstock was 87% in Category A (perfect union).

The *Cupressaceae* family includes 21 genera and around 130 species of anemophilous trees and shrubs from the northern and southern hemispheres [10 and 11]. In the Mediterranean region, this family is widely represented both by native species, typically in woodlands, and by non-native species used for ornamental purposes and in reforestation programs. Albania is very rich in terms of flora: it is estimated that there are around 3,200 species of vascular plants, and the *Cupressaceae* family is quite widespread.

Incense cedar *(Calocedrus decurrens* (Torr.) Florins) is the only example of the small genus *Calocedrus* [12], (*syn. Libocedrus decurrens* Torr.). It belongs to the *Cupressaceae* family native to western North America, with the bulk of its range in the United States, from central western Oregon through most of California and the extreme west of Nevada. It grows at altitudes of 50-2,900 m.

Incense cedar was recently introduced in Albania as an ornamental tree in public and private urban green spaces and is now much in demand [13].

Its foliage is produced in flattened sprays with scale-like leaves 2–15 mm long; they are arranged in opposing decussate pairs, the successive pairs spaced at increasing distances; the facial pairs are flat, while the lateral pairs fold over their bases [14]. As an ornamental plant, '*Aureovariegata*' is a broadly columnar, evergreen, coniferous tree with exfoliating, red-brown to grey-green bark, slightly fragrant, flat sprays of linear, glossy, dark green and golden-yellow variegated leaves and, occasionally, erect, oval, red-brown female cones. Common propagation methods are seed and semi-hardwood cuttings.

Geographic variability in cone and seed production is great. There is considerable information available on incense cedar seedling production. Although a prolific seed bearer, it does not produce seeds every year; germination under controlled conditions may reach 98%, but usually averages from 20 to 40% [15].

Although incense cedar does not reproduce vegetatively in nature, it can be stimulated to do so in a nursery greenhouse: rooting, assessed in spring, is best (92% rooting) with 2500 p.p.m. NAA [16]. *Chamaecyparis lawsoniana* scion graft success is moderate with *C. decurrens*; there is little information regarding incense cedar grafting on rootstocks of other genera.

Research was, therefore, carried out to evaluate the effects of intergeneric grafting on the quality of ornamental incense cedar, which is little known in Albania.

II. MATERIALS AND METHODS

The experiment was carried out in an ornamental nursery, in a plastic greenhouse in the Laknas area (41° 22' 36" N, 19° 44' 14" E, Tirana, Albania).

Scions were prepared by taking 8-10 cm apical shoot from young *C. decurrens* ‘*Aureovariegata*’ plants. They were individually transplanted in 30 cm diameter containers filled with a mix of Thumanes torfe (Albania) base and perlite (v:v=3:1)

Rootstocks were prepared from one-year-old Mediterranean cypress (*Cupressus sempervirens* L.) seedlings produced by seeds harvested from natural plants of the Kruja region (AL). Seedlings were grown in pots filled with a mixture of peat and perlite (v:v=3:1). The thickness of the rootstock was slightly greater than 1 cm; the length of the bark cut was 1.5 - 2.5 cm.

Grafting was carried out on 3 February 2015; in order to better connect rootstock and scion, the grafted point was tied down with raffia of the *Sagustaedinera* tropical palm bark. Five hundred grafted plants were grown under controlled conditions (T=22°C and UR 90%).

To assess grafting progress, outgrowth measurements were carried on grafted plants in the laboratory of the Horticulture and Landscape Architecture Department of the University of Tirana in three different periods: March 3 (30 days after grafting, DAG), April 3 (60 DAG) and May 3 (90 DAG) 2015.

The following bio-morphological parameters of the scion were measured: plant height (cm), leaf surface (cm^2), aboveground fresh and dry weight (g) (shoots were air-dried and then placed in an oven at T=70 °C). Ten plants were sampled. The aboveground fresh and dry weights (g) of the rootstock were measured.

The Growth index Relative Growth Rate (RGR) was calculated using dry weight data, according to Hunt (2002), as follows: $RGR = d_W/W * 1/dt$; in mg g^{-1} day^{-1}, where W = the sample’s dry weight and dt= d2 - d1 is the interval of time. RGR was calculated for scion (RGR Scion), root system (RGR rootstock) and the whole plant (RGR-Plant).

Scion and rootstock RGR values were assessed throug ANOVA (Shapiro-Wilk Test). At the end of the experiment (90 DAG), graft compatibility was calculated as a percentage.

III. RESULTS

Figure 1 shows how the leaf surface area of grafted plant tends to increase slowly from 5.4 cm^2 (30 DAG) to 7.2 cm^2 (90 DAG).

Figure 2 shows that the scion height tends to increase slowly by 4.9 %, from 22.2 cm (30 DAG) to 23.3 (90 DAG); this slow growth may be ascribed to the time it takes (several days) for the grafted plant to connect scion and rootstock.

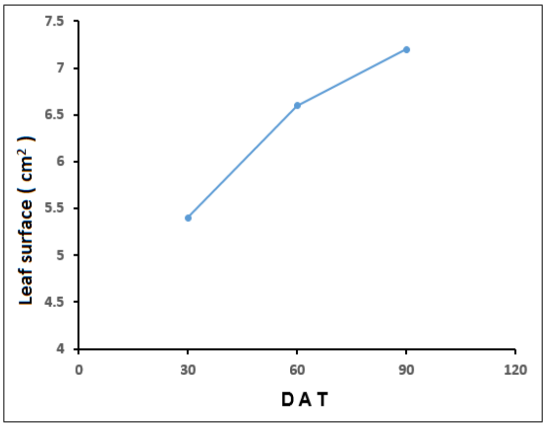

FIGURE 1: Leaf surface area of grafted plant at 30, 60 and 90 DAG.

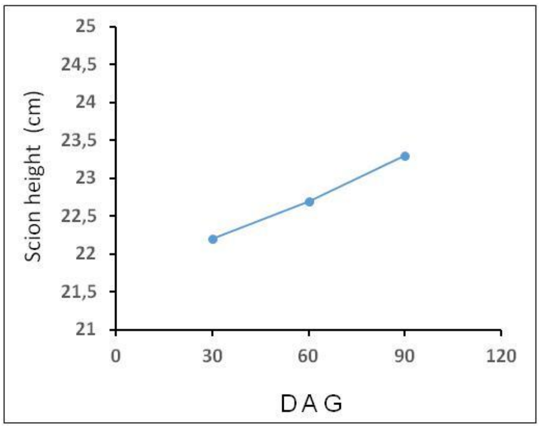

FIGURE 2. Scion height at 30, 60 and 90 DAG.

As for the Relative Growth Rate, the RGR of scion (RGR-sc) is higher than that of rootstock (RGR-rst), which even yield negative values at 60 DAG (Figure 3).

This trend grows after 90 DAG (Figure 4).

The difference is thought to be due to genetic variation in components deriving from different species.

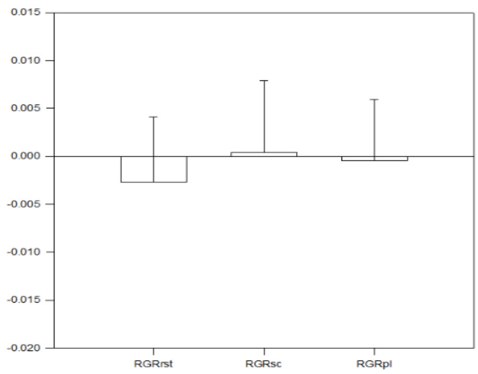

FIGURE 3. Height Relative growth rates of plant (RGR-Pl), relative growth rates of scion (RGRsc) and relative growth rates of rootstock (RGRrst) at 90 DAG.

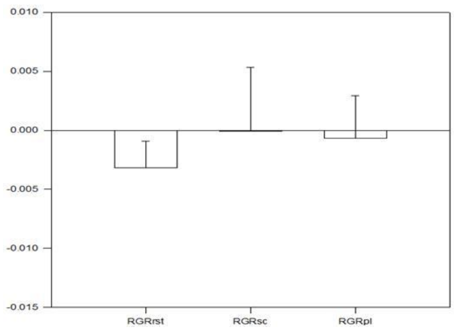

FIGURE 4. Relative growth rates of plant (RGR-Plant), relative growth rates of scion (RGRst) and relative growth rates of rootstock (RGRrst) 90 DAG.

This trend was statistically estimated by means of RGR analysis of variance (Shapiro-Wilk Test) $P < 0.050$.

TABLE 1
THIS RELATIVE GROWTH RATE OF ROOTSTOCKS (RGRRST), RELATIVE GROWTH RATE OF PLANTS (RGRPLANT) AND RELATIVE GROWTH RATE OF SCIONS (RGRSC) 60 AND 90 DAG (MEAN VALUES ± STD.DEV)

	Measurement 60 DAG	Measurement 90 DAG
RGR rst	-0.00267±0.00681	-0.00319±0.00226
RGR sc	0.000421±0.00746	-0.0000473±0.00539
RGR plant	-0.000410±0.00637	-0.000689±0.00360
Probability (P)	0.589	0.192

The differences in the mean RGRrst, RGRsc and RGRplant values among the processed groups are not great enough to exclude random sampling variability, i.e. differences (P1 = 0.589, P2 = 0.589) are not statistically significant.

Another interesting statistical finding is that the Standard Deviation (StdDev) value is large, indicating that growth rates vary widely among plants: when all growing conditions are the same, this may be ascribed to variations in the quality of manual grafting. At the end of our experimental work, we obtained 410 plants (representing an 82% grafting success rate) ready for planting in Albanian different soils. Estimated RGR rootstock values tend to decrease over time, even reaching negative values, a trend that grows stronger more than 90 DAG. It's thought that changes in RGRrst are influenced by changes in the physiological grafted component; in our case, this is even more pronounced because we joined two different genetic species. This is of particular concern for plant viability in the future, although the grafting success rate seems satisfactory.

IV. DISCUSSION

Some studies have reported similar or better success rates for scions grafted onto different rootstocks [17 and 18]. In some cases also taxonomically distant species have been grafted successfully [19].

Intergeneric grafts are rarely used in conifers. Great variations in anatomy, physiology and morphology among some genera often prevent successful grafting [19].

Nevertheless grafting of Nootka cypress [*Chamaecyparis nootkatensis* (D. Don) Spach] cultivars onto Chinese arbor-vitae (*Thuja orientalis* L.) stocks has yielded up to 94% success rates [20].

White cedar (*Thuja occidentalis* L.) rootstocks are used commercially for grafting Lawson cypress scions [21].

As for intergeneric grafting, two *Cedrus* species on *P. pinea* L. (Stone pine) rootstocks were incompatible, although several grafts survived for two years [22].

There is evidence that rootstocks can alter the scion growth rate in conifers: several studies [23-25] report slower scion growth when scions are grafted onto other species.

V. CONCLUSIONS

Grafting success requires specialists with long experience in the field. For best results, rootstock and scion should have a similar consistency to not compromise the quality of the plants produced.

In order to produce quality seedlings suitable for growth in Albania's soil with abundant skeleton and to meet growing market demands, it is important to develop suitable methods, such as grafting, for producing plants quickly.

Estimated RGR-rootstock values tend to decrease over time, even reaching negative values, a trend that grows stronger more than 90 DAG.

This is of particular concern for plant viability in the future, although grafting success seems satisfactory.

We will therefore continue our experimental work by monitoring the intergenerically grafted plants to ensure their success.

AUTHORS CONTRIBUTION

Conceptualization, G.V.; methodology, B.D. and G.C.; software, H.K.; validation, G.V., G.C. and B.D.; data curation, GC.; writing—original draft preparation, G.V.; writing—review and editing, BD and GC; funding acquisition, B.D. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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