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Morphogenic Competence of Vegetative Buds and Its Effect in Select Adult Trees Cloning

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Abstract: Cloning adult trees by conventional techniques carry a difficulty grade which it is directly related with physiologic and ontogenic age of materials. The objective of this study was to evaluate efficacy of plant grafting and to analyze the relation between physiological aging state of vegetative bud and its response in grafting process of *Cedrela odorata* L. Basal portion caulinar, middle and apical from adult trees were grafted by "lateral slit" technique on two years old juvenile rootstock. The buds located in basal zone of donor tree showed the best results in efficacy of grafting, survival, proliferation and elongation of buds. By contrast, buds from apical zone registered low percentage in evaluated variables and the grafting showed in their phenotype and aspect of premature aging. This research permits to stablish a relation between the vegetative bud position inside of the donor tree canopy and its response as an optimal germplasm source for the supply of a forest genetic improvement program.

Key words: tropical forest, forestry plant breeding, forestry germplasm

1. Introduction

The cedar (*Cedrela odorata* L.) is one of the tropical timber species tropical whose commercial value reaches up to 15 times in respect to other forestall species. The above is principally to the characteristics of its wood for example: hardness, color and scent [1]. However, populations of this species have been severely affected fragmenting and diminishing its natural population due to the dysgenic selection and deforestation [2]. This problem has diminished the seed dispersion capacity in that species, originating natural regeneration problems. Thus, it is important to explore efficient alternatives of propagation by asexual way that permits to rescue and to conserve and improve the germplasm quality of this species.

Asexual propagation of adult individual has many difficulties as happen in the most tree species [3]. Due to the above, to select traits of interest in maturity phase is a big problem, while in vegetative propagation is possible in juvenile phase due to the long maturation, a descent is produced in morphogenic capacity generating a barrier in plant regeneration [4].

Vegetative propagation method by grafting permits the physiologic and ontogenic adult vegetative buds reinvigoration on juvenile rootstock [5]. Furthermore, this method has the capacity of revert the processes of phenotypic aging and to facilitate the morphogenic capacity manipulation and therefore, cloning of selected adult materials [6, 7]. Another characteristic of this method is that it maintains maternal traits in the offspring which is an aspect of great importance in improvement cycle and silvicultural planning to achieve the capture of genetic gain additive and not additive [8].

However, utilization and introduction of vegetative material as germplasm source for vegetative propagation has been due to effect of age of the donor plant [9]. Because of the above, factors that determine the optimum quality of explant and its association with physiologic and ontogenic ripening were necessary to

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analyze. The objective of this paper was to stablish and to analyze the relation between vegetative bud extraction source by its location in the donor tree and the response in grafting conditions on juvenile rootstock of *Cedrela odorata* L.

2. Materials and Methods

2.1 Study Site

This study was realized in San Felipe Bacalar experimental site which belongs to Instituto Nacional de Invesgitaciones Forestales, Agrícolas y Pecuarias (18°46´N y 88°17´O), Quintana Roo, México.

2.2 Vegetal Material

The vegetal material was collected in 2018 mid-march from three adult trees of *Cedrela odorata* L. previously evaluated and selected by genotype. Total length of the tree was divided in three zones: basal (0-8 m), mid (8.1-12 m) and apical (12.1-16 m). Then, 100 scions from 40-50 cm and from 2-2.5 cm were collected in each one of the zones. Each scion contained three vegetative buds of average which were healthy, vigorous and an intense green color. The scions were piled in a unicel coller and marked with a unique code of identification. Then, the scions were covered with paper and sealed with transparent film paper. Finally, each sealed and piled scion was wrapped with flannel and covered with hydrogel packs to maintain fresh environment inside of coller during its transfer to the grafting site.

2.3 Rootstock Plant Production

The rootstock plant was produced in greenhouse from recollected botanic seed from pest and diseases vigorous free trees. Each rootstook plant was grown in polietylene black bags of 40 × 20 cm height by width respectively. The substrate was a combination of sugarcane, sawdust and black earth cacha compost in proportions of 2: 2: 1, respectively. Furthermore, cultural labors were proportioned properly and timely (weeding, irrigation and fertilization). Finally, healthy and vigorous plants were selected to use as rootstock plant. Plants selected as rootstock plant had 2.5 cm in stem plant and develop of meristematic tissue.

2.4 Grafting

The grafting was carried out by side slit technique. Three cuts were done to the rootstock, two in vertical form and separated by a third of the thickness of the stem and a third cut horizontally at the top (joining the vertical cuts). Then, using the same shave, the tree peel was separated pulling it down to form an elongated (tongue-shaped) cut. Then, a scion with one bud (grafting) was implanted and covered with plastic band. The plastic band was wrapped from the bottom up completely covering and carrying out a tie up to the graft union. During the grafting process, a fungicide mixture was prepared at a rate of 5 g of promyl (fungicidal product) per liter of water; then, the scions were submerged in a diluted mix of that fungicidal for 10 minutes. Finally, a piece of flannel was soaked with the same solution and it was impregnated on the wound of the rootstock plant.

2.5 Statistical Analysis

Data obtained were analyzed by analysis of variance (ANDEVA) and the significant differences were determined by Tukey ($p \leq 0.0$) test. To obtain this analysis, the SAS (*Statistical Analysis System*) software statistical was used.

3. Results and Discussions

Buds from medium and basal zone of tree donor showed high percentage of detachment and Survival, in contrast, buds from apical zone exhibited low percentage of detachment and Survival (Table 1).

The differences in the percentages of survival and survival observed are attributable to the physiological and ontogenic conditions of the vegetative buds in the different collection areas. Specifically, the high percentages in graft yield are attributable to the physiological state of the mother plant and to the

Table 1 Position effect in vegetative buds inside of donor tree on detachment and Survival percentage of *Cedrela odorata* L. grafting.

Bud origin	Number of grafts made	Detachment percentage	Survival percentage
Basal zone	100	94	88
Medium zone	100	86	80
Apical zone	100	36	30

endogenous hormonal levels at the time of collection of plant tissue. In addition to the above, the juvenile characteristics of plant tissue are retained and persist in the basal areas of the trunks and branches, while the highest and peripheral parts are the first to manifest adult characteristics. The results demonstrate the existence of a gradient of morphogenic competence that decreases from the base to the top of the tree, which is related to the number of cell divisions that separate each meristem from the original embryo [10]. On the other hand, in the main meristems there are certain cells with a low mitotic activity whose mission is to maintain a group of juvenile cells, while in the areas with a higher activity (apical meristems) the cells divide more frequently and in each of these divisions, they become more and more mature.

3.1 Grafts on Youth Rootstocks

Buds of the apical area grafted on juvenile patterns showed a vigorous growth attributable to cell divisions, which resulted in a temporary reversal of physiological aging (Table 2). The above suggests that grafting has the potential to induce processes of reversal of adult phenotypic characters to juvenile states. In this regard, Wendling and Xavier (2001) [11] pointed out that vegetative propagation through the graft induces rejuvenation of the grafted adult portion which is evidenced by the ability of cell proliferation that characterizes the vegetative growth in the juvenile phase.

3.2 Growth Habit

The habit of oblique growth was observed more frequently in grafts that were conceived using buds of the apical zone (Fig. 1F). This quality is attributable to the different physiological conditions of meristems located in the apical area of the individual (physiological aging). The above suggests that the type of growth is determined by the position that meristems occupy within the crown of an adult tree.

According to the ANDEVA results, the treatment factor observed statistically significant differences ($P < 0.05$) on the response variables “detachment percentage” and “survival percentage”. For this reason, a comparative test between means was carried out using the Tukey honest significant difference test procedure. This analysis confirmed that the buds located in the middle and basal areas of the tree, were the study factors that observed statistically significant differences on the variables “percentage of yield” and “percentage of survival” (Table 3). Therefore, it is statistically affirmed that there is a gradual effect of morphogenic competition and the position of the bud on the graft’s survival and survival. However, the buds of the apical zone showed a vigorous growth which

Table 2 Effect of bud position within the donor tree on the expressed vegetative vigor and graft growth habit of *Cedrela odorata* L.

Bud position	Number of grafts made	Vegetative vigor expressed	Growth habit
Basal zone	100	Medium	Straight
Apical zone	100	Strong	Oblique

Table 3 Comparative test between means of the percentage of yield and percentage of graft survival of *Cedrela odorata* L. generated by the test procedure of Tukey’s honest significant difference.

Study factor	Detachment percentage	Survival percentage	Vegetative vigor expressed	Growth habit
Basal zone	$90,5^a$	$81,5^a$	Medium	Straight
Medium zone	$82,5^a$	$76,5^a$	Medium	Straight
Apical zone	$32,5^b$	$34,5^b$	Strong	Oblique

Different letters indicate statistically significant differences ($P \leq 0.05$).

is attributable to the reversal of physiological aging processes related to cell division, growth and development. Specifically, the vigor expressed evidenced the capacity of cell proliferation, a characteristic that matches the vegetative growth in the juvenile phase of woody species.

Fig. 1 Grafting process of *Cedrela odorata* L. A) select adult tree, B) collection of stitches with vegetative buds, C) 1.5-year-old standard plant, D) grafting process (steps from a to l), E) two-month-old grafts and F) oblique growth habit.

4. Conclusions

The physiological and ontogenic ages of the vegetative buds of adult trees of *Cedrela odorata* L., play a key role in the percentages of graft survival and survival. In this regard, it is recommended to use physiologically and ontogenetically juvenile buds located in the basal areas of the trunk and branches of the mother tree. The practical utility of the results of the present study was to determine and establish the optimal position of the buds in adult trees with total expression of their genotype as sources of germplasm to supply a grafting program via grafting, a key aspect for the multiplication of individuals Elite on a large scale.

Conflict of Interest

The authors do not have conflict of interest in terms of the products mentioned in this paper.

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