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RESEARCH ARTICLE

EVALUATION OF TWO METHODS OF GERMPLASM COLLECTION IN MEXICO SOUTHEAST

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Abstract

The implementation of good farming practices in seed collecting is fundamental key to guarantee the physiological and genetic quality of forest seeds. The objective of this work was to evaluate the implementation of two methods to collect seedsfrom *Cedrelaodorata* L.toguarantee seeds of high physiologicalquality. Seeds from 40 superior phenotypically trees of *Cedrelaodorata* L. in Yucatan Mexico peninsula region were collected from March to May 2019.The collect methods applied in this research werethe traditionalcollectand the climb trees. The physiological quality of seeds wasevaluated by germination and tetrazolium test. The laboratory analysis confirmed 88% of seeds pure with 94% of germination in climbing trees method, in contrast in the traditional method showed 66% of seeds pure with 72% of germination. The results by conventional germination it was not showed significative statistical differences. These results are part of novel contributions in collectmethodology using tools to climb on trees which to be complement of superior phenotypically trees selection, contribute comprehensively to obtain physiological and genetic quality forest seeds.

Introduction:-

Genetic quality seed collect and production are activities not common which limits the development and quality of commercial forest plantations. Those plantations usually require continue and abundant supply of seeds with high yielding (Fuentes *et al*., 2009) and the best traits (Holliday *et al*., 2017). Furthermore, introduction of forest plants in some areas under completely different environment conditions to require by each species could cause development of plagues and diseases (Vanegas, 2016), alteration in trophic relationships (Liebhold*et al*., 2017) and loss of biodiversity (Fernández-Pérez *et al*., 2013). Seeds are the principal reproductive organ in the most of superior land plants, those are product of biologic processes which start with flowering and finish with fruit ripening, besides represent a remarkable evolutionary advance in plant sexual reproduction (Coen and Magnani, 2018) andcontain genetic variability essential source to supply a forest genetic improvement program (Louwaars, 2018).

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Consequently, genetic quality of seedsimplies the precise knowledge of parental source and the individual phenotypic characteristics, which are essential to design effective strategies in plant selection breeding (Zhang *et al*., 2020; Thomas *et al*., 2017). One of the important factors to obtain quality seeds is the collect method.Youngentob*et al*. (2016) mentioned that seed samples are required for a wide range of research and management activities,howeverobtaining these materials from the branches of tall trees can be extremely difficult (Barker andPinard, 2001).The production and utilization of high-quality seeds are the basic keys for success in plant forest breeding programs also are requisites for control of seed industry quality (França-NetoandKrzyzanowski, 2019).On the other hand, to sure that seeds are of quality with high germination rate is necessary to apply tools that permit to identify defective and unviable seeds.This kind of analysis it is necessary to obtain information about physiological and biological quality of seeds (Xia *et al*., 2019).One of the most traditional tests for analyzing seed quality is the tetrazolium test, whose effectiveness is known becauseprovide information of physiological characteristics of seeds (Salazar Mercado *et al*., 2020).Tetrazolium test indirectly determines the respiratory activity in the cells of seed tissues (França-Neto and Krzyzanowski, 2019) and is a widely used method to evaluate the quality of seeds in different species (França-Neto and Krzyzanowski, 2019; Pereira *et al*., 2019; Belniaki*et al*., 2019; Oliveira *et al*., 2016;Espitia-Camacho *et al*., 2017).

The objective of this research was to evaluate the implementation of two methods to collect seedsfrom superior trees of *Cedrelaodorata* L. with the purpose to optimize the process to selectphenotypicquality germplasm from trees and provide sources to commercial forest plantations in tropic.

Materials and Methods:-

Seed collect location

The study was carried out in tree of *Cedrelaodorata*L. distributed in Mexico, Yucatán peninsula region.

Trees selection

Evaluation and selection of trees were by individual assessment method (Ipinza, 1998). This method is based in evaluation and registration of two groups of variables.The quantitative variables were total height (m), height of clean stem (m) and normal diameter (m).Qualitative variables wereshaft shape (straight = 6, slightly crooked = 4, crooked = 2, very crooked = 1), forks (none = 6, top 1/3 = 4, middle 1/3 = 2, bottom 1/3 = 1), branch insertion angle (60^o to 90^o = 6, 30^o to 60^o = 4, 0^o to 30^o = 2), crown shape (circular = 6, irregular circular = 5, half circular = 4, less than half a circle = 3, few branches = 2, only sprouts = 1), position within the canopy (dominant = 6, codominant = 4, intermediate = 2, suppressed = 1), health (no pests = 7, bark cutters = 6, borers = 5, defoliators = 4, parasitic plants = 3, fungi = 2, physical damage = 1) and cup vigor (good = 6, fair = 4, bad = 2). Data obtained from evaluation were added and then defined in three categories: 1= from 33 to 49 points, 2= from 25 to 32 points and 3= less than 25 points. Finally, the trees in category 1 were selected to harvest the seeds. Theequipments used to measure were clinometer and diametric tape, furthermore, a global positioning system to georeference each individual.

Collect Methods

The seed collect from 40 previously selected *Cedrelaodorata* L. trees were carried out from March to May 2019.Two methods of collect were implemented in field:1. - traditional method and 2. - Tree climbing method. Traditional method consisted in collecting inferior phenotypic trees (short trees with a high number of branches) without tools. This method was carried out in natural stands and unknown geographic origin. The tree climbing method consist in collect seeds with tools (climbing harness, climbing rope and slings) to rise to the top of superior phenotypically trees (sturdy, straight and low-branching trees).

Seeds analysis

Analysis of seeds were carried out in Forest Biotechnology Laboratories located in Forest Biotechnology Laboratory of the National Center for Disciplinary Research in Conservation and Improvement of Forest Ecosystems (CENID/COMEF, by its acronym in Spanish) which belongs to National Institute of Forestry, Agricultural and Livestock Research (INIFAP, by its acronym in Spanish). Seeds were evaluated in laboratory considering number and weight of seeds, viability test with tetrazolium, germination percentage and humidity contain per each seed lot accord International Seed Testing Association (ISTA, 2014) standards.

Seed variability test with tetrazolium

Seeds were immersed in distillate water for 24 hours, thena transversal cut with scalpel was carried out in embryos to separate the seed coat and reserve tissues. Posteriorly, embryos were placed in test tubes with distillated water and 2, 3 and 5 of 1% of triphenyltetrazolium (Merck) taking care that solution covers all seeds. The test tubes were placed in incubation room at 30 ± 1°C of temperature in darkness condition. Finally, solution was drained, and seeds were evaluated with stereoscopemicroscope (Vista Vision®).

Statistical analysis

Variance analysis was carried out in all experiments and significative differences were determined accord Tukey test ($p \leq 0.05$). The software used for all analysis was the statistic software SAS (Statistical Analysis System, 1999).

Results and Discussion:-

Analysis of method type

Laboratoryanalysis of seeds confirmed 88% of purity and 94% of germination in seeds collected by climbing trees, in contrast the traditional method showed 66% of purity and 62% of germination. The variance analysis showed significative statistical differences ($p \leq 0.05$) between study factors and germination percentage of seeds. Tukey test in collect method of tree climbing factor showed significative statistical differences(Table 1).

Table 1:- Comparative test between percentage average of purity and *Cedrelaodorata* L. seeds germination generated by Tukey test.

Collect method	Variables		Trees number
	Purity percentage	Germination percentage	
Traditional	66.0[a]	72.0[a]	40
Tree climbing	88.0[b]	92.0[b]	40

Different letters indicate significative statistical differences accord Tukey test ($p \leq 0.05$).

This difference could be that seed collector realized this activity with tools to get up on treetop (Figure 1) which helped it directly collect and obtain better visibility to select seeds with the best physiological characteristics, health, coloration, size, maturation and weight.

Size and weight of seeds were characteristics that showed the best association with germination percentage which were 92% (Table 1). This response could be attributable to high contain of carbohydrates inside of seed endosperm which contain high availability of nutrients and energy to stimulate the germination, emergency and seed surviving(Khuranaand Singh, 2001). It is fundamental to consider the interaction with other factors, for instants: genetic quality, physiological maturity of trees and seeds and climate conditions of fruit development (Ribeiro*et al*., 2012) which could influence inthe capacity of seeds to develop and obtain high germination rates.Donoso*et al*.(1999) mentioned that fruits and seeds should be collected before its dispersion. This is accord of our results due to that this work showed the efficacy of tree climbing collect method as astrategy which permitted obtain vigorous, health and better condition seeds with high germination percentage (Figure 1).

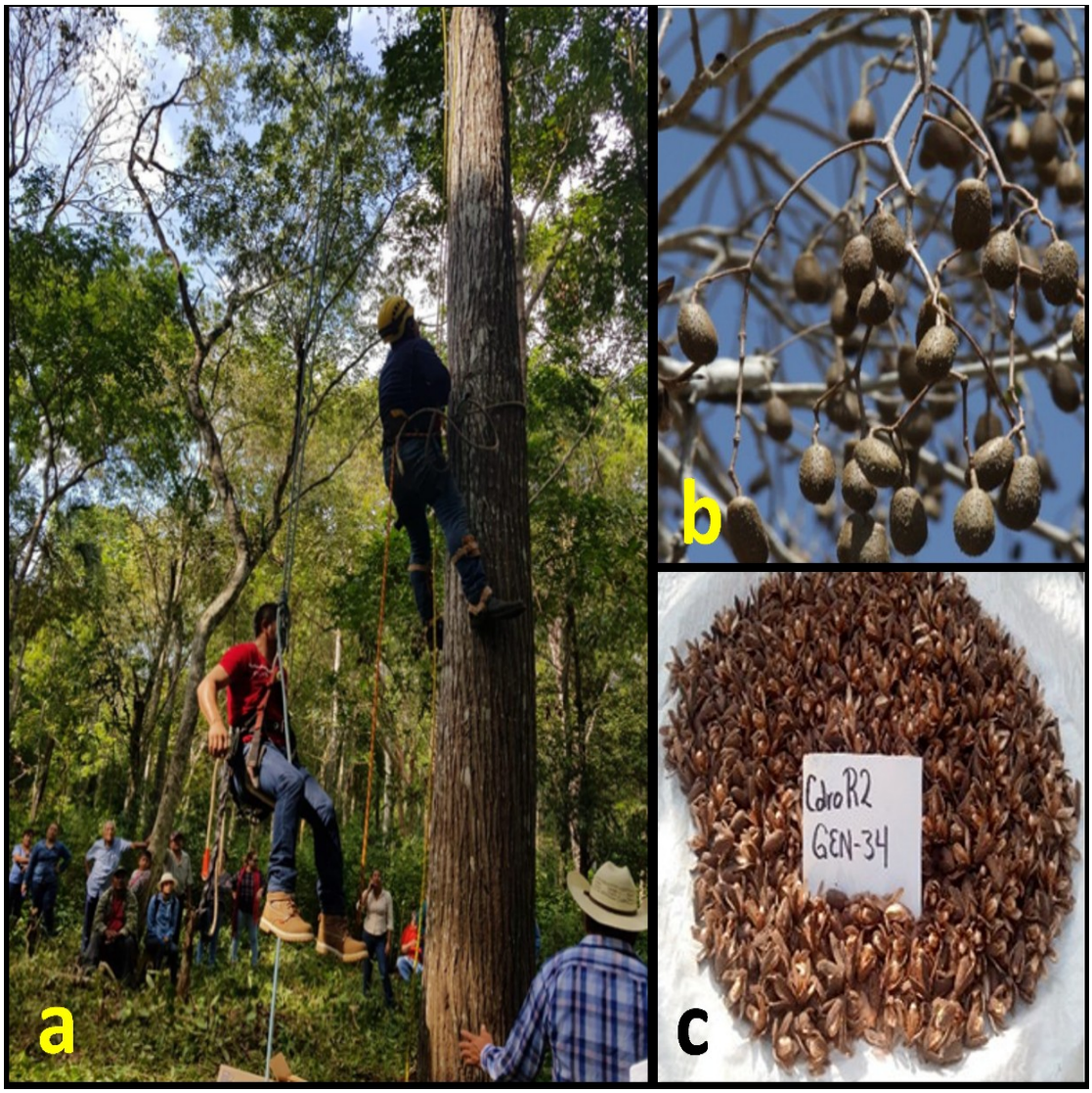

Figure 1:- Fruits and seeds collected from superior phenotypicallytrees of *Cedrelaodorata* L. by climbing method. a. - collect brigade starting the climb process, b. - fruits with optimal characteristics for collecting and c. - vigorous, healthy and physiological maturity fruits.

In contrast, traditional collect method is used by collectors to collect seeds with unknown origin and uncontrol of select the trees. This method is usuallyapplied by collectors due to its easyproccessing and the high quantity of fruits harvested. The only limiting is that collectors do not consider the seed parameters quality (Table 1)

Tetrazolium test

No coloration, scattered pale pinks and intense pink color were observed in tetrazolium test. Embryos which showed more than three quarters with an intense pink color were determined as viable, contrary, embryos not viable showed a pale red or white coloration (ISTA, 2014) (figure 2).

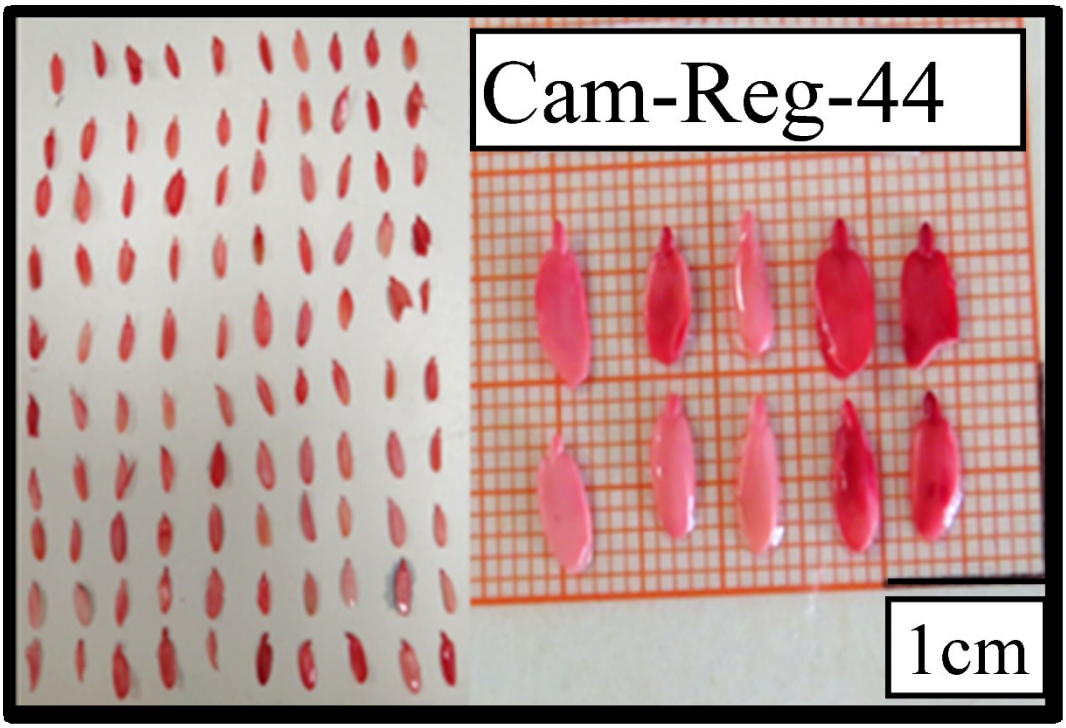

Figure 2:- Embryos of *Cedrelaodorata*L. evaluated with tetrazolium test. Cam-Reg-44: code of superiorphenotypically cedar tree.

These results indicated that concentration of tetrazolium and tissue exposition time permitted the best stain because of the oxide-reduction reactions in cell of embryos.

Germination test

Germination test in germinative chamber showed plant averages germinated between 72 and 92 %. Variance analysis confirmed that results obtained by germination conventional test was not showed significative statistical differences. On the other hand, unviable seeds presence could be to degradation in cellular membranes because of peroxidation of lipids and peroxidation no enzymatic (Ravikumar*et al*., 2002). Stain intensity of embryos confirmed that tetrazolium concentration of 1% and stain time of 1.5 hours were enough to evaluate the seed quality. Lima *et al*. (2010) mentioned that first hours of water absorption of seeds is fundamental because of enzymatic activity which is related with final color.

Conclusions:-

Optimization of a collect methodology based in climbing of superior phenotypically trees, showed to be effective to obtain vigorous seeds of genetic and physiological quality. The characteristics that showed the best association with germination percentage were seed size and weight. Nevertheless, it is fundamental to consider the interaction of other factors as: genetic quality and physiological maturity of trees and seeds. This research provides a fundamental technology to supply physiological and geneticalquality seeds for commercial forest plantations.

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Declaration of interests

All authors declare that they have not conflicts of interest to disclose.

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