Research And Development Of Vegetative Propagation Techniques For *Pinus* Sp. In The Northeast Region Of Argentina

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ABSTRACT

Exotic pine plantations in Misiones and Corrientes provinces, northeast region, represent more than 70 percent of the industrial forested land in Argentina. An intensive breeding program has been developed for these species and resulted in the identification of a large number of outstanding families and individual phenotypes. However, the availability of genetic improved seedling is scarce and expensive Therefore, in 1998, a research project to develop *in vitro* and *ex vitro* techniques leading to a cost effective system for mass propagation of genetically improved material of loblolly pine and slash x caribbean pine was initiated. Current results indicated that, our propagation strategy, combining tissue culture techniques and macropropagation, has the potential to deliver more than 12,000 trees from a single seed in a 2.5 years period, for loblolly and slash x caribbean pine in northeast Argentina.

Key words: Loblolly pine, slash x caribbean pine, Argentina, tissue culture, organogenesis, micropropagation, macropropagation, rooted cuttings, vegetative propagation.

INTRODUCTION

Exotic pine plantations in the northeast region of Argentina (Misiones and Corrientes provinces) represent more than 70 percent of the industrial forested land in the country. Pine seedling production is about 40 millions/year, of which 30 millions belongs to loblolly pine (*Pinus taeda L.*). An intensive breeding program has been developed for these species and resulted in the identification of a large number of outstanding families and individual phenotypes. It is expected by the year 2002 local control pollinated seed production will start. However, the availability of genetic improved seedling is scarce and expensive, and to date 80 % of the seed is imported from United States and only 10 to 20 % comes from local seed orchards. Slash x caribbean pine (*Pinus elliottii var. elliottii x P. caribbaea var. hondurensis)* is another specie of interest in the region and its demand for industrial forest plantation has been increased in the last five years. Similar to loblolly pine situation as exotic, slash x Caribbean pine seeds are mostly imported from Queensland Australia and it is also, locally scarce and expensive.

The development of vegetative propagation techniques, offers the opportunity for mass propagation and deployment of scarce superior families and clones in larger areas. Therefore, a research project to develop *in vitro* and *ex vitro* techniques leading to a cost effective system for mass propagation of *Pinus sp* was initiated in 1998. This paper will outline our research strategy and most recent results to accomplish this goal.

GENETIC IMPROVEMENT AND PROPAGATION STRATEGY

As in any forest tree improvement program, because of the long life cycle of trees, it is important that the genetic gain per unit time is maximized. There is therefore, a need to reduce the length of the breeding cycle: selection of individuals, progeny testing, grafting, seed orchard establishment through to seed harvesting and finally planting of genetically improved material in commercial forests. Vegetative propagation can be used to shorten the length of the breeding cycle between the identification of superior families and the planting of genetically improved stock (Fletcher, 1992), as well as increasing the availability of highly improved genetic propagule and consequently the genetic quality of each planted hectare. Genetic gain is maximized by increasing the selection intensity of the production population, capturing the non additive genetic variance, eliminating pollen contamination, selecting crosses or clones that have most desirable trait combinations (Fletcher, 1992). Final gain will depend upon the genetic quality available in the program, and on the number of hectares planted with different genetic material (Balocchi, 1997).

In this way, mass propagation of elite families by means of an efficient vegetative propagation system will further increase the expected genetic gain at harvest time. Our propagation strategy integrates tissue culture with macropropagation techniques to produce rooted cutting for out planting. Organogenesis (from mature embryo) and micropropagation (seedling based) are included in our tissue culture system to produce stock plants for cutting production. Rooted cuttings will be used as planting material in the field or new stock plants for further propagation (serial propagation).

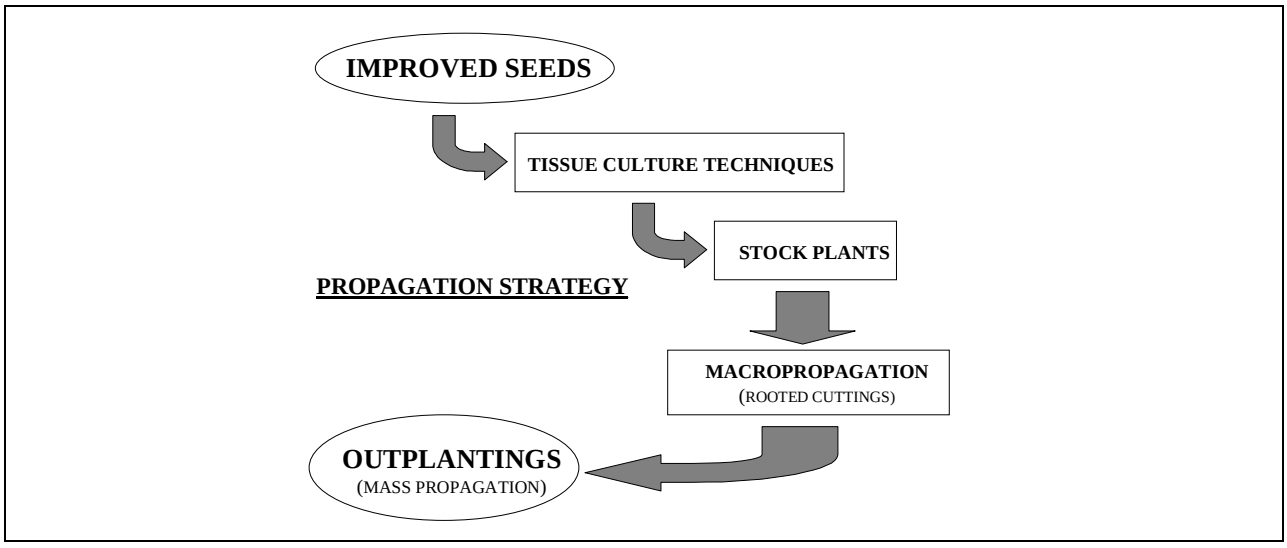

Figure 1. Vegetative propagation strategy for loblolly pine and slash x caribbean pine in northeast Argentina.

TISSUE CULTURE TECHNIQUES

Tissue culture protocols based on an organogenic process and seedling based model using shoots removed from decapitated seedlings have been developed for pines with different degree of success and multiplication rate. (Mott and Amerson, 1981; Amerson et al. 1988; Handley, 1995; Aitken Christie, 1988; Sen et al., 1989; Tang, 2001; Lapp et al, 1996; Mehra Palta, 1983;

Sommer and Brown, 1974; Abo el Nil, 1982; Abdullah, 1986). In our studies both techniques were addressed and our recent results indicated that by integrating both techniques a reasonable high multiplication rate is possible through a wide range of genotypes.

Organogenesis

The predominant morphogenic route reported for adventitious shoot formation in conifers has been through direct organogenesis without the involvement of an intermediate callus stage (Thorpe and Biondi, 1984). Shoot apices, needles, hypocotyls, epicotyls, cotyledons, zygotic embryo, dormant buds, needles fascicles, and laterals buds have been used as explants for the induction of adventitious bud meristems (John, 1983; Thorpe and Biondi, 1984).

Our approach with organogenesis for loblolly and hybrid pine has been the development of direct organogenesis from mature embryo. For explants pretreatment open pollinated mature seed were cold stratified for 30 to 40 days at 4 °C and thereafter treated with agitated 0.3% water peroxide for up to 15 days, to allow germination in aseptic conditions, without the need of seed mechanical scarification (nick micropilar end). For adventitious shoot initiation we first studied the appropriate embryo development stage to be used as explants, as well as which portion of the embryo will have the highest frequency of adventitious bud formation. Three different nutrient media were tested (GD1, Gresshoff and Doy 1972; LP, Von Arnold and Erikksson 1979; WV5, Coke 1996) and three different concentrations of benzyladenine (BA), as well as the combination of BA and absicic acid (ABA) were studied. Proliferation and differentiation were performed on WV5 growth regulator free media.

The results obtained, indicated that an average of 20 elongated adventitious shoots/embryo, with a 70-80% frequency of induced embryo, in a six month period, can be obtained when mature embryo in the appropriate developmental stage are induced on WV5 media supplemented with BA and ABA for no more than 15 days period.

Micropropagation

Micropropagation utilizing axillaries meristems differ from adventives shoot production only in the ontogenesis of the shoot. After axillary bud stimulation and elongation, rooting and acclimation procedures are essentially the same as those used with shoots adventives organogenic origin (Schwarz 1994). Axillary shoots produced in *Pinus* species generally arise from preexisting quiescent meristems. These meristems are located adaxial to juvenile needles, in the axils of cotyledons, in the normally dormant short-shoot surrounded by needle primordial that make up a fascicular budlet, and primarily, in older plant material adaxial to scale leaves (David 1982; Lanner 1978; Stomp 1985; Toribio and Pardos 1989). Axillary and fasciculary shoot activation has been usually accomplished with hormonal treatment of the explants tissue. However, selection of the explants is probably the most important factor for successful micropropagation of conifers (Thorpe et al. 1991). Based on this consideration, our initial approach was to test different portions of an adventitious shoot to be used as explant.

Our results indicate that the most productive explants in terms of percent of explants forming shoots and the mean number of shoots per test explants were: a) shoot explants (15-20 mm long) with their apical meristem surgically removed (detopping) and; b) 3-4 mm thick cross sectional (shoot segment) slices. After 8 weeks on the shoot–forming medium (WV5 growth regulator free medium), an average of 2 shoots were produced per 3-4 mm thick cross section, and 4 shoots per 15-20 mm apical shoots portions. Therefore, up to 10 - 15 new shoots can be obtained from each 4 cm long original donor shoot. Removal of the apical meristem significantly increased the production of secondary buds. The frequency of explants that formed shoots was 90-100%. The

multiplied shoots were elongated on full strength WV5 media. Rooting induction treatment studies are underway. Preliminary results indicated that 90 % rooting induction could be accomplished in vitro in the presence of ANA or IBA and subsequent root development ex vitro in greenhouse environment. Up to 50 – 60% of the micropropagated shoots were successfully converted into plantlets ready to be transferred to outdoor conditions.

MACROPROPAGATION

Rooted cutting technology proved to be an efficient propagation system for many forestry species. System for large-scale production of rooted cuttings has been developed for many conifers like pine (Pinus radiata D. Don) and Norway spruce (Menzies, 1986; Foster, 1990). Loblolly pine considered to be the most economically important of the southern pines, has the added distinction of being one of the most difficult conifers to propagate by rooted cuttings. Most attempts to root loblolly pine have generally reported successes within a range of 0% - 60% (Wise and Caldwell, 1994). On the other side, for slash x caribbean pine it has been reported rates of 89% rooting for a wide range of families (Haines and Walker, 1993).

Successful uses of rooted cuttings propagation system for large scale production on loblolly and slash x caribbean pine depends on the understanding of genotype, maturation, and environmental factors influences on rooting capacity of stem cuttings. Rooting potential can be further manipulated by appropriate management of the stock plant (Haissig, 1986; Moe and Andersen 1988). Based on these considerations, we studied the influences of factors such as: a) rooting environment (mist controlled irrigation scheme; cutting fungicide and cold storage pretreatments); b) shoot morphology (primary needles presence, size and diameter of cuttings); c) age of donor hedged (4, 8, 12 and 36 month old) and; d) stock plant management (pruning, decapitation height treatments and frequency, container size, light condition, fertilization management and field growth), have on the rooting capacity of loblolly and slash x caribbean pine. These factors were optimized for both species, to develop a reliable, cost effective and genotype independent rooted cutting system.

Our current results indicate that an average of 40-60 cuttings/stock plant/year, 3-4 harvest/year, with an average of 80% rooting capacity are common, for both species, when: a) outdoor, containerized 4-5 months old seedlings are used as stock plant; b) cuttings with juvenile morphology are harvested; c) cold storage of cuttings for up to 15 days; d) cuttings fungicide treatments prior to sticking; e) rooting environmental conditions included controlled mist irrigation, 80 %relative humidity, no use of heated benches or rooting hormones, and f) composted pine bark is used as rooting substrate.

CONCLUSIONS

Our current research results indicate that through the integration of tissue cultures techniques (organogenesis and micropropagation) and macropropropagation, a reasonable high multiplication rate through a wide range of genotype is possible for loblolly pine and slash caribbean pine. At present, our propagation strategy has the potential to deliver more than 12,000 trees from a single seed in a 2.5 years period. Interaction of vegetative propagation techniques with on going tree improvement programs in the region, will allow the deployment of highly improved genetic material to a large number of hectares maximizing genetic gain at harvest time.

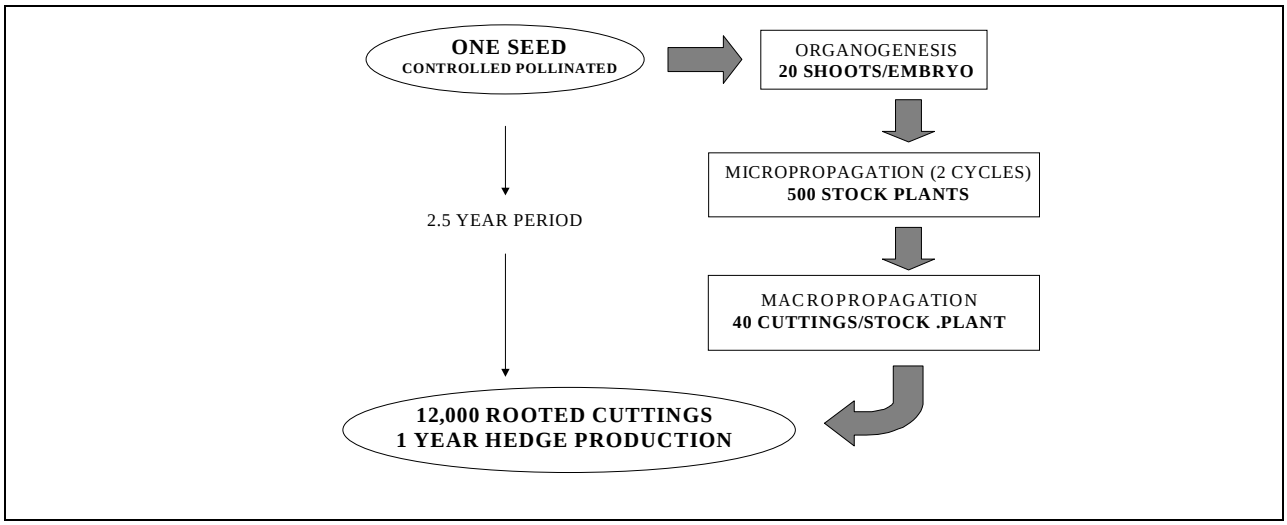

Figure 2. Potential multiplication rate applying the vegetative propagation strategy developed for loblolly pine and slash x caribbean pine in northeast Argentina. Final Multiplication rates presented (12,000 rooted cuttings) include 40 % losses throughout the entire process.

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