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Rejuvenation of a 100 yr old giant sequoia (*Sequoiadendron giganteum* Buchholz) through *in vitro* meristem culture

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Introduction

Vegetative propagation is currently recognized as a powerful tool for forest tree improvement to increase forest plantation yield (Zobel and Talbert, 1984). However, selected trees – the ortets – must develop enough to reach a sufficient size for reliable evaluation of their genetic potential, which is accompanied in most cases by a significant decrease of their capacities for cloning by asexual propagation methods. In this context, the possibilities of cloning selected mature tree genotypes true-to-type remain greatly influenced by the prior rejuvenation of the ortets. This problem was investigated at AFOCEL using *Sequoiadendron giganteum* Buchholz.

Materials and Methods

Plant material

The mature material originated from a 100 yr old *Sequoiadendron giganteum* selected *in situ*. The easy-to-root juvenile clone used as the control consisted of young cuttings derived from a 2 yr old seedling.

Experimental methods

The mature and the juvenile materials were concurrently compared regarding their respective capacities for vegetative propagation, using propagation by cuttings, grafting and subsequently *in vitro* methods. These included subcultures with sequential BAP (benzylaminopurine) treatments (Fouret *et al.*, 1986), micrografting (Monteuuis, 1987a) and meristem culture (Monteuuis, 1987b, 1988). Rejuvenation of the mature material was evaluated through morphological – especially leaf form – and organogenic capacity criteria, with reference to known juvenile material. In addition, these observations were supported by biochemical investigations (Bon, 1988).

Results

Under nursery conditions, the mature material failed to root, while the juvenile clone rooted but rooting ability denoted seasonal variations. Moreover, it was shown that leaf form may be a reliable marker for rooting (Monteuuis, 1985). The rejuvenation of the apical meristem of the scion resulting from grafting onto a young seedling and expressed through a morphological juvenile type reversion, was shortlived and did not induce any improve-

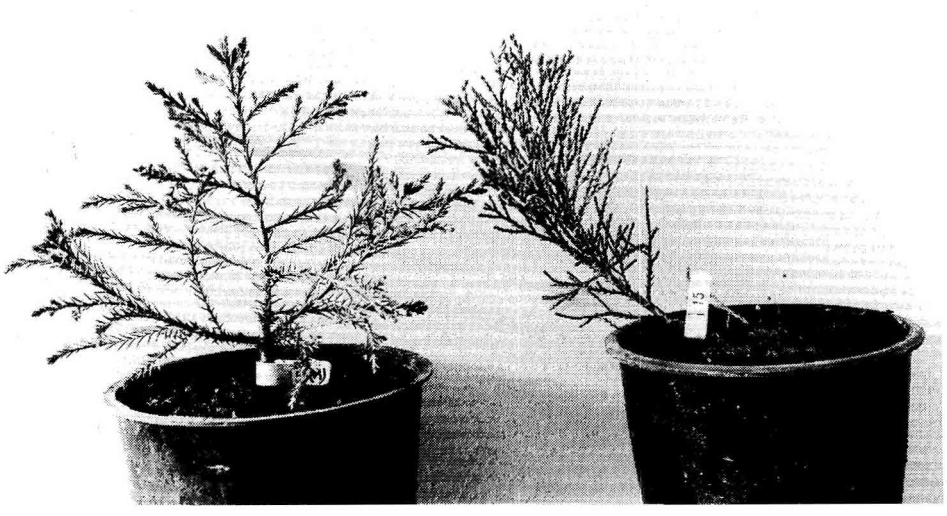

Fig. 1. Rejuvenation of a 100 yr old giant sequoia through *in vitro* meristem culture. On the left: rejuvenated rooted cutting originating from meristem culture. On the right: the same 100 yr old clone non-rejuvenated by grafting.

ment of the rooting ability of the mature material. Similarly, despite using scions as small as 200–300 μm, rejuvenation attempts through *in vitro* micrografting led to only temporary rejuvenation (Monteuuis, 1987a). Nevertheless, the fleeting morphological rejuvenation corresponded with the meristem protein pattern associated with juvenile material (Bon and Monteuuis, 1987).

Subcultures of microcuttings using BAP treatments appeared to be less effective than for other species (Fouret *et al.*, 1985). Morphological and organogenic observations conducted over a 3 yr period showed that *in vitro* medium macronutrients and, more particularly, the physiological state of the explant caused variations even under stable environmental conditions (Monteuuis and Bon, 1986; Monteuuis *et al.* 1987). Taking into account this physiological parameter susceptible of fluctuating greatly under outdoor conditions (Monteuuis and Gendraud, 1987) proved to be determinant in ensuring successful meristem culture of the mature material. Thus, when removing the meristems at bud-break, it was possible to regenerate a truly rejuvenated line. The rejuvenated plantlets exhibited the same morphological characteristics and organogenic potentialities, including rooting abilities, as the juvenile clone. This rejuvenation has been maintained for more than 2 yr for *in vitro* as well as for outdoor cultivated rooted cuttings. In addition, the rejuvenated material produced a 16 kDa protein found in juvenile *Sequoiadendron giganteum* (Bon, 1988).

Discussion and Conclusion

For giant sequoia, like most forest tree species that do not sprout from stumps, the only possibility indeed to clone selected mature trees is to rejuvenate them

through manipulations of ramets taken from the crown. In this context, propagators must be aware of the very promising potentialities of shoot apical meristems, which may theorically be capable of totipotency (Margara, 1982), although some specialists state that irreversible maturation processes occur within apical meristems of aborescent species in proportion to the number of mitotic divisions their cells undergo (Fortanier and Jonkers, 1976).

Our results, based concurrently on physiological, biochemical, histocytological, organogenic and morphological investigations (Bon, 1988; Monteuuis, 1988), support the hypothesis that shoot apical meristems of trees should be able to express, according to a reiterative pattern, juvenile potentialities during a period that shortens with increasing ontogenic development, the maximum intensity corresponding to bud-break. This opinion is in total accordance with Krenke's (1940) conception of maturation, although this juvenile state remains invisible most of the time, probably as a consequence of the inhibitory correlative systems. But it should be noted, as reported by Edelin (1987) that, in certain cases, meristems do not need to be excised from the mature ortet in order to develop ontogenetical reversions exhibiting the juvenile traits that characterize the first phases of ontogeny.

In contrast to the truly rejuvenated plants obtained from meristem culture, the ephemeral morphological rejuvenation induced by micrografting (Monteuuis, 1987) could be interpreted as a simple and temporary transfer of hypothetical rejuvenating substances from the juvenile tissues of the seedling rootstock to the mature scion meristem, rather than a self-juvenile-status functioning.

As a concluding remark, it should be added that *Sequoiadendron giganteum*, due to its specific characteristics including foliar dimorphism that reflects the juvenility degree of its apical meristems, appears to be a good model for the study of phase change phenomena of forest trees. In this way, most of the investigation procedures, and especially biochemical techniques (Bon, 1988), proven to be powerful analytical tools for giant sequoia, are actually being applied at AFOCEL to other promising forest species in order to enhance their ability for true-to-type cloning.

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