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Maturation of woody plants: a review of metabolic and genomic aspects

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Summary — The first part of this review consists of an evaluation of the bibliographic data on maturation studies in woody plants. It reports on the existing knowledge and the remaining questions relating to the events which control the transition phase between the juvenile and the mature phase, as well as the causes of the relative stability of these 2 phases. The physiology and molecular biology aspects are then considered for listing biochemical markers of maturation in woody plants. These markers occur as part of the primary and secondary metabolism (mineral and carbon nutrition, growth regulators, polyamines, phenolic compounds, peroxidase activity) and gene expression (nucleic acids, transcription, proteic synthesis). The results considered show that maturation is accompanied by variations in different – more or less linked – parameters. The discussion on the intervention of these parameters in the control of maturation and their use as maturation criteria shows that determination of the mature state should be multifactorial. These considerations point to a new "system" approach to physiology, based on the relations between the different metabolic systems of plants, and designed for the correlative study of tree development. This approach is intented to further the understanding of the phenomenon in question, and the determination of reliable maturation criteria.

juvenility / maturation / criterion / metabolism / genetic expression

Résumé — Maturation chez les plantes ligneuses : synthèse sur les aspects métaboliques et génomiques. *La première partie de cette revue consiste en l'évaluation des données bibliographiques concernant les études sur la maturation chez les ligneux. Elle rapporte l'ensemble des acquis et des questions concernant les événements physiologiques contrôlant la transition du stade juvénile au stade mature, ainsi que les causes de la relative stabilité de ces 2 phases. Les domaines de la physiologie et de la biologie moléculaire sont ensuite considérés afin d'inventorier les marqueurs biochimiques de la maturation des plantes ligneuses. Ces marqueurs interviennent dans le cadre des métabolismes primaires et secondaires (nutrition minérale et carbonée, régulateurs de croissance, polyamines, composés phénoliques, peroxydases) et de l'expression du génome (acides nucléiques, transcription, synthèse protéique). Les résultats considérés montrent que la maturation*

Abbreviations: IAA, Indole-acetic acid; GAs, gibberellins; CKs, cytokinins; NAA, naphthalene-acetic acid; ABA, abscisic acid; ATP, adenosine triphosphate; NTP, nucleotide triphosphate; GDP, guanosine diphosphate; GTP, guanosine triphosphate; RNA, ribonucleic acid; DNA, desoxyribonucleic acid.

s'accompagne de variations au niveau de certains paramètres plus ou moins liés entre eux. L'intervention possible de ces paramètres dans le contrôle du processus de la maturation et leur utilisation comme critères de maturation sont discutés. Il apparaît que la détermination de l'état mature d'une plante ligneuse serait multifactorielle. Ces considérations débouchent sur l'évocation d'une nouvelle approche de la physiologie, de type «système», basée sur les relations existant entre les différents systèmes métaboliques des plantes, et préconisée pour l'étude corrélative du développement des arbres. Cette démarche est proposée pour avancer dans la compréhension du phénomène et la détermination de critères fiables de la maturation.

juvénilité / maturation / critère / métabolisme / expression génétique

THE MATURATION PHENOMENON

It has been known for many years that chronological measurement of plant age does not allow universal interpretation of the different phases of physiological development in plants, and that plant aging has 2 different aspects: physiological aging (senescence), which corresponds to the increase in size and/or structural complexity of the plant (Borchert, 1976a), and ontogenetic aging, which is localized in the meristem, at the level of the individual cell or of the entire meristem (Hackett, 1985).

Maturation is a developmental process, described in woody plants in particular, and characterized by a reduction in the growth rate and rooting aptitude of cuttings, by changes in morphological parameters and by the onset of flowering. The reliability of this last parameter is discussed due to its dependence on environmental conditions (Wareing, 1971). The usual plant used to illustrate the maturational phenomenon is *Hedera helix*, which has often been used to study this process since Doorenbos (1954) described the morphogenetic changes between the juvenile and mature phases of this plant. The cuttings of *Hedera helix* retain the morphological and physiological characteristics of the mother plant (Doorenbos, 1965). In contrast, the mature characteristics are eliminated during the formation of zygotic or nucellar embryos (Borchert, 1976a), which is considered as the means of maximum rejuvenation for trees (Bonga, 1982). Many other woody species exhibit this process, which has been reviewed by several authors (Borchert, 1976a; Fortainer and Jonkers, 1976; Chouard, 1977; Bonga, 1982; Hackett, 1985; Zimmermann *et al,* 1985; Greenwood, 1987).

Distinction between growth and maturation

Most of the studies on this subject are descriptive; comprehensive studies are still rare and the events which regulate maturation are therefore not yet known (Greenwood *et al,* 1989). The authors have discussed (Hackett, 1985) whether maturation corresponds to ontogenetic (Greenwood, 1984) or to physiological aging (Borchert, 1976a). These 2 processes should affect both plant development and determine its lifespan (Fortainer and Jonkers, 1976). But the general definition of the juvenile and mature phases as a "full-vigor" phase (Assman, 1970), where annual growth increment reaches a maximum value followed by a mature phase where annual growth increment declines and then stabilizes does not allow determination of the role of tree size or maturation state as a basis for these 2 phases (Greenwood, 1989). Greenwood *et al* (1989) reported that while increasing size and complexity

may affect the onset of phase change in Loblolly pine, they are not required for the maintenance of mature shoot growth characteristics resulting from changes in the cells of the apical meristem.

Actually, annual growth increment should be in part determined by the maturation state of the tree, which in turn is a function of size (Greenwood, 1989). This definition leads to a discussion on the relation between juvenile state and vigor, which have often been associated (Greenwood, 1984; Legocka, 1989). In Eastern larch, the vigor of a shoot, measured by the proportional growth increment, is associated with a quantitative contribution on the part of the root system, depending on the distance between the shoot and the root (Greenwood, 1989). On the other hand, the mature characteristics, such as growth increments and chlorophyll content, foliar morphology and reproduction competence (Greenwood *et al,* 1989) are associated with an inability of the shoot depending on its age, to use the root system inputs (Greenwood, 1989). This is in agreement with the hypothesis that the system receiving the signals for vegetative programming of the meristem is located within the meristem itself, but that meristem function is also sensitive to signals received from the environment and from elsewhere in the plant (Sussex, 1989).

Thus, whether the cause of maturation is self-programming of the meristem or signals from the other parts of the plant, the expression of maturation occurs through changes in the activity of the apical meristem (Borchert, 1976a) and cambia (Bonga, 1982).

Determination of the mature state in the meristem

Maturation has long been considered as an irreversible process with regard to the difficulty in propagating some species (Franclet, 1979; *Araucaria, Sequoia*). This is in agreement with the genetic determination of maturation in the meristematic cells, instead of the correlative control of the phenomenon by other differentiated parts of the plant. However, the use of *in vitro* methods has resulted in the propagation of some species which were recalcitrant to classical propagation methods: 7-month old *Eucalyptus* (De Fossard *et al,* 1973), wild cherry tree (Riffaud and Cornu, 1981) and *Hevea* (Dublin *et al,* 1991). This led Hackett (1983) to consider the level of juvenility or maturation of a plant as an equilibrium than can be reversed under certain conditions instead of an irreversible state. Thus, the efficiency of the propagation technique must be questioned in any discussion on the propagation aptitude of one plant. Nonetheless, there are few methods for regeneration of plants from tissues of coniferous trees once they have passed the embryonic or seedling stage (Greenwood, 1987). Some recent studies even show that tissue culture plantlets derived from embryonic tissue of pines behave like mature trees (McKeand, 1985; Greenwood, 1987). Then, we do not yet know whether maturation occurs in all the woody and non-woody plants or whether the phenomenon is characteristic of only the woody plants, even if its expression can greatly differ between certain woody species (for example, coniferous and other woody plants often do not behave in a similar manner).

The intrinsic determination of the meristematic cells in the mature phase should be either biochemical or biophysical, and should correspond to a different ability of the cells to react to developmental signals emitted by differentiated parts of the plant. This change in competence of the meristematic cells persists in the absence of an initiating stimulus and could be related to the number of divisions the apex has under-

gone (Hackett, 1980). In animals, there is ample evidence that the decline in ability of cells to divide with increasing age is a controlled developmental phenomenon, proportional to the number of cell divisions (Greenwood, 1984). Sussex (1976) discussed the existence of systems measuring the developmental time, associated with the cell cycle of plants, and Greenwood *et al* (1989) agreed with the possibility that a developmental time-clock resides in the meristem, although their data on Loblolly pine do not directly indicate that maturation is proportional to the amount of mitotic activity that has occurred in the apical meristem. In the same manner, Bonga (1982) reported that the differences in degree of juvenility between different shoot apical meristems in the tree could be related to the number of cell divisions that separate each meristem from the original embryo shoot apex.

Thus, plants could assume some loss in juvenility in each successive division, but only up to a point (Bonga, 1982). As a matter of fact, even though mature characteristics may be transmitted through the first generation of vegetative propagules, later generations do not become progressively more mature. Furthermore, the application of certain technics of propagation, especially in vitro methods, to some species leads to rejuvenation (Mullins *et al,* 1979 in *Vitis*).

Genetic stability of the juvenile or mature state

The genetic stability of the juvenile or mature phase in certain species may be due to the more or less important presence of weak mitotic activity cells in the main meristem (Bonga, 1982). The areas of strong mitotic activity may age more quickly, undergoing progressive maturation with each cell division, whereas areas of weak mitotic activity may age more slowly. The areas of weak mitotic activity would be very stable genetically because of their low mutation rate, related to the small number of cell divisions.

As a matter of fact, Charlesworth (1989) supports the idea that long-lived plant species show high mutation rates because of the great number of divisions before gamete formation. Plants have characteristics allowing the accumulation of somatic mutations: lack of a separated germline, open system of growth, flexible meristem organization and the fact that most somatic mutations are not immediately life-threatening (Klekowsky, 1988). This led Klekowsky and Godfrey (1989) to recall that this is one reason for the accumulation of mutations in the meristematic initials as the plant ages.

So the question of genetic stability in plants concerns the mechanisms available to reduce the impact of mutational load. The critical point is whether mutant cells are maintained in apical or even in cambial meristems or whether the mutant cells are lost to tissues and organs that soon become metabolically moribund (Klekowsky, 1988). Many characteristics of the apical meristems can affect the loss or fixation of somatic mutations: number of cell divisions undergone by the initials per node of growth, organization in "méristème d'attente" or in "tunica-corpus" or unstratified organization, number of periclinal divisions resulting in the movement of cells between the different layers of the meristem, and the changes in these parameters during growth (Klekowsky, 1988).

Apical meristems also have characteristics that can modify the effective mutation rate. Mutation rate per biological time unit is in part a function of the number of times a genome has been replicated and chromatids divided during the biological time

unit. Thus, the maintenance of cell pools within the meristem which seldom divide but which give rise to meiocytes (such as the "méristème d'attente") may reduce mutation rates (Klekowsky, 1988).

Consequently, the balance between the appearance, the fixation or the loss of somatic mutations within the apical meristem could interfere in the determination of the juvenile or mature phase in the apical meristem, the persistence of these phases through generations of vegetative propagation, the reversion of the mature phase to the juvenile phase for certain vegetatively propagated species especially by *in vitro* culture, and the differences in juvenile state between different parts of one plant. Finally, one of the most significant losses of mutation occurs during sexual reproduction (Klekowsky, 1988) which is also the means of maximum rejuvenation in trees.

Then, if genes control the ontogeny and the final form of an organism, both the specific pattern of ontogeny and the final form of an organism may have repercussions upon the integrity of the genes (Klekowsky *et al,* 1989). The objective of comprehensive studies of maturation should concern occurrences during sexual rejuvenation (Bonga, 1982).

METABOLIC CRITERIA OF MATURATION

Morphological, physiological, and histocytological parameters have been used in the descriptive of juvenility in many species (Wareing and Frydman, 1976; Zimmermann *et al,* 1985). Biochemical parameters related to general metabolism or/and genetic expression could allow a quantitative approach to the phenomenon. As a matter of fact, factors such as the physiological state or histocytological structure of a plant, the concentration, distribution, and type of active substances in plant metabolism are affected by environmental factors, and the levels of these substances differ between juvenile and adult plants (Zimmermann *et al,* 1985). The analysis can even reach the molecular level concerning mechanisms of gene expression in plants (Bon, 1988c). The evolution of these parameters during maturation and their possible use as criteria are reviewed below.

Carbohydrates and other parameters of carbon metabolism

In 1967, Wareing and Seth showed that carbohydrate synthesis varies during maturation. More recently, maturational variations of cellulose and lignins have been characterized in *Pinus radiata* (Uprichard and Llyod, 1980). Observations on leaves of *Sequoiadendron giganteum* (Monteuuis and Genestier, 1989) have shown that parietal polysaccharides of the mesophyll, particularly cellulose and hemicellulose, increase in mature trees.

Apart from variations in carbohydrate levels, maturation is characterized by changes in the levels of many enzymes (Zimmermann *et al,* 1985), generally related to carbon metabolism (amylase, catalase, cytochrome *c* oxidase, alkaline and acid phosphatases, ascorbic acid oxidase). The juvenile phase of *Hedera helix* is characterized by weaker photosynthetic activity (Bauer and Bauer, 1980), associated with a reduction in the activity of the photosynthetic apparatus (activity of the ribulose 1,5-diphosphate carboxylase) and with anatomical features of the leaves (reduction of stomatal frequency, number of chloroplasts per cell, leaf thinness). Lastly, during the growing phase of hybrid walnut, the pentose phosphate and the amino acid degradation pathway function in a synchronous and moderate manner in juvenile

plants, whereas the 2 pathways function in an asynchronous but accelerated manner in adult plants (Drouet *et al*, 1989).

Thus, all the levels of carbon metabolism seem to be implicated in maturation, so the determination of one or several criteria connected with this field would be rather long and tedious.

Mineral elements

The concentrations of chlorine, potassium, and sodium increase with dry weight in buds of mature *Picea abies* (Von Arnold and Roomans, 1983). Sodium increases more quickly than potassium, which is why the K/Na ratio decreases with physiological age (Von Arnold and Roomans, 1983). In the same manner, buds of *Sequoia sempervirens* have different calcium and potassium levels depending on their position on the parent plant and the K/Ca ratio decreases with age (Vershoore-Martouzet, 1985). The potassium level is known to decrease in aged tissues in favor of the new tissues (appeal mechanism) and, in contrast, the aged tissues accumulate calcium. Thus, the K/Na and K/Ca decrease could be associated with the increased size of the aged trees rather than with maturation.

On the other hand, in Douglas fir, rejuvenation produced by *in vitro* subculturing is characterized by a decrease in the K/Na ratio (Bekkaoui, 1986). More recently, the K/Ca ratio has been recommended for use as marker of juvenility in Douglas fir and eucalyptus cultivated *in vivo* under very precise conditions, and with other criteria for *in vitro* culturing (Dechamps, 1986). These data should be in favor of a relation between the increase of these ratios and maturation of the meristem. Furthermore, the interaction of the potassium metabolism with growth regulators (Erdei *et al*, 1989) and with anthocyanin synthesis (Rembur and Nougarede, 1989), a parameter that can vary with maturation, as well as the relation of the concentrations of zinc (Cakmak *et al*, 1989) and manganese (Tomasewski and Thimann, 1966) with auxin metabolism, could be in agreement with this.

Thus, the K/Na and K/Ca ratios inversely evolute during the increase in tree size and in the course of meristem maturation. They could then be used to distinguish between these 2 maturation processes.

Polyamines

Recently a role in rejuvenation has been attributed to polyamines by Georges *et al* (1989), who reported that putrescine and spermidine increase when *Asparagus* is rejuvenated by micropropagation and that there is a close correlation between the level of endogenous polyamines and the length of the rejuvenated phase. On the contrary, polyamines have been implicated in the loss of totipotency during maize tissue cultures (Tiburcio *et al*, 1989), and the ratio of putrescine to spermine in internodes has been found to increase with stem age in this plant (Schwartz *et al*, 1986).

The results of these studies are confused and do not concern woody plants. But the role of polyamines in the cell cycle and their suggested role in the regulation of senescence and morphogenesis (Galston and Sawhney, 1990) indicate that polyamines could be implicated in the regulation of maturation in woody plants.

Phenolic compounds and anthocyanins

Many studies indicate a direct relationship between phenolic compounds and juvenili-

ty, rejuvenation, and maturation. Qualitative variations of polyphenols occur during plant ontogenesis (Vieitez and Vieitez, 1976). Moreover, the accumulation of hydroxycinnamic amides is related to the flowering and differentiation rate, and there is a difference (unconfirmed) between the juvenile phase, which is lacking in amides, and the mature phase, possessing amides (Cabanne *et al,* 1981). The number of phenol compounds increases with maturation in the chestnut (Garcia *et al,* 1980). But phenol compounds cannot be used as morphogenic markers in giant sequoia because of large clonal variations in polyphenol levels and a lack of synchronization in the physiological states of the plant material (Monteuuis and Bon, 1986; Bon *et al,* 1988).

The mature phase appears to be characterized by inactivation of one or more enzymes involved in biosynthesis of active polyphenols and flavonoids (Hackett *et al,* 1989). For instance, if the specific activity of phenylalanine ammonia lyase (PAL) in mature tissue of *Hedera helix* is twice that observed in juvenile tissues, the accumulation of anthocyanins in mature tissue of this plant may be due to inactivation of dehydroquercetin reductase (DQR).

Severe pruning of walnut tree strongly affects the phenol content in new shoots, which is similar to that in juvenile individuals (Jay-Allemand *et al,* 1987). In the same manner, rejuvenation in hybrid walnut has also been characterized by 3 ratios of 5 different polyphenols during the growth period (Jay-Allemand *et al,* 1988). The study also examined 2 phenol compounds, hydrojuglone glucoside and myricitrine, which show significant differences depending on physiological state in walnut (Jay-Allemand *et al,* 1989). The first one is presumed to act as a biological accelerator and the second as a brake system. Moreover, these 2 polyphenol markers of hybrid walnut rejuvenation accumulate in sclerenchyma and to an even greater extent in phloem. Hydrojuglone glucoside accumulates at the beginning of the growing phase in rejuvenated individuals, while myricitrine accumulates in mature individuals along with PAL (Claudot, 1989). The authors concluded that the organogenetic capacity of different tissues and their activities can be modified during maturation by variations in the levels of phenol compounds involved in different biological processes. Thus, phenol metabolism varies in walnut during aging, so that maturation is qualitatively and quantitatively characterized by different phenolic compounds.

Hormones

Phytohormones are involved in regulating maturation. For instance, maturation phase changes in birch are related to large phytohormone changes in buds and apical part of the stem (Galoch, 1985). Furthermore, rooting potential, which is directly related to juvenility, appears to be controlled by relative phytohormone levels (Gaspar *et al,* 1977; Okoro and Grace, 1978; Baz *et al,* 1984a; Berthon *et al,* 1989; Chin *et al,* 1989).

The action of auxin is related to rejuvenation. For instance, auxin has a negative effect on plagiotropy in conifers, which is associated with maturation (Chaperon, 1979); mature cuttings of *Ficus pumila* require twice as much auxin for rooting as juvenile cuttings (Davies, 1984). This indicates that the auxin level decreases in the mature parts of the tree: either the mature meristem supply decreases or/and the auxin transport cannot reach the roots because of the increased size of the tree. But during maturation the auxin level seems to decrease less rapidly than the cytokinin level, because Douglas fir maturation is characterized by a decrease in the zeatin/indole acetic acid (Z/IAA) and zeatin-

riboside/indole acetic acid (ZR/IAA) ratios (Maldiney *et al,* 1986). On the contrary, maturation of *Sequoia sempervirens*, characterized by a fall in its cloning capacity, is accompanied by an increase in the abscissic acid/indole acetic acid (ABA/IAA) ratio (Fouret *et al,* 1986).

Gibberelins (GAs) are implicated in the morphological reversion of adult leaves of *Hedera helix* to the juvenile type (Rogler and Dahmus, 1974). The natural gibberellic substance GA3 participates in rejuvenation (Rogler and Hackett, 1975a; Wareing and Frydman, 1976; Hackett, 1985). The direct effect of GA3 appears to concern elongation, depending on the dose, whereas its indirect effect is connected with morphological changes related to rejuvenation (Wallerstein and Hackett, 1989). Thus, GA3 seems to induce rejuvenation without affecting the persistence of the juvenile phase, perhaps in relation with the auxinic metabolism (Wallerstein and Hackett, 1989). The role of GAs in maturation is still under discussion, because although they reverse the mature phase of *Hedera helix* (Zimmermann *et al,* 1985), this group of substances promotes flowering in conifers, which remains a mature characteristic. Moreover, the primary role of GAs in controlling maturation is questioned by Greenwood *et al* (1989), because while GAs promote flowering in many conifers, their application often cannot offset a genetic indisposition of trees to flower. Lastly, with regard to development, GAs are associated with the vigor of apple trees *in vivo* (Lonney *et al,* 1988). Vigor has often been related to juvenility (Greenwood, 1984; Looney *et al,* 1988).

The relation between cytokinins (CKs) and maturation is also still open to discussion. CKs affect the reactivity and growth of buds in many species, either directly (Von Arnold and Tillberg, 1987; Label *et al,* 1988; Pilate *et al,* 1989; Young, 1989) or *via* root activity related to juvenility (Franclet, 1981). CKs induce apex rejuvenation in mature trees of the species *Pseudotsuga menziesii* (Bakkaoui, 1986) and *Picea abies* (Tsogas and Bouriquet, 1983), characterized by reactivation. On the contrary, a relationship has been demonstrated between an increase in CK level and lack of rooting capacity in poplar (Okoro and Grace, 1978). Finally, in conifers, Benzyl-adenine (BA) has been implicated in both promotion and reversal of maturation (Greenwood, 1987). With regard to development, CK levels decrease in more vigorous cultivars of apple (Looney *et al,* 1988).

Abscissic acid (ABA) indirectly affects senescence, exerting its action on mature and older organs by inducing the production of a senescence factor that controls ethylene synthesis (Milborrow, 1974). In addition to its effect on senescence, ABA is involved in maturation. The mature phase is characterized by higher ABA levels than the juvenile phase (Hackett, 1985; Galoch, 1985; Fouret, 1987), the Z/ABA and ZR/ABA ratios decrease with maturation (Maldiney *et al,* 1986). Rogler and Hackett (1975a) have reported that the GA3/ABA ratio has more importance than the absolute values of the 2 substances in controlling reversion from the adult to the juvenile phase in *Hedera helix* and that stabilization of the mature form by ABA probably occurs *via* regulation of the GAs level in the plant (Rogler and Hackett, 1975b).

Thus, even if the role of the root-produced plant growth regulators (GAs and CKs) in the process of maturation is still confusing (Greenwood *et al,* 1989), the auxins, gibberellins, cytokinins and abscissic acid are related to the maturation phenomenon. Furthermore, the ratios between these 4 phytohormones seem to better determine the induction and the stabilization of the phase change than their respective

absolute values. Moreover, their interaction and their transport from the synthesis site to the active site probably make them interfere in the physiological as much as in the ontogenetic ageing processes.

With regard to rejuvenation, elevated ethylene levels in the culturing atmosphere of *Hemerocallis* plantlets have been correlated with transition from the juvenile to the adult phase, which is accompanied by histological changes (Smith *et al,* 1989). Moreover, difficult-to-root petioles of mature *Hedera helix* produce more ethylene than juvenile petioles (Georges *et al,* 1989; Geneve *et al,* 1990a, b). These authors indicate that ethylene does not seem to play a significant role in the different rooting responses of juvenile and mature petioles treated with naphthalene acetic acid (NAA). In contrast, it appears to have an inhibitory effect during adventitious root elongation on juvenile petioles. The role of ethylene in maturation has still been insufficiently studied, but these results indicate that these phytohormones should be considered as a parameter of maturation.

Peroxidasic activity

Peroxidases are considered as markers of rooting potential (Quoirin *et al,* 1974; Ranjit *et al,* 1988; in *Prunus*; Moncousin and Ducreux, 1984 in *Cynara scolymus*; Berthon *et al,* 1987 in *Sequoiadendron giganteum*; Gus'kov *et al,* 1988; De Klerk *et al,* 1989 in apple) and some authors also consider them to be good biochemical markers of juvenility and rejuvenation of *Cynara scolymus* (Moncousin, 1982; Moncousin and Ducreux, 1984). However, the conclusions of Dalet and Cornu (1989) on *Prunus avium* do not agree with the other findings.

The peroxidase content in some plants has been correlated with the potential for grafting, micropropagation by cuttings, and growth (Poessel *et al,* 1982). This makes it possible to base the selection of cuttings for propagation on their peroxidase content (Quoirin *et al,* 1974 in *Prunus* species; Mosella-Chancel, 1980 in *Prunus persica*). The narrow relation between peroxidasic activity and rooting makes this parameter a good criterion of the physiological state of certain species with regard to their propagation capacity, but no relation with the meristem maturation has yet been demonstrated in woody plants.

GENOMIC CRITERIA AND GENETIC EXPRESSION OF MATURATION

Nucleic acid composition varies between the juvenile and mature phases (Zimmermann *et al,* 1985). After considerable controversy with regard to desoxyribonucleic acid (DNA) differences, it would appear that the DNA levels of 2c cells do not differe in juvenile and mature tissue of *Hedera helix* (Zimmermann *et al,* 1985). On the other hand, several authors have found differences in total, soluble, and ribosomal ribonucleic acid (RNA) levels in the 2 phases (Zimmermann *et al,* 1985). Wareing and Frydman (1976) have reported quantitative and qualitative RNA variations during maturation of *Hedera helix.* In *Ficus pumila*, total RNA levels are higher in juvenile individuals (Davies, 1984). In addition, the RNA levels and cambial activity in this plant increase during maximum rooting, and these phenomena are more pronounced in juvenile individuals. Thus, the transcription of specific genes should interfere in the determination of the juvenile and mature phases.

In studies of gene expression, it has been found that DNA coding for ribosomal RNA in the 2 forms shows no differences in redundancy (Dommoney and Timmis, 1980; Hackett, 1985). In contrast, it is possible to isolate cDNA clones specific to the

juvenile and mature phases (Hackett, 1985). It has been proposed that only a few genes are active in the mature phase (Zimmermann *et al,* 1985). Consequently, the RNA transcribed from these genes would only represent a small portion of total DNA, suggesting that the molecular basis of phase change depends on an alteration of the transcription rate of certain DNA sequences (Zimmermann *et al,* 1985). This alteration of the transcription rate could be associated with the methylation of cytosine in DNA, since older trees of *Picea abies* showed a greater cytosine methylation than the youngest ones (Wescott, 1987). But this is not the case for *Larix laricina*, in which the morphological foliar differences related to age were not associated with any difference in the cytosine methylation in DNA (Greenwood *et al,* 1989). However, the methods used would not detect methylation of only one or a few genes.

Moreover, in *Larix laricina*, the purification of RNA did not show any difference between juvenile and mature trees, suggesting that maturation is not the result of a general decline in the level of transcription in meristematic tissues (Hutchinson et al, 1987). But differential gene expression, associated with development, could be masked by variations in genetic background. On the other hand, maturation is accompanied by variations in the levels of chlorophyll and ribulose 1,5-diphosphate carboxylase activity (Bauer and Bauer, 1980; Hutchinson *et al,* 1987). Now, if no maturation-related expression of the gene of the small subunit of this enzyme has been observed, the chlorophyll a/b binding protein is differentially expressed in juvenile and mature plants (Hutchinson et al, 1987). In addition, the gene coding for the cab-protein, which is associated with photosystem II, is expressed differently depending on the maturation status of larch (Greenwood, 1984). These facts led Hutchinson *et al* (1987) to feel they would succeed in identifying sequences that were differentially expressed between juvenile and mature plants.

On the energetic level, in giant sequoia, the higher RNA/DNA ratio in juvenile apex during vegetative dormancy is associated with a higher adenosine triphosphate (ATP)/nucleotide triphosphate (NTP) ratio (Monteuuis and Gendraud, 1987). During growth reactivation, juvenile and mature apices of giant sequoia show no differences with regard to the RNA/DNA ratio, whereas the ATP/NTP (Monteuuis and Gendraud, 1987) and guanosine diphosphate (GDP)/guanosine triphosphate (GTP) (Bon, 1988a) remain high in the juvenile individuals. The stimulation of protein synthesis in buds of giant sequoia at the time of bud burst persists in juvenile buds, but is quickly repressed in mature buds, in correlation with a deficit in energy derivatives such as GTP (Bon, 1988a).

Wareing and Frydman (1976) and Aghion (1978) have observed qualitative and/or quantitative differences in proteins during maturation in *Hedera helix*. However, the differences were not great enough to distinguish juvenile and adult clones of walnut (Drouet *et al,* 1989). Recently, Hackett *et al* (1989) have shown that the juvenile and mature phases of *Hedera helix* can be characterized by 2 polypeptides; and Bon and Monteuuis (1987) have reported that rejuvenation of *Sequoiadendron giganteum* by micrografting is accompanied by a decrease in meristem proteins. A membrane protein (J16) specific to juvenile individuals and individuals rejuvenated by apical micrografting has been detected by 2-dimensional electrophoresis (Bon, 1988b). Moreover, culturing of meristem from a 100-year-old individual produced protein J16 along with juvenile organogenetic properties (Bon, 1988c). Two-dimensional

electrophoresis of proteins has also been used to distinguish vegetative, prefloral, and reproductive apices of *Prunus* on the basis of certain polypeptides (Ranjit *et al*, 1988).

Thus, the genetic expression seems to be different in the juvenile and mature phases. This is observed by differences in RNA, proteins and energetic componds and will soon probably be related to differences in the transcription rate and genomic state of the 2 phases. Furthermore, maturation should not only be determined by the nucleus, but also by the accumulation of self-replicated DNA, located in the organelles of the cytoplasm, which could be transmitted over many cell generations (Bonga, 1982).

CONCLUSION

The present review of criteria for maturation shows that expression of the juvenile or mature phase of a plant might be controlled by different related factors. The carbon, mineral and phytohormonal metabolisms and the secondary metabolism are implicated in the regulation of maturation in woody plants, at the levels of physiological aging of the tree as well as maturation of the meristem. The variations in all these parameters are the result of the genetic expression (protein synthesis, transcription, genes), in which the basis of the maturation phenomenon is found. However, the phenomenon is complex in woody plants (Monteuuis, 1988): it depends on a physiological context, can vary and even be reiterated (Nozeran, 1978) and can differ in various plant parts (Chaperon, 1979).

There is a temptation to simplify the study of maturation by trying to obtain a single biochemical marker of juvenility unrelated to physiological markers (Bon, 1988c). But the control mechanisms for maturation are probably the result of the products or regulatory activities of many different genes. The question remains of whether the maturational changes for one species vary independently or not. Borchert (1976b) suggested that they vary independently of one another. Results of Greenwood *et al* (1989) concerning larch do not necessarily support this view, but rather suggest that a single process may affect groups of traits during maturation. In any case, the maturation changes are expressed through the physiological state of the plant, which could not be characterized by a single biochemical parameter.

The control of development, *ie* maturation, is often considered to be a process that is accessible by a simple experimental approach, whereas metabolism, cells, tissues, and the whole plant form systems with many interconnections or "networks" (Trewavas, 1986). Data concerning the control of these systems are limited and very controversial. Trewavas (1986) showed a new "systems" approach to developmental physiology. The relationships between metabolic networks form the basis of this approach, which soon was suggested in a correlative study of maturation in which a "systems" theory was applied to trees (Borchert, 1976b).

An illustration of the "systems" approach is given by the fact that at least the mineral elements and especially the phytohormones appear to be implicated in both physiological aging of the tree and ontogenetic aging of the meristem. The meristem can be considered as a "system", whose behavior depends on its self-programming and on the signals received from the other plant organs (Sussex, 1989). Hence, determination of the maturation status of a whole or fractionated plant and the possibility of predicting its behavior under particular conditions will probably depend on the combined development of several criteria which remain consistent with physiological criteria.

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