XII COLLOQUE DE LA SECTION FRANCAISE DE L'IAPTC

MONTPELLIER - 16/17 novembre 1993

"L'embryogenèse somatique : approches cellulaire et moléculaire"

INFLUENCE OF WALNUT LIQUID ENDOSPERM ON SECONDARY SOMATIC EMBRYOGENESIS IN WALNUT HYBRIDS SOMATIC EMBRYO CULTURE

LABEL P., LUCAS I., DENG M.-D. and CORNU D.

INRA, Station d'Amélioration des Arbres Forestiers, Ardon, F-45160 Olivet, France.

SUMMARY

Vegetative propagation of walnut is difficult through traditional techniques like cuttings (1) but more powerful techniques like somatic embryogenesis offer a good solution to produce vegetatively selected trees (2).

The expression of the competence to somatic embryogenesis (SE) is presently possible only from immature zygotic embryos and in a very short time-window during their development. Furthermore the process of induction is irregular, especially with respect to the genetic origin of the material.

Production of somatic embryos is performed through an adventive secondary SE and secondary somatic embryos (SSE) produced from the "primary" somatic embryo (PSE) are isolated, thus becoming PSE and subcultured iteratively. These steps occur without any exogenous plant growth regulator complementation.

Physiological mechanism underlying SE are unknown. Our purpose is to study the regulation of this SSE production in order to control the onset and outset of the culture. Therefore, we tested the effect of a natural liquid tissue occuring during the formation of the zygotic embryo : the endosperm. The aim of the experiment is to complement biphasically the gelified culture medium (2,3) with walnut liquid endosperm and to study the effect of this liquid endosperm on the formation of SSE and on the growth of PSE.

Walnut liquid endosperm was collected from immature nuts (0.6 ml/nut) when maternal structures just fully developed and before the zygotic embryo started to grow (1st week of July in Orléans, France). This endosperm was centrifuged (6000 x g, 20 min.) and the supernatant was filtered through a 0.2µm-polysulfone Millex filter (Millipore,USA). Six hundred microliter of the supernatant were added to each PSE at subculture. We counted the formation of SSE at several dates and PSE were also weighted at the beginning and the end of the culture after excision of all SSE.

The walnut liquid endosperm strongly reduced the formation of SSE while promoting an increase in fresh weight of PSE. The experiment also showed that the liquid endosperm was rapidly absorbed by PSE (within 1 week) whereas control culture complemented with liquid medium remained in its original biphasic state. After liquid endosperm was absorbed, SSE reformed at the surface of PSE. This was not true if PSE were complemented again with liquid endosperm.

Results also showed that double-phasing the culture medium with liquid medium, without totally immersing PSE, significantly enhanced the production of SSE. These results are linked with previous experiments dealing with hormonal quantitation of walnut endosperm (4).

(1) Cornu D. 1977. La multiplication végétative du noyer hybride : résultats d'une première campagne, perspectives d'avenir. Rev For Fr **29** : 457-463.

(2) Cornu D. 1988. Somatic embryogenesis in tissue cultures of walnut (*Juglans nigra*, *J. major* and hybrids *J. nigra* X *J. regia*). In Somatic Cells Genetics of Woody Plants. Edited by M. Ahuja. Kluwer Academic Publishers, Amsterdam, pp. 45-49.

(3) Driver JA and Kuniyuki AH. 1984. In vitro propagation of paradox walnut rootstock. HortScience **19** : 507-509.

(4) Label P. and Cornu D. 1988. Determination of plant growth substances in liquid endosperm of immature walnut (*Juglans regia*) nuts by an ELISA technique. Plant Growth Regulation **7** : 209-215.