THE EFFECT OF DEVELOPMENTAL STAGES OF THE ZYGOTIC EMBRYO AFFECTING THE EMBRYO INDUCTION EFFICIENCY IN *QUERCUS FRAINETTO*

Timofte Adrian Ioan*, Lazăr Andra Nicoleta**

** University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania, e-mail: adi_timofte@yahoo.com*
*** University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania, e-mail: ienciuandra@yahoo.com*

Abstract

The somatic embryogenesis is an advanced method for clonal propagation and a useful tool for ex situ conservation of genetic resources.
In this paper, an experimental device was designed, composed of two provenances in Q. frainetto and four types of explants (developmental stages of the zygotic embryo).
The aim of the paper is to assess the explant upon the embryogenic induction.

Key words: *Q. frainetto, in vitro, embryo induction, germination, developmental stage*

INTRODUCTION

The somatic embryogenesis in oak, as an alternative to propagation by cuttings, provides the possibility of mass production of cotyledonary embryos that can be either cryopreserved, or maintained by long-term culture as stable embryogenic somaclones (Wilhelm et. al., 2000).

Starting with the first successful embryogenic inductions (Chalupa, 1987, 1990, Gingas and Lineberger, 1989; Jorgensen, 1988, 1993), the method was continuously improved. As a result of many experiments, nowadays is rather easy to obtain a big amount of somatic embryos out of juvenile explants, and also to conserve the embryogenic ability of the selected somaclones for long periods (Wilhelm et al., 1996). The embryogenesis occurs mostly direct on the explant, without a callus phase, and the stabilisation of embryogenic cultures is based on the serial adventitious embryogenesis.

The somatic embryogenesis can be considered an efficient mean of clonal propagation, when the following conditions are accomplished:

- the stable embryogenic cultures constantly produce a big amount of somatic embryos;
- the embryogenic ability of such cultures can be maintained for long time by serial adventitious embryogenesis;
- the somatic embryos can be efficiently converted into acclimatable plants.

MATHERIAL AND METHOD

The immature acorns harvested at 4 different dates were dissected and used as sources of explants. The explants were represented by immature zygotic embryos in different developmental stages or fragments of more advanced embryos. The developmental stages of zygotic embryos have been defined and correlated with the morphological characteristics of acorns (Palada-Nicolau, Hausman, 2001):

Stage 1 – acorn has a diameter of 6-8 mm and the cup covers it almost completely;

- the zygotic embryo of 1 – 1.2 mm is inside the acorn in an early cotyledonous stage, with the endosperm in a cenobial stage.

Stage 2 (more advanced cotyledonous but non-cumulative stage)

- acorn has a diameter of about 10 mm and is at the level of the cup;
- the zygotic embryo of 2 -2.4 mm has also translucid but thicker cotyledons,
- the endosperm, well represented, is in a cell stage.

Stage 3 is characterized through the pronounced increase and thickening of the cotyledons:

- acorn is elongated having dimensions of 10 / 12 mm and exceeds the level of the cup;
- the embryo which is also elongated (5/3 – 8/3 mm) has thickened but still translucid cotyledons and presents only pieces of endosperm.

Stage 4 is characterized by the beginning of starch cumulation in cotyledons:

- acorn is elongated, having dimensions of 10 / 14 mm and exceeds the cup level;
- the embryo, which is also elongated (5/3 – 8/3 mm) has thickened, opaque cotyledons and presents only pieces of endosperm.

Stage 5 defines the immature but completely formed embryos:

- acorn of 12 – 13 / 16 – 18 mm is only half covered by the cup;
- the embryo of 10 / 7 mm has thick and opaque cotyledons and obvious meristematic areas (apical and radicular), and is completely without endosperm.

The young embryos, in stages 1, 2 and 3 **(**pre-cumulative stage) achieved in harvests 1 and 2, were wholly extracted through the dissection of the fructifications and the removal of the embryo's cover. The extraction

of the embryos without being injured is very important to prevent the necrosis and the release of phenols that blacken the medium.

More advanced embryos, achieved in harvests 3 and 4 are in a cumulative stage. Only embryos in stages 4 and 5 are recommended for embryogenesis, the most advanced ones being useful only for germinations in aseptic conditions.

Embryos in stages 1, 2 and 3 were integrally used while a part of the cotyledons were removed from the embryos in more advances stages.

Structure of the experiment was: two provenanceand four developmental stages of explants (4 explant types)

Three replications consisting of ten explants have been counted for each parameter.

Explants (the explants) represented by immature zygotic embryos were inoculated according to the stage of development: stage 1 embryos were inoculated entirely, the stage 2 embryos were inoculated with only one third of the cotyledons, and for the more advanced embryos (stages 3 to 5), cotyledons were removed almost entirely because of the environment's oxidation, thus affecting the viability of explants.

At the end of the first passage with a duration of 21 days, the following phenomena were recorded:

- germination (for more advanced embryos)
- growth of embryonic roots and formation of adventitious roots on hypocotyl axis
- compact callus formation, especially on the section surfaces and at the place the insertion of the cotyledons.
- embryogenic induction.

RESULTS AND DISCUSSIONS

Observations in an experimental device comprising the testing of several factors that could influence the embryogenic capacity (explant type, genotype, culture medium, growth regulator substances) showed that the strongest influence on the efficiency of embriogenic induction has the explant type, that is the zygotic stage of development of the embryo. It works by juvenile cell level and as the embryo is younger, the incidence of embriogenic cells in response to the signals transmitted through growth regulator substances is higher.

Embryogenic capacity varied widely depending on the stage of development of immature zygotic embryo when inoculation, the induction yields decreasing from over 47% in stage 1 and about 20% in the second stage, to below 10% in stage 3 and 0-5% in stage 4, for all provenances. (Fig. 1).

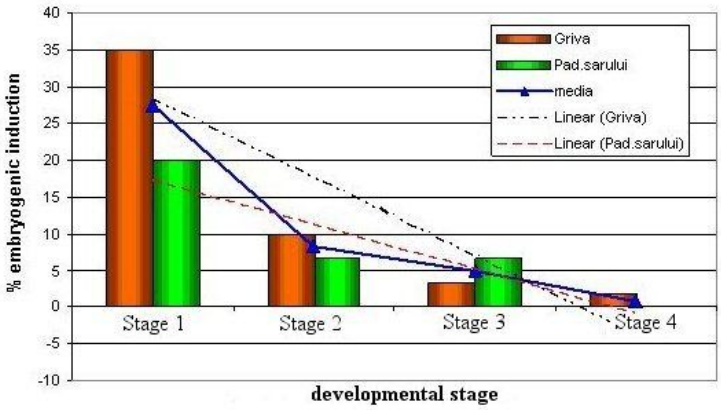

Fig. 1 The effect of explant type (developmental stage of zygotic embryo) upon the embryogenic ability, in *Q. frainetto*

The most pronounced decrease of the embriogenic capacity coincided with the emergence of starch accumulation in the cotyledons of the explants.

The general tendency is to reduce the embriogenic capacity and frequency of callogenesis with the development and maturation of zygotic embryo, and at the same time increasing the organogenic trend (root formation) and the emergence of germination capacity during the embryonic maturation.

The analysis of the following graphs (Fig. 2-5) highlights some deviations from the general trend.

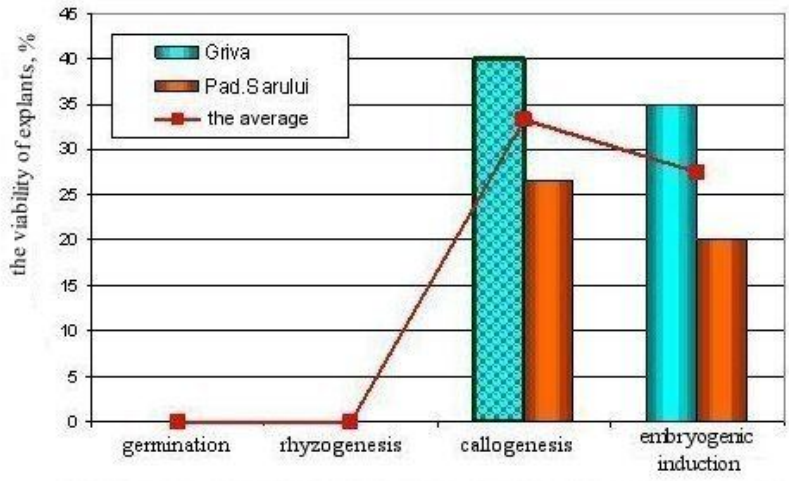

Fig.2 The effect of genotype (provenance) upon the reaction of *Q. frainetto* zygotic embryo explants in stage 1

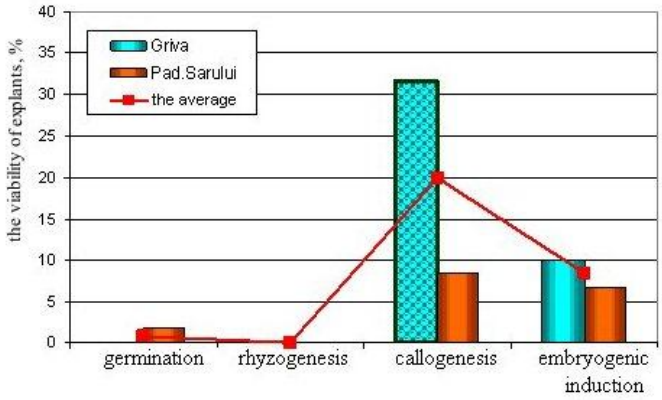

Fig.3 The effect of genotype (provenance) upon the reaction of Q. frainetto zygotic embryo explants in stage 2

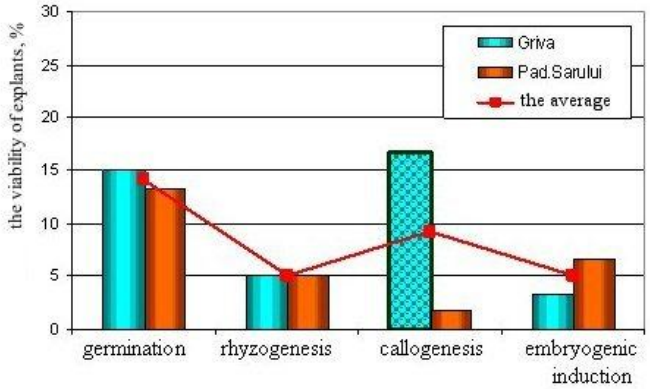

Fig. 4 The effect of genotype (provenance) upon the reaction of Q. frainetto zygotic embryo explants in stage 3

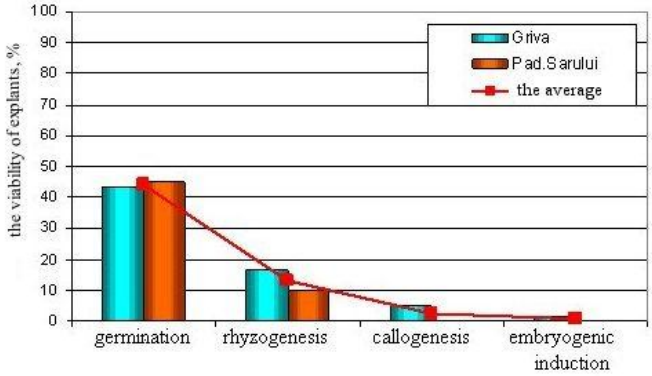

Fig. 5 The effect of genotype (provenance) upon the reaction of Q. frainetto zygotic embryo explants in stage 4

CONCLUSIONS

It is very important to harvest the acorns for somatic embryogenesis experiments in early stages, and when it is not possible, to plant on culture medium a big number of explants (5 fold more than usually), in order to ensure the somatic embryo induction, despite of the very low efficiency.

At *Quercus frainetto*, the return of the embriogenic induction in stage 1 for Griva provenance had an average value of 35%, and for Sarul Forest provenance, of only 20%. Embryogenic induction in later stages presented conflicting values: stage 3, Şarul Forest provenance presented an embryogenic yield almost double than Griva provenance (6.67% vs. 3.33,%), while in stage 4, embryogenic induction occurred only for Griva provenance, with a yield of 1.67% embriogenic explants.

The general trend for all provenances, was represented by obtaining the highest yield from embriogenic induction for the explants represented by zygotic embryos in stage 1 and its constant decrease in the embryos found in more and more advanced stages

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