STATISTICAL ANALYSE OF THE RESULTS CONCERNING THE EMBRYO INDUCTION EFFICIENCY AND THE OTHER SPECIFIC REACTIONS BY THE AID OF CORRELATION COEFFICIENTS IN THREE *QUERCUS* SPECIES

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Abstract: *The in vitro clonal propagation in oaks is an important step in the breeding activity using clonal strategies, and in the same time a tool for the application of somaclonal selection procedures in the improvement of adaptability traits. The use of somatic embryogenesis in oak clonal micropropagation is preferable in the applications concerning somaclonal variation and selection, because the plants regenerated from somatic embryos usually have monocellular origin and consist in clones of long-term culture, submitted of in vitro selection procedures. The somatic embryogenesis in oak, as an alternative to propagation by cuttings, provides the possibility of mass production of cotyledonary embryos, which can be either cryopreserved, or maintained by long-term culture as stable embryogenic somaclones. An experimental device was designed, composed of three oak species and 6 provenances (three of Quercus robur, one of Quercus petraea and two of Quercus frainetto), 4 types of explants (developmental stages of the zygotic embryo) and 4 variants of culture medium. The investigation of factors affecting the embryo induction efficiency, as well as other specific reactions of explants in culture (callogenesis, rhyzogenesis and germination) and of the relationship among these factors acting simultaneously. Plant material: As a genetical background, the plant material was represented by three oak species, in each of them one or more provenances being investigated: Quercus robur (three provenances), Q. petraea (one provenance) and Q. frainetto (two provenances). 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. Culture medium: Four media were tested for the induction of embryogenesis, representing combinations between two nutrient recipes and two combinations of growth regulators. Structure of the experiment: 3 oak species: Quercus robur, Quercus petraea, Quercus frainetto; 6 provenances: 3 of Quercus robur, one of Q. petraea and two of Q. frainetto; 4 developmental stages of explants (4 explant types): stages 1, 2, 3 and 4. Three replications consisting of five to ten explants have been counted for each parameter. The most important factor affecting the embryo induction efficiency was the developmental stage of the zygotic embryo used as explant. The best results were obtained with the youngest embryos (stage 1), with a linear decrement towards the stage 4. Inside the same developmental stage, important differences concerning the embryo induction efficiency were observed among species and also among the provenances of the same species. It was found that the most important factor affecting the somatic embryo induction efficiency was the developmental stage of the explant. In all oak species, the embryogenic ability decreased constantly from the youngest to the more advanced zygotic embryos used as explants. The same trend was recognized in the case of the provenances inside the species, regardless the culture medium used for the induction. Correlations coefficients for all 48 pairs of values were calculated, for the ten combinations of the characters, in all species and provenances. The significance of the results was done for P5% and P1%. The simple correlations studied showed that most characters are closely correlated. There are differences among provenances concerning the relationship between the pairs of characters. In Q. robur the provenience has an extremely evident effect. In Q. petraea, all of the correlations are highly significant between all the five characters, positives or negatives. Also in Q. frainetto, for the first provenance, the correlation is significant (for example the rhyzogenesis x the embryo induction efficiency) or highly significant for all the others characters. of characters.*

Key words: *Quercus, in vitro, embryo induction, correlations coefficients, somatic embryogenesis*

INTRODUCTION

The *in vitro* clonal propagation in oaks is an important step in the breeding activity using clonal strategies, and in the same time a tool for the application of somaclonal selection procedures in the improvement of adaptability traits.

The use of somatic embryogenesis in oak clonal micropropagation is preferable in the applications concerning somaclonal variation and selection, because the plants regenerated from somatic embryos usually have monocellular origin and consist in clones of long-term culture, submitted of *in vitro* selection procedures.

The somatic embryogenesis in oak, as an alternative to propagation by cuttings, provides the possibility of mass production of cotyledonary embryos, which can be either cryopreserved, or maintained by long-term culture as stable embryogenic somaclones (WILHELM et al., 1996, 2000).

An experimental device was designed, composed of three oak species and 6 provenances (three of *Quercus robur*, one of *Quercus petraea* and two of *Quercus frainetto*), 4 types of explants (developmental stages of the zygotic embryo) and 4 variants of culture medium (TIMOFTE, 2007).

The investigation of factors affecting the embryo induction efficiency, as well as other specific reactions of explants in culture (callogenesis, rhyzogenesis and germination) and of the relationship among these factors acting simultaneously.

MATERIALS AND METHODS

Plant material:

As a genetical background, the plant material was represented by three oak species, in each of them one or more provenances being investigated: *Quercus robur* (three provenances), *Q. petraea* (one provenance) and *Q. frainetto* (two provenances).

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).

Culture medium:

4 culture media (M1, M2, M3, M4), represented by:

2 variants of nutritive medium: ½ MS (MURASHIGE-SKOOG, 1962) and DCR.(DURZAN, GUPTA, 1987)

2 variants of growth regulator combinations, for each nutritive medium

Four media were tested for the induction of embryogenesis, representing combinations between two nutrient recipes and two combinations of growth regulators.

In order to establish the effect of culture medium upon the efficiency of somatic embryo induction, comparisons were made between nutritive media (QE1 + QE2, versus QE3 + QE4), on one hand, and between the two combinations of growth regulators (QE1 + QE3, versus QE2 + QE4), on the other hand.

Structure of the experiment:

3 oak species: *Quercus robur, Quercus petraea, Quercus frainetto*

6 provenances: 3 of *Quercus robur*, one of *Q. petraea* and two of *Q. frainetto.*

4 developmental stages of explants (4 explant types): stages 1, 2, 3 and 4

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

RESULTS AND DISCUSSIONS

Because some experimental data had values "0", the transformation $x' = \sqrt{x+1}$ was used, all calculations being performed with transformed values (Ardelean et al., 2005).

Correlations coefficients for all 48 pairs of values were calculated, for the ten combinations of the characters. The significance of the results was done for P5% and P1% (Table 1-6).

The most important factor affecting the embryo induction efficiency was the developmental stage of the zygotic embryo used as explant. The best results were obtained with the youngest embryos (stage 1), with a linear decrement towards the stage 4.

Inside the same developmental stage, important differences concerning the embryo induction efficiency were observed among species and also among the provenances of the same species.

Table 1

Simple correlation coefficients among the various characters studied in *Quercus robur* – provenance no. 1

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0.57**	0,24	-0.02	-0.26
The germination	-	-	0.48**	-0.47**	-0.65**
The rhyzogenesis	-	-	-	-0.22	-0.41**
The callogenesis	-	-	-	-	0,40**
The embryogenesis	-	-	-	-	-

r = 0,29 for P= 5%
r = 0,37 for P= 1%

Table 2

Simple correlation coefficients among the various characters studied in *Quercus robur* – provenance no. 2

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0,60**	0,38**	-0,41**	0,04
The germination	-	-	0,60**	-0,51**	-0,50**
The rhyzogenesis	-	-	-	-0,29*	-0,31*
The callogenesis	-	-	-	-	0,22
The embryogenesis	-	-	-	-	-

Table 3

Simple correlation coefficients among the various characters studied in *Quercus robur* – provenance no. 3

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0,21	0,18	-0,19	-0,06
The germination	-	-	0,06	-0,51**	-0,61**
The rhyzogenesis	-	-	-	-0,04	-0,13
The callogenesis	-	-	-	-	0,43**
The embryogenesis	-	-	-	-	-

Table 4

Simple correlation coefficients among the various characters studied in *Quercus petraea*

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0,63**	0,52**	-0,52**	-0,65**
The germination	-	-	0,42**	-0,68**	-0,82**
The rhyzogenesis	-	-	-	-0,39**	-0,38**
The callogenesis	-	-	-	-	0,64**
The embryogenesis	-	-	-	-	-

Table 5

Simple correlation coefficients among the various characters studied in *Quercus frainetto* – provenance no. 1

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0,56**	0,42**	-0,42**	-0,44**
The germination	-	-	0,59**	-0,57**	-0,45**
The rhyzogenesis	-	-	-	-0,37**	-0,34*
The callogenesis	-	-	-	-	0,57**
The embryogenesis	-	-	-	-	-

Table 6

Simple correlation coefficients among the various characters studied in *Quercus frainetto* – provenance no. 2

The character	The viability of explants	The germination	The rhyzogenesis	The callogenesis	The embryogenesis
The viability of explants	-	0,59**	0,33*	-0,47**	-0,59**
The germination	-	-	0,49**	-0,53**	-0,36*
The rhyzogenesis	-	-	-	-0,32*	-0,29*
The callogenesis	-	-	-	-	0,42**
The embryogenesis	-	-	-	-	-

It was found that the most important factor affecting the somatic embryo induction efficiency was the developmental stage of the explant. In all oak species, the embryogenic ability decreased constantly from the youngest to the more advanced zygotic embryos used as explants. The same trend was recognized in the case of the provenances inside the species, regardless the culture medium used for the induction

CONCLUSIONS

The simple correlations studied showed that most characters are closely correlated. There are differences among provenances concerning the relationship between the pairs of characters.

In *Q. robur* the provenience has an extremely evident effect.

In *Q. petraea*, all of the correlations are highly significant between all the five characters, positives or negatives.

Also in *Q. frainetto*, for the first provenance, the correlation is significant (for example the rhyzogenesis x the embryo induction efficiency) or highly significant for all the others characters.

BIBLIOGRAFY

1. ARDELEAN, M., R. SESTRAŞ AND MIRELA CORDEA (2005). Tehnica experimentală horticolă. Editura Academicpres, Cluj Napoca, pg.18.
2. DURZAN, D.J. AND P.K. GUPTA (1987). Somatic embryogenesis and polyembryogenesis in Douglassfir cell suspension cultures. Plant Sci. 52: 229-235.
3. MURASHIGE, T. AND F. SKOOG (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.
4. PALADA-NICOLAU, MAGDALENA AND J-F. HAUSMAN (2001). Comparison between somatic and zygotic embryo development in *Quercus robur* L. - Plant Biosystems, 135 (1) x-xx, 2001, pg. 1-9.
5. TIMOFTE, A.I. (2007). Cercetări privind variabilitatea somaclonală în embriogeneza somatică la stejar. Teză de doctorat, USAMV Cluj-Napoca.
6. WILHELM, E. (2000). Somatic embryogenesis in oak (*Quercus* spp.). *In Vitro* Cell Dev. Biol. Plant, 36:349-357.
7. WILHELM, E., A. BURG, M. BERENYI, M. ENDELMANN and R. RODLER (1996). Plantlet regeneration via somatic embryogenesis and investigations on Agrobacterium tumefaciens mediated transformation of oak (*Quercus robur*) - In: Ahuja et al. (Eds.), Somatic Cell Genetics and Molecular Genetics of Trees, Kluwer press: 125-130.
8. WILHELM, E., M. ENDEMANN; K. HRISTOFOROGULU, C. PREWEIN, M. TUTKOVA (2000). Somatic embryogenesis in oak (*Quercus robur* L.) and production of artificial seeds. In: Espinel, S.; Ritter, E. eds. Proceedings of application of biotechnology to forest genetics. Biofor 99. Vitoria-Gasteiz, Spain, 22-25 September 1999:213-225.