II Seminário de Corantes Naturais para Alimentos
I Simpósio Internacional de Urucum

THE TISSUE CULTURE RESEARCH ON URUCUM (*Bixa orellana*)

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INTRODUCTION

The main purpose of researching on "in vitro" culture is to perfect a new method of vegetative propagation. The advantages are reduction in the general heterogeneity found in the progeny produced by the conventional method (e.g. seed sowing), and above all, in the case of urucum, reduction in the fall in productivity of bixine compared to the mother-plant.

Considerable increase in productivity is therefore expected by using homogeneous plants with high individual qualities.

Direct organogenesis, indirect organogenesis and somatic embryogenesis are the three processes generally applied to achieve "in vitro" micropropagation. Although Organogenesis and Embryogenesis are fundamentally two different lines of research in this field, in time they might prove to be complementary with regard to the objectives of "in vitro" culture.

METHODOLOGIES INVOLVED

Direct Organogenesis: This is the simplest method of micropropagation and is generally utilized for obtaining clean material for further manipulations, or for medium-scale multiplication of desired clones/individuals of commercial interest. It involves basically five "in vitro" stages:

a) culture initiation (obtaining clean material),
b) shoot initiation,
c) plantlet multiplication,
d) rooting,
e) hardening off.

At each of these stages, the correct culture parameters have to be developed empirically for each species. This involves the correct nutrients, vitamins and plant growth regulators, as well as physical parameters. The advantage of this method of micropropagation is that the resultant plantlets are genetically identical to the mother material — and thus guarantees clonal integrity. Hence for urucum this is probably the approach of choice, in that clones/individuals known to produce high yields of bixine may be multiplied true to type.

Indirect Organogenesis: This methodology is somewhat more complex in that it involves a callus induction stage and this may introduce some genetic variation. However the considerable higher multiplication rates than those obtained using direct organogenesis, can make this method desirable to commercial concerns. In this procedure callus is initiated from tissue of the desired clone/individual. This process involves the dedifferentiation of the mother tissues using plant growth substances. The cells of the callus are then manipulated so that shoot formation is initiated. A large number of shoots can be produced from each portion of callus. The shoots are then isolated from the callus tissue and rooted in a separate pre-determined medium. Hardening follows as in the direct organogenesis.

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Somatic Embryogenesis: This technique is recommended for the production of plants on a very large scale, thus it is the most economical. It also has the advantage of inducing the full rejuvenation: i.e. going back to embryo stage means that young plants display identical morphology to those produced from seeds. This is very important in all perennial crops for a perfect architecture of the root system and thus for productivity too. This methodology however also involves a callus stage, as in the indirect organogenesis, and this may introduce unwanted variations. In this method rather than the formation of shoots and roots, the cells of the callus are manipulated so that embryos are created. These embryos are physically identical to the zygotic embryos of seeds. The high multiplication rates obtained from this procedure can be desirable in some cases, particularly if the passage through the callus stage is rapid enough to minimize genetic variation. Additionally, the screening of variants eventually developed from this procedure may, yield some highly desirable individuals. This procedure also has applications in the development of artificial seeds.

THE APPROACH USED FOR URUCUM:

Basically, both the organogenic and the embryogenic approach have been implemented as regards the investigations for a successful "in vitro" micropropagation of urucum.

A fair amount of encouraging results however have been obtained in the embryogenic methodology, whereby somatic embryos have been established. Once it is in the seed that bixine is commercially extracted from, seed coat had been initially chosen and proven as the most promising explant in this case. It has been realized though, that careful handling to dissect out the embryo and cotyledons must be done and only the seed coat used. This would ensure that the genetic identity of the mother-plant is maintained in the explant.

The callus initiates in the inner tegument of the seed coat, therefore that is the part to be put against the medium. MS salts + 2,4 D + low levels of kinetin are initially used to induce callus formation. After enough multiplication of the undifferentiated callus is achieved, 2,4 D is dropped to zero. Cytokinin and giberelic acid are now added to the culturing medium to initiate the callus differentiation process. The successful process of somatic embryogenesis is monitored by its typical sponge-like callus, which is formed of characteristic embryogenic cells that are microscopically examined. All this operation so far is basically carried out under dark incubation conditions. Given that time and all the other conditions are adequate, embryos are formed and germination is then carried out under the light.

So far, embryo formation has been successfully achieved, though at low frequencies. Experiments are under way to increase the frequency of successful embryo formation, as well as to improve the entire process to a shorter time. That is to say, the research is continuing on mastering the expression of somatic embryogenesis of urucum, so the complete differentiation of somatic embryos and their development into plantlets can be achieved under commercially acceptable principles.

AREAS OF INTEREST:

The callus stage of the "in vitro" micropropagation is also used in the initiation of cell suspension culture (which gives the highest multiplication rates) and in batch cultures.

In the cell suspension culture technique, the callus is broken up into its constituent cells by physical rotation. Theoretically, each cell in the suspension has the potential to develop into an individual plant with the same characteristics of the mother-plant. After multiplication of

the cell numbers in the liquid suspension, the individual cells are cultured on solid media to produce somatic embryos and subsequently plantlets. The development of this technology is expensive and time-consuming. Ultimately, it may probably not be the most practical technique for the specific case of urucum.

However, this may not be the case of using the callus stage to progress into batch cultures. Specially by using the seed coat as the explant, the callus formed from the mother-plant is induced to produce a desirable product (such as bixine) rather than plants. The callus is then cultured in large volumes (usually as a suspension) and the desired product either extracted directly from the growing medium or from the callus itself.

Although the development of such bio-reactors is expensive and time consuming, this technique may in the end prove to be well worthwhile, once it will totally replace the agricultural phase of the operation.

This technology however, is still in its infancy and perhaps may be beyond the scope of most developing countries, where the cultivation of urucum should predominate for many years to come.

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