

ARTICULO BREVE

Establishment of morphogenic rice cell suspension cultures (*Oryza sativa*) in Costa Rica

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Resumen: Se establecieron cultivos morfogénicos de células en suspensión de arroz del cultivar costarricense CR-5272 (*Oryza sativa*) a partir de callos de siete semanas. Estos callos se obtuvieron del escutelo de embriones maduros, cultivados en un medio de Murashige y Skoog modificado, suplementado con 2,4-D. Las suspensiones se subcultivaron semanalmente por más de dos meses y se tamizaron cada cuatro semanas. Se obtuvieron doce plántulas luego de transferir los microcallos a un medio de regeneración MS de base suplementado con BAP y ANA. Dichas plántulas se transfirieron al invernadero y serán evaluadas próximamente en términos de fertilidad.

Key words: *Oryza sativa*, *indica* rice, *in vitro* culture, cell suspension, plant regeneration.

Abbreviations: 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine; NAA: naphthalene acetic acid.

The optimization of the *in vitro* culture of cereals forms the basis for the genetic transformation of rice. The establishment of embryogenic cell suspension cultures is essential for the development of more advanced methods of genetic engineering, such as genetic transformation of plant cells and somatic hybridization through protoplast fusion. These biotechnological tools can complement conventional plant breeding, and hence benefit agriculture (Vasil 1990).

Although there are several reports concerning the regeneration of plants from protoplasts isolated from primary callus (Coulibaly & Demarly 1986, Kyojuka *et al.* 1987, Lee *et al.* 1989), the greatest success has been achieved with cell suspension cultures (see Hodges *et al.* 1991). Despite the large number of *indica* rice genotypes that produce morphogenic primary callus, only a few genotypes (IR-54 and Chin-

surá Boro-II) generate embryogenic cell suspension cultures from which protoplasts can be isolated that regenerate into fertile plants (see for example Lee *et al.* 1989, Datta *et al.* 1990).

The objective of the present work was to develop experimental conditions for the establishment of embryogenic rice (*Oryza sativa* L.) cell suspension cultures from the Costa Rican *indica* cultivar CR-5272.

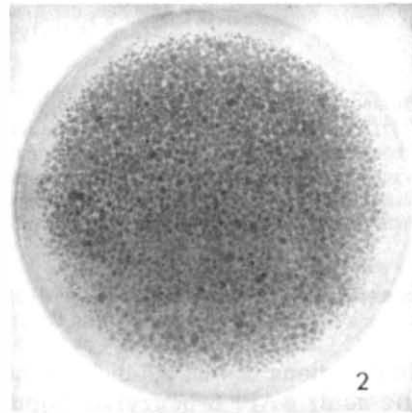
Seeds of the cultivar CR-5272 were obtained from the Rice Office of Costa Rica. Between 200 and 300 were used to initiate callus cultures in each experiment. Methods for disinfecting explants and for inducing calluses were similar to those established by Valdez *et al.* (in this same volume). The callus derived from the scutellum were maintained for seven weeks in MSC callogenesis medium (Murashige & Skoog 1962), supplemented with 2.5 mgL⁻¹ of 2,4-D. Small (1.0-1.5 g fresh weight) fragments of embryogenic callus were placed in 250 ml Erlenmeyer flasks containing 100 ml liquid MSC medium. The cultures were placed on an orbital shaker (Lab Line Instruments) at a velo-

city of 90 rpm in a dark growth room at 25°C. They were subcultured weekly, sedimenting the cells at the bottom of the flask and replacing half of the liquid volume with fresh medium.

After four weeks, the cellular aggregates, consisting of microcalli of about 20-50 cells, were filtered through gauze (SIGMA, 40 mesh) to eliminate excessively large calluses and retain those of smaller size. The cultures were subcultured and filtered on this schedule for two more months. The morphogenic capacity of these suspensions was investigated by inoculating 1 ml of suspension on a petri dish (100 x 15 mm) containing 40 ml of semi-solid callogenesis MSC medium, supplemented with 0.3% Phytigel™ (w/v). These microcalli grew and produced large undifferentiated calli (about 2 cm diameter) after a month of culture, which were ultimately transferred to MSR regeneration medium (Murashige & Skoog 1962) supplemented with 0.5 mgL⁻¹ BAP and 0.05 mgL⁻¹ ANA, to test their ability for plant regeneration. When the regenerated plantlets reached 10 - 15 cm in height, they were transferred to plastic pots, protected with plastic bags to conserve the humidity levels and allowed to acclimatize to greenhouse conditions.

Cell suspension cultures have to be handled with care, because of the frequent contamination problems encountered when working with aseptic techniques and liquid media. In three independent callogenesis experiments a total of 22 cultures were started (Fig. 1), of which only four survived after two months of subculturing (Fig. 2). These cell lines are being multiplied to produce a large number of embryogenic cellular cultures which can be used to isolate and manipulate protoplasts. Table 1 shows the results obtained in the establishment of rice cell lines, as well as the number of plants regenerated in each experiment. A total of 12 plants could be regenerated (Fig. 3) after two months of culture of the calli on regeneration medium. These are currently being grown to maturity, to evaluate their fertility and morphological integrity.

Monocots are in general recalcitrant to *in vitro* culture methods, so their morphogenic ability is very low and dependent on the genotype. *Indica* rice varieties display lower rates of callus induction and plant regeneration when compared with *japonica* cultivars (Abe & Fut-



Figs. 1-3. Morphogenic rice cell suspension cultures of the Costa Rican rice cultivar CR-5272. 1- Flask containing a cell suspension culture. 2. Bottom of a flask containing an established cell suspension. 3. Regenerated plant from cell suspension cultures.

TABLE I

Callogenesis experiments with mature embryos of indica rice CR-5272 for the establishment of cell suspension cultures

N° Exper	N° embryos	N° Erlens	Results	N° regenerated plants
4113	300	17	11 contaminated flasks 4 flasks with necrosed calli 2 flasks with small cell aggregates	— — 5
4288	200	2	1 flask with microcalli 1 flask with small cell aggregates	— 6
4296	200	3	1 contaminated flask 1 flask with necrosed calli 1 (line 4296-01) flask with fast growing small cell aggregates	— — 1

suhara 1986). This can explain the low rate of plant regeneration obtained in our experiments. Consequently, more experiments are being carried out to develop a reliable protocol for the establishment of embryogenic rice cell suspensions. These involve the use of amino acids and also maltose instead of sucrose as a source of carbohydrates.

The establishment of cell suspension cultures of rice constitutes a great technological advance. It underpins the use of more sophisticated technologies of genetic manipulation, such as the culture and transformation of protoplasts, which could lead to the production of rice cultivars resistant to diseases, such as that caused by the rice hoja blanca virus, which is one of the most important diseases currently affecting rice cultivation in Costa Rica.

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