

***In Vitro* ANTHHER CULTURE OF RICE HYBRIDS**

Noraida Pérez[✉], Elena Admetlla and M. Aguilar

ABSTRACT. In the Laboratory of Rice and Maize Breeding, belonging to CIFA "Las Torres", Seville, Spain, and sponsored by an investigation project, a rice breeding program began to be developed, including the anther culture of hybrids in its objectives. For this purpose, different media were evaluated for callus induction and green plant regeneration as well as F_1 hybrids originated from 15 crossings. Results showed that the highest percentages of calluses and green plants as a continuous process were obtained by N_{6m} and MS media respectively. The best response to callus formation was obtained by Puntal/Thaibonnet crossing and Lemont/Newbonnet and Doñana/V7876 green plant regeneration.

Key words: rice, anther culture, plant breeding

INTRODUCTION

Rice is one of the most important cereals cultivated in the world, as it provides food for more than two billion persons (1). In Spain, this crop occupies about 100 000 ha mainly in Seville, Valencia, Tarragona and Badajoz, Andalusian rice representing around 40 % of the complete growing area (2); however, most varieties come from the introduction and selection of foreign materials.

Since 1968, rice is considered the first cereal managing *in vitro* regeneration, so that plantlets started to appear from undifferentiated somatic cells of japonica seeds; since then, several tissues have been used to induce callus formation and further plant differentiation (3).

By the rise of these techniques, phenotypically and genotypically uniform plants were obtained, arising a wide perspective to plant breeding for developing better varieties in a short time (4).

In this sense and as a consequence of a research project, a program for obtaining varieties was initiated, with the purpose of using hybrid anther culture, whose results are presented in this paper.

Noraida Pérez, Investigador Auxiliar de la Estación Experimental del Arroz "Los Palacios, Instituto Nacional de Ciencias Agrícolas, Gaveta Postal 1, San José de las Lajas, La Habana, Cuba, CP 32 700; Lic. Elena Admetlla y Dr. M. Aguilar, Investigadores del Departamento de Arroz y Maíz del Centro de Investigación y Formación Agraria "Las Torres Tomejil", Alcalá del Río, Sevilla, España.

✉ nory@inca.edu.cu

RESUMEN. En el Laboratorio de Mejoramiento de Arroz y Maíz del CIFA "Las Torres", en Sevilla, España, auspiciado por un proyecto de investigación, comenzó a desarrollarse un programa de obtención de variedades, que incluía en sus objetivos la utilización del cultivo de anteras de híbridos. Para ello se evaluaron diferentes medios para la formación de callos y regeneración de plantas verdes así como anteras de híbridos F_1 , provenientes de 15 cruzamientos. Los resultados mostraron que los más altos porcentajes de callos y plantas verdes como proceso continuo fueron obtenidos con los medios N_{6m} y MS respectivamente. La mejor respuesta a la formación de callos fue obtenida por el cruce Puntal/Thaibonnet y regeneración de plantas verdes Doñana/V7876 y Lemont/Newbonnet.

Palabras clave: arroz, cultivo de anteras, fitomejoramiento

MATERIALS AND METHODS

At the Laboratory of Rice and Maize Breeding from CIFA "Las Torres", Seville, Spain, F_1 hybrid anthers from 15 crossings were *in vitro* cultivated after being submitted to a temperature treatment between 8 and 10°C for seven to eight days. The liquid media applied to callus induction from immature pollen were N_{6-1} , recommended by Li Mei Fang and used by others (5), N_{6-m} and NL, recommended by CIAT (International Center of Tropical Agriculture), Colombia (6).

Seeding and disinfection followed CIAT's methodology (6) and the cultures were kept away from light at a temperature of $26\pm 1^\circ\text{C}$ for four to six weeks; once calluses reached 1 to 2 mm high, they were transferred to a MS regenerative medium containing Murashige & Skoog salts, vitamins and growth regulators (AND and kinetine), except NL-formed calluses, which were transferred to $\frac{1}{2}$ MS, containing half of MS inorganic salts but $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ that is fully added when using Phytigel as a solidifying compound. Incubation temperature was kept at $26\pm 1^\circ\text{C}$ during a 16-hour-light photoperiod.

Percentages of callus formation and plant regeneration were determined and processed by comparing proportions and means through Duncan's Multiple Range Test at 5 %.

When plantlets reached a full leaf and root development, they were taken out from the flask, besides removing residues of calluses and culture media. They were later immersed in water for two days and then transplanted to trays to be kept under semicontrolled conditions at a temperature between 28 and 30°C, directly protected from the sun to avoid dehydration.

After eight days, they were fertilized by a complete formula containing nitrogen, phosphorus and potassium. Two weeks later, they were transferred to the farm along with Doñana parent and submitted to an appropriate rice crop management.

RESULTS AND DISCUSSION

Table I presents anther culture response in F₂ hybrids by a high percentage of calluses obtained for some hybrid combinations. Similar results were achieved by other researchers (5) when using this breeding technique; thus, callus formation appeared in 15.6 to 24.1 % of anthers seeded whereas for others (7) the response was even lower, just 1.7 to 7.7 calluses per 100 anthers.

Table I. Callus formation

Crossings	Calluses					
	N _{6m}		N ₆₋₁		NL	
	P	ESx	P	ESx	P	ESx
Bahía/Puntal	0.00	0.01 i			0.00	0.04 g
Doñana/Estrela A	0.01	0.01 i	0.02	0.01 d	0.10	0.01 ef
Doñana/V 7876	0.09	0.01 ef	0.06	0.01 bc	0.07	0.00 g
Estrela A/Doñana	0.14	0.01 c				
Estrela A/Puntal	0.12	0.00 cd	0.04	0.00 cd	0.07	0.02 fg
Lemont/Bahía	0.17	0.01 b	0.07	0.01 b	0.12	0.02 def
Lemont/Newbonnet	0.19	0.01 b	0.18	0.02 a	0.13	0.02 cde
Lemont/Puntal	0.12	0.01 cde	0.16	0.01 a	0.31	0.01 b
Newbonnet/Doñana	0.03	0.00 h				
Puntal/Doñana	0.23	0.01 a			0.16	0.02 cd
Puntal/Newbonnet	0.07	0.01 fg	0.09	0.01 b	0.15	0.01 cd
Puntal/Thaibonnet	0.10	0.01 de	0.06	0.02 bc	0.50	0.02 a
Thaibonnet/Estrela A	0.11	0.01 cde				
Thaibonnet/Puntal	0.06	0.01 g	0.07	0.01 b	0.19	0.02 c
V 7876/Estrela A	0.07	0.01 fg	0.06	0.01 bc	0.00	0.01 g
F	74.17***		15.14***		88.00***	

Moreover, the table shows up the differentiated response of hybrid material by means of anther culture technique, Puntal/Thaibonnet crossing being the best in NL medium, followed by Lemont/Puntal in the same medium and Puntal/Doñana in N_{6m}.

In studies conducted with japonica and indica varieties, it was found that callus formation has a variable frequency and the ability for vigorously-developed calluses, which is better appreciated in japonica lines than in indica ones (8).

The highest percentages of callus formation were obtained when using NL medium and indica hybrid combinations (50.2 calluses/100 anthers to Puntal/Thaibonnet and 31.0 to Lemont/Puntal) whereas N_{6m} medium, although less efficient to form calluses, was effective in seven hybrid combinations.

N₆ medium has been widely adapted to japonica rice anther culture but the modification to N₆ containing half the ammonium, twice the level of phosphorus and 1/5 magnesium (N_{6m}) increases indica genotype callus induction frequency; this kind of rice is highly susceptible to ammonium and magnesium. Subsequent modification to N_{6m} (NL) increased callus induction three times (6).

Table II shows green plant regeneration in MS medium with calluses from N_{6m} and N₆₋₁ whereas ½ MS with calluses from NL. It also presents that the best results, despite low percentages, were recorded by MS medium with calluses from N_{6m}, achieving 11-cross green plants and six albino plants.

No green plants were regenerated in calluses from N₆₋₁, just those calluses from three crossings induced in NL.

In this regard, literature states that folded haploid production by anther culture is even higher in japonicas than in indicas, not only due to a better response of japonicas but also to the fact that most laboratories have used them as research patterns; unlike the results of this study, they got a substantially higher indica response by NL and ½MS media (9).

Albinism is a widely stated phenomenon in the rice anther culture of indica, which restricts the use of this breeding technique (10); however, it was not an important handicap in this study and the best green plant regeneration was recorded by Doñana/V7876 crossing, followed by Lemont/Newfonnet.

Table II. Green plant regeneration per every 100 anthers seeded in different media

Crossings	Regenerated plants									
	Green				Albino					
	N _{6m}		NL		N _{6m}		N ₆₋₁		NL	
	P	ES	P	ES	P	ES	P	ES	P	ES
Bahía/Puntal	0.00	0.12 c	0.00	0.02	0.00	0.02	-	-	0.00	0.01
Doñana/Estrela A	0.00	0.06 c	0.00	0.02	0.00	0.01	0.00	0.02	0.00	0.01
Doñana/V 7876	0.55	0.04 a	0.04	0.01	0.02	0.00	0.00	0.01	0.00	0.00
Estrela A/Doñana	0.01	0.03 c	-	-	0.00	0.00	-	-	-	-
Estrela A/Puntal	0.01	0.03 c	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.01
Lemont/Bahía	0.12	0.02 c	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00
Lemont/Newbonnet	0.33	0.03 b	0.02	0.01	0.00	0.01	0.00	0.01	0.00	0.01
Lemont/Puntal	0.26	0.02 bc	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
Newbonnet/Doñana	0.00	0.01 c	-	-	0.00	0.02	-	-	-	-
Puntal/Doñana	0.14	0.02 c	0.01	0.02	0.00	0.01	-	-	0.01	0.00
Puntal/Newbonnet	0.04	0.11 c	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01
Puntal/Thaibonnet	0.01	0.02 c	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.00
Thaibonnet/Estrela A	0.01	0.02 c	-	-	0.01	0.00	-	-	-	-
Thaibonnet/Puntal	0.06	0.03 c	0.00	0.01	0.00	0.01	0.03	0.01	0.00	0.00
V 7876/Estrela A	0.00	0.02 c	-	-	0.00	0.01	0.00	0.02	-	-
F	85.78***		3.68 NS		1.66 NS		1.15 NS		0.91 N	

Evidently, N_{6m} to callus formation and further green plant regeneration in MS were the best for *in vitro* cultured F_2 hybrids in this trial as well as the conditions for this technique; nevertheless, this study should be deepened to obtain plants more efficiently, which constitute homocigous lines, as a result of applying anther culture technique, that is considered the essential advantage of this method when the breeding cycle is shortened.

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