

Full Length Research Paper

In Vitro Regeneration of Nigerian Rice Varieties: Callus Culture from Caryopsis of Suakoko 8 and FARO 55

Abolade S. Afolabi^{1*}, O. Oyebanji¹, O. Odusanya¹, M. E. Abo², M. Misra³ and G. H. Ogbadu¹

¹Biotechnology Advanced Laboratory, Sheda Science and Technology Complex, PMB 186, Garki, Abuja FCT, Nigeria.

²National Cereal Research Institute, Badegi, Bida, Niger State, Nigeria.

³M. Misra, Institute of Frontier Sciences and Biotechnology, Baramunda, Bhubaneswar-751003, India.

Accepted 4 October, 2024

The regeneration protocol for rice plants from callus culture obtained from dehusked and sterilized whole rice seeds (caryopses) of a popular Nigerian local cultivar Suakoko 8, and a NERICA cultivar FARO 55 is described. Utilizing a modified Nippon Barre medium (NBm) consisting of: Macro N6, Micro B5, Fe-EDTA, B5 vitamin and 30 mg/l of locally produced St Louis® sugar (to save cost) supplemented with casein hydrolysate and proline, and with cytokinin and auxin hormones (ratio 1:10), profuse and friable calli were obtained at 25°C in the darkness. The friable calli produced were transferred to fresh medium once every week for three weeks before transferring them to pre-regeneration medium containing ABA and NAA (replacing 2-4,D in the callus induction medium), and incubated for 9 days in the darkness at 25°C. Finally the creamy calli were transferred into regeneration medium containing high cytokinin (3 mg l⁻¹ BAP) with incubation at 28°C and 12 h – photoperiod. Regeneration of plantlets was obtained between two to four weeks of transfer on the regeneration medium. The regeneration frequency (efficiency) of 53 and 42% were obtained for Suakoko 8 and FARO 55. Regenerated plants also produced viable seeds and hence fertile plants.

Key words: Embryogenic calli, regeneration, Suakoko 8, FARO 55, new rice for Africa (NERICA), *Oryza sativa*, *Oryza glaberrima*

INTRODUCTION

Rice genetic transformation technologies appear to hold great promise for increasing rice productivity, especially in areas where conventional breeding lacks solution and farmers have little means to counter damage caused by pests and disease. Since the mid 1990s, *Agrobacterium*-mediated transformation of rice and other major cereals like maize, wheat and barley have been achieved (Kathuria et al., 2007; Ishida et al., 1996; Aldemita and Hodges, 1996). But, improvement of rice production in Nigeria, using marker-free transformation biotechnology, has been significantly impaired due to lack of a suitable regeneration protocol for the locally preferred rice cultivars.

In Nigeria, one of the locally preferred cultivars Suakoko 8, has excellent agronomic characteristics, but is

Abbreviations: ABA- abscisic acid, BAP-Benzylaminopurine, 2, 4-D - 2, 4-dichloroacetic acid, ENU- embryogenic nodular units, NAA- naphthalene-acetic acid, NBm – Nippon Barre medium, NERICA- new rice for Africa, CIF: callus induction frequency; RF: regeneration frequency, RN: regeneration medium, MS: Murashige and Skoog medium, FARO: Federal Agricultural Research Oryza (FARO).

highly susceptible to rice blast fungus and other biotic and abiotic stresses. *Agrobacterium* mediated transformation of cv. Suakoko 8 with useful genes, including the chitinases and glucanases genes, could have solved this problem, if it has not been recalcitrant to regenerate *in vitro*.

FARO 55, one of the new rice cultivars for Africa (NERICA), an interspecific hybrid between *Oryza glaberrima* Steud. and *Oryza sativa* L., is also a favourite elite variety. While it possesses some good agronomic characteristics, pyramiding of useful traits using marker-free

*Corresponding author. E-mail: abolade.afolabi@gmail.com, aboladeafolabi@yahoo.co.uk.

Table 1. Experimental profile of Suakoko 8 and FARO 55 rice varieties: CIM = callus induction medium, PRM = pre-regeneration medium, RM = regeneration medium.

Rice Variety	No of Seeds on CIM (A)	No of callus obtained (B)	No of callus transferred to PRM (C)	No of callus transferred to RM (D)		Callus Induction Frequency (CIF) B/A*100	Regeneration frequency (RF) (E/B*100)
Suakoko 8	25	23	23	17	16	92%	53%
FARO 55 (NERICA)	25	24	24	18	10	96%	42%

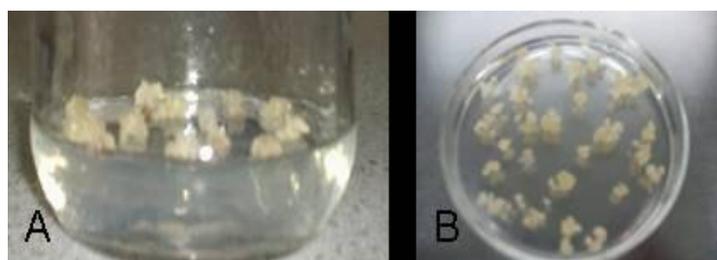


Figure 1. Calli of (A) Suakoko 8 and (B) FARO 55 (NERICA) rice varieties on callus induction medium

transformation biotechnology would also be extremely useful. However, in general, *in vitro* regeneration is also a prerequisite for marker-free transformation with useful genes.

To solve these biotic and abiotic problems using transformation biotechnology in Nigeria, it is crucial therefore, to formulate and optimize a simple and robust regeneration technique for both Suakoko 8 and FARO 55 rice cultivars. The ultimate goal, however, is to transform these preferred varieties with useful genes for abiotic and biotic stresses using the available marker-free technology.

MATERIALS AND METHODS

Dehusked seeds (caryopses) of both cv. Suakoko 8 and cv. FARO 55 (a NERICA variety) were surface sterilized first with 90% Ethanol for 1 min and rinsed with sterile distilled water followed by immersion of the caryopses in commercial bleach (-JIK® Reckitt Benckiser™ Nigeria Ltd - 3.5% m/v sodium hypochlorite) for 30 min and rinsed three times with sterile distilled water.

The sterilized caryopses were plated onto modified Nippon Barre medium (NBm of Vain et al., 2002) with N6 macro-element, B5 microelements, Fe-EDTA, 30 g l⁻¹ locally available sugar source (St. Louis sugar®) in lieu of sucrose and 2 mg l⁻¹ 2, 4-dichloro-roxyacetic acid (2, 4-D). The basal medium was supplemented with 300 mg l⁻¹ casein hydrolysate, 500 mg l⁻¹ l-glutamine, 500 mg l⁻¹ l-proline with 2.5 g l⁻¹ Phytigel as solidifying agent and pH adjusted to 5.8 with 0.5 M KOH. Filter-sterilized vitamins B5 were added to autoclaved media. Callus induced in this medium within 2 weeks at 25°C in the darkness was transferred into fresh medium every seven days. Friable calli of about 1 mm diameter, were separated by rolling them onto the gelling agent, each time the callus transfer took place. The calli were cultured for an additional 7 days onto fresh NB

medium to produce embryogenic nodular units (ENU) (Bec et al., 1998).

These calli were transferred to pre-regeneration medium (PR) (NB medium containing 2 mg⁻¹ benzylaminopurine (BAP), 1 mg l⁻¹ naphthalene acetic acid (NAA), 5 mg l⁻¹ abscisic acid (ABA), but without 2,4-D. The plates were incubated in the darkness at 28°C for 9 days.

After which the cream colored calli, were transferred to regeneration medium RN (NB medium without 2, 4-D but with 3 mg l⁻¹ BAP, 0.5 mg l⁻¹ NAA) incubated in the light (Light was supplied by the Plant Germinator Incubator Model HOT-COLD®- 4000700 with six fluorescent 40 W tube lights fixed at 25 cm lateral to the culture plates (Lamps are situated at the inside side of the incubator door and are lateral to the incubated calli and plantlets). The day/night timer was set for 12 h / 7 days (12 h photoperiod) at 28°C for 2–3 weeks. After 10–14 days, shoots began to emerge from the greenish spots on the calli. These developed into plantlets within 6–10 days.

The plantlets were transferred to hormone-free MS based medium MSR6 (Vain et al., 2002) for 2–4 weeks at 28°C in the light (as indicated above- 12 h photoperiod) before transferring the young plants into pots containing soil for growth to maturity.

RESULT

Profuse callus formation was obtained in both Suakoko 8 and FARO 55 (NERICA) rice varieties with the modified callus induction medium (NB) supplemented with 500 mg/L proline, glutamine and 2, 4-D (Figure 1). The callus induction frequency (CIF) - calculated as “Number of calli / Number of inoculated whole seed” x100% (Table 1) was 92 and 96% for cv. Suakoko 8 and cv. FARO 55 (NERICA), respectively.

The calli turned cream coloured within 9 days of transfer into the pre-regeneration medium (Figure 2). Shoots

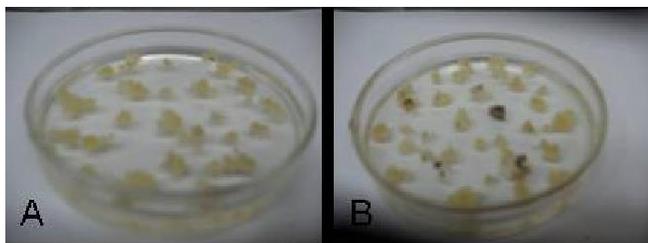


Figure 2. Calli of (A) Suakoko 8 and (B) FARO 55 (NERICA) rice varieties on pre-regeneration medium (PR)

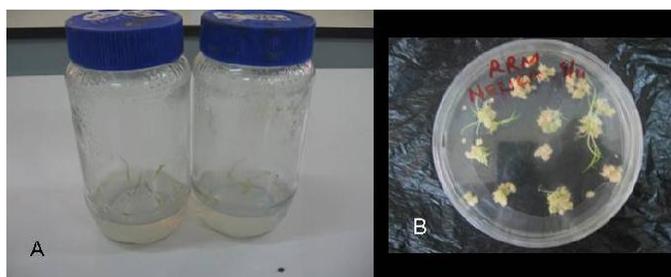


Figure 3. Plantlets of (A) Suakoko 8 and (B) FARO 55 (NERICA) rice varieties on regeneration medium



Figure 4 (b) . Suakoko 8 (Nigerian local) Amplified to show seeds setting (hence fertile).



Figure 4 (a). Suakoko 8 (Nigerian local) seeds setting shown by arrow



Figure 5. FARO 55 (NERICA) rice varieties in pots - with seeds (arrowed).

appeared from these calli (Figure 3) 9 - 12 days after transferring them to regeneration medium. The shoots became plantlets, which were later transferred into pots in the net – house and gradually shifted to the open field conditions. These later grew into healthy plants with profuse tillering and did produce viable seeds (hence the plants are fertile) within 3 months (Figures 4a, 4b and 5). Regeneration frequency (RF), calculated as “Number of regenerated plantlets/Number of calli inoculated on regeneration medium”, was 53 and 42% for Suakoko 8 and NERICA, respectively.

DISCUSSION

The regeneration of complete plants via tissue culture is a necessary requirement in the production of novel varieties of plants using transformation methodology. Without this, it is virtually impossible to introduce foreign genes into plant cells and recover transgenic plants.

Morphogenesis could occur directly from the explants or indirectly via the formation of a dedifferentiated callus. Until recently, it is generally not easy to culture and regenerate monocot plants, including agronomically important

crops such as rice, wheat, and maize. In rice, an efficient culture system using mature seeds has been established with model varieties such as Nipponbare (*Japonica*) and Kasalath (*Indica*). However, many leading varieties used for food production, such as Koshihikari in Japan, IR64 in tropical countries (including Suakoko in Nigeria), are highly recalcitrant to regeneration especially in the mature seed culture system, resulting in a serious obstacle to efficiently improve them through transgenic biotechnology (Yu and Pauls, 1993; Takeuchi et al., 2001). In this paper we have successfully developed a protocol for regeneration of callus derived from caryopses with a frequency as high as 53% for cv. Suakoko 8 and 42% for the NE-RICA cv. FARRO 55. The regenerated plants also set viable seeds. Hence fertile plants could be generated and established in open field conditions through this protocol. This is the first step towards the initiation of Nigerian local rice germplasm improvement through genetic manipulation.

ACKNOWLEDGEMENT

We thank Mr. Akinwale Adewumi for the photographs in this work.

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