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Optimization of Plant Growth Regulators in Indirect Plant Regeneration in Rice Variety Nerica 3 Using Mature Seeds as Explant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study is the first attempt to highlight about callus induction and plantlet regeneration in rice variety 'Nerica 3' using mature seed as explant and using five regeneration media with plant growth regulators (BA, IAA, and 2,4-D) at different concentrations in other to establish the efficient indirect plant regeneration protocol. Among the five regeneration media tested, R3 medium (2 mg/L BA), showed greater percentage of plantlet regeneration (45.31%) followed by R2 medium (0.5 mg/L BA + 0,1 mg/L IAA; 29.68%), whereas, R4 medium showed the lowest (2 mg/L 2,4-D; 0%). These results will be used for genetic improvement programs of the variety 'Nerica 3'.

Keywords: Rice variety 'Nerica 3'; mature seed; plantlet regeneration; Plant growth regulators.

1. INTRODUCTION

In vitro tissue culture is a crucial technique in rice (*Oryza sativa* L.) research and breeding programs [1,2]. It involves the cultivation of rice cells, tissues, or organs under sterile and controlled environmental conditions. This technique is widely used for various purposes, including genetic transformation, breeding, conservation of germplasm, pathogen-free plant production, and research and functional genomics [2,3]. However, *in vitro* tissue culture faces many challenges including contamination, genetic variability and optimized protocol.

In rice, callus induction and plantlet regeneration (indirect regeneration system) are essential techniques in plant biotechnology and breeding [4-7]. The success of these techniques depends on optimizing various factors, including environmental conditions, the type of explant and culture media composition [8]. Among the elements which constitute the culture media, we find exogeneous plant growth regulators. In fact, plantlet regeneration in rice using callus culture involves the exogenous application of plant growth regulators to facilitate various stages of plant development [9] especially that it was noticed that the quantity of callus produced from explants did not positively correlate with the subsequent shoot induction [10]. Plant growth regulators, also known as plant hormones, play a crucial role in tissue culture by influencing cell division, elongation, and differentiation [11]. Their functions can be summarized as follows: i) auxins (IAA, NAA, 2,4-D, etc), are pivotal in the initiation and proliferation of callus, as well as in root formation; ii) cytokinins (BAP, Kinetin, TDZ, etc), are critical for shoot induction and proliferation; iii) others plant growth regulators (Gas, ABA, etc) can be used to enhance shoot elongation and overall plantlet growth [12].

The objective of this study was to define plant growth regulators suitable for plantlet regeneration of rice variety 'Nerica 3' using callus from mature seed as explants. However, studies on *In vitro* plantlet regeneration in rice using mature seed as explant are lacking although mature seed has the advantage of being available throughout the year, easy to handle and in bulk quantities.

2. MATERIALS AND METHODS

2.1 Plant Materials and Explants Preparation

Field grown seeds (matured caryopses) of rice variety 'Nerica 3' [13] were used as the source for mature seed culture. The seeds were procured from Garoua Multipurpose Research Station of Institute of Agricultural Research for Development (IRAD), Sanguéré - Paul, Cameroon.

The seeds were then surface sterilized by washing in ethanol 70% (v/v) for 3 minutes, followed by a bath of 100% commercial bleach plus a drop of Tween 20 for 30 minutes with agitation. Thereafter, they were rinsed four times in sterile distilled water [13].

2.2 Callus Induction and Maintenance

The disinfected seeds were aseptically placed in Petri dishes containing the induction medium based on MS basal medium with vitamins [14], sucrose (3%), agar (0,8%) and 2,4-D (2 mg/L). The embryos were incubated on the medium at 27°C for six weeks in the dark [9] to obtain embryogenic callus. Subculture was performed at 21 days at the same conditions, but the induction medium was modified by adding BA (1 mg/L).

2.3 Plantlet Regeneration

After six weeks, embryogenic calli were transferred to five regeneration media (R1 to R5)

based on MS medium with vitamins, sucrose (3%), agar (0,8%) and which differed respect to concentrations of plant hormones: R1 (without hormones; [15]), R2 (0.5 mg/L BA + 0.1 mg/L IAA; [16]), R3 (2 mg/L BA; [17]), R4 (2 mg/L 2,4-D; [18]) and R5 (2 mg/L IAA; [19]). Embryogenic calli were then incubated in the light (16 h per day) at 27°C. The regeneration rate was calculated eight weeks after transfer of the callus. Percentage of plants regeneration was calculated as follows: (the number of calli which regenerated plantlets / the number of calli transferred to the regeneration medium) × 100 [19].

2.4 Experimental Design and Statistical Analysis

A randomized complete block design (RCBD) was used with one variety and 5 regeneration media. The treatments consisted of 10 replications of each medium, each replication with 10 embryogenic calli. For the analysis of the regeneration rate, Analysis of Variance (ANOVA) was performed using R version 4.1.3 software. ANOVA results were considered significant at $P < 0.05$ and means of treatments were compared using Tukey's HSD test.

3. RESULTS AND DISCUSSION

Callus initiation started four days after incubation of mature seeds as explants. At the end of the callogenesis, six weeks after incubation of the explants, the percentage of callus induction was 90%. At this stage, calli were characterized by a friable, granulated, and smooth texture and a whitish to cream colour (Fig. 1).

Calli were then transferred to the regeneration medium. After 8 weeks of the culturing, the plantlets regeneration (Fig. 1) was recorded (Table 1). The regeneration media used had a significant effect on plantlets regeneration (F value = 8.93 at P< 0.001). Among the five regeneration media tested, R3 medium, showed greater percentage of plantlet regeneration (45.31%) followed by R2 medium (29.68%), whereas, R4 medium showed the lowest (0%; Table 1).

Our results clearly showed that plantlet regeneration was strongly influenced by the type of plant growth regulators in the medium and confirm many other studies [8,9,17,20]. R3 medium (2 mg/L BA) yielded the highest plantlet regeneration followed by R2 medium (0.5 mg/L BA + 0.1 mg/L IAA). These results indicate that plantlet regeneration improved by the presence of BA which is a cytokinin. In fact, cytokinin affects competent cells in the shoot-regeneration process, leading to cell-mass generation and cell-fate transformation. Cytokinin can induce adventitious shoots alone and cooperates with auxin to reinforce proliferation in chosen cells" [21].

Increasing concentration of BA from 0.5 mg/L to 2 mg/L in regeneration media (as in the case of R2 and R3 respectively) increased the rate of plantlets produced. This result agreed with the findings of Noor et al. [20] which suggested that increase in concentration of BA increased regeneration frequency of callus from mature seed of Himalayan rice genotype SR-4 as explant.

On the other hand, R4 medium containing auxin 2,4-D (2 mg/L), did not induce regeneration. Auxin is the most important determinant of somatic embryogenesis for many species in tissue culture. Exogenous auxin promotes callus formation from cultured materials by inducing the production of endogenous precursors of ethylene synthesis, including 1-aminocyclopropane-1-carboxylic acid [22]. 2,4-Dichlorophenoxyacetic acid (2,4-D), a synthetic auxin, is widely used in many species, especially cereal crops and medicinal plants. The concentration of 2,4-D affects callus formation, and the optimal concentration varies for different species or tissues. The general principle is that a low concentration promotes embryonic callus formation, whereas a high concentration inhibits its formation [12]. This may explain the non-regeneration of plantlets due to prolonged exposure of embryogenic calli to 2,4-D in our study.

Table 1. Effect of medium on plantlets regeneration of rice variety 'Nerica 3'

Media	Plantlet regeneration (%)
R1	9.37^{bc}
R2	29.68^{ab}
R3	45.31^{a}
R4	0^{c}
R5	4.68^{bc}

Studentized Range (q) = 4.06 at alpha = 0.05 according to the Tukey's HSD test; R1 to R5 are the regeneration media used. For composition of media, please refer to Material and Method

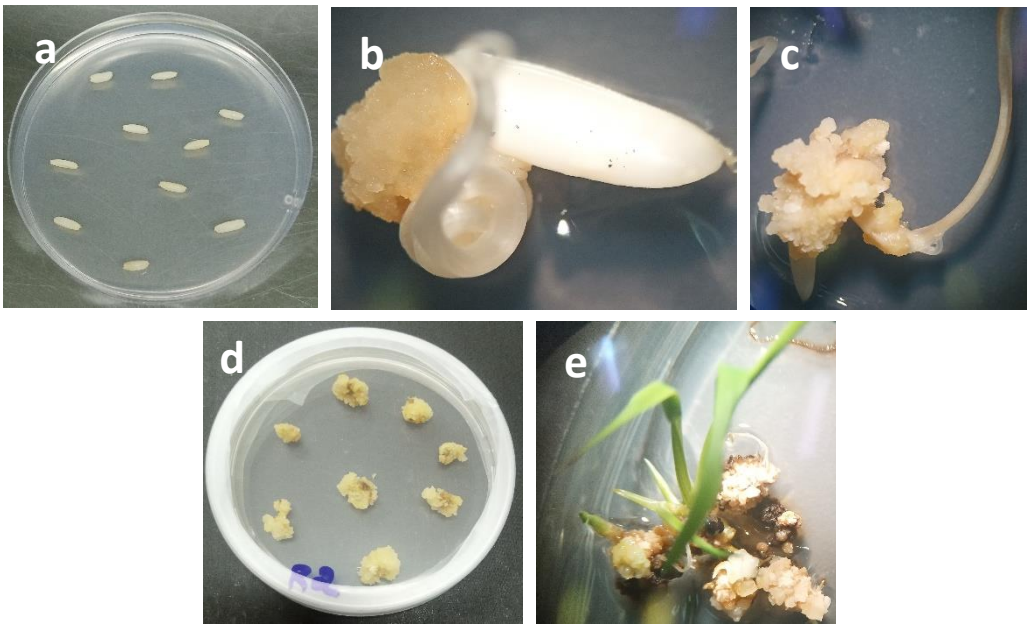

Fig. 1. Embryogenesis and plant regeneration from mature seed of rice variety 'Nerica 3': (a) Inoculation of mature rice seed into the callus induction medium; (b) Callus formation 12 days after incubation; (c) Embryogenic callus formation after 6 weeks of culture; (d) Inoculation of embryogenic calli into the regeneration medium; (e) Plantlet regeneration on the regeneration medium after 4 weeks of transfer

4. CONCLUSION

In the present study, we investigated the effect of exogenous plant growth regulators (BA, IAA, and 2,4-D) in plantlet regeneration using callus from mature seed as explant of rice variety 'Nerica 3' for the first time. We reported that cytokinin BA is one the most exogeneous plant growth regulators which promoted plantlet regeneration from callus. These results will be used for genetic improvement program of this variety.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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