Track: BD (Conservation of Biodiversity and North Eastern States)

In vitro callus induction from endosperms of Indian black rice (*Oryza sativa* L.)

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Black rice, also known as 'forbidden rice or emperor's rice', is a native Asian rice variety. In India few popular black rice varieties are the 'Kalabat' of Odisha, 'Chakhao' variety of Manipur and 'Mamihangar' of Tripura (Pal et al. 2019). 'Chakhao' and 'Mamihangar' black rice varieties have short cropping growing period, therefore can be harvested twice a year. Recently the 'Chakhao' black rice variety of Manipura has got geographical indication tag. In north eastern region of India different black rice varieties are grown in both wetland and upland. The black rice varieties have got increasing attention because it enhances life longevity due to its high nutritional, antioxidant, and nutraceutical properties (Ito and Lacerda 2019). The dark purple pigmentation is due to the presence of high anthocyanins in the pericarp which act as antioxidants and have vast applications in industries as colorant, food supplements and nutraceuticals (Silva et al. 2017). Black rice contains carbohydrate, fat, fibers and proteins, vitamin E, iron, copper and zinc. Generally, black rice is heirloom and open pollinated rice, posing greater genetic variations (Kushwaha 2016). Moreover, propagation of black rice is through seeds, availability of which is limited and restricted to few farm growers only. The Indian black rice is a lesser-known variety, locally cultivated in a few states of India. Since the rice yield is less, the variety is not much cultivated by the farmers though it has high demand in the market. Thus, improved high yielding black rice will encourage the farmers for its cultivation. Through tissue culture technique, such as culture of anther, embryo, protoplast, ovule, somaclonal variations can be produced that leads to development of new varieties (Brown and Thorpe, 1995). Tissue culture-based study is limited in Indian black rice due to rare availability. In the present study, the objective was to develop an effective protocol for optimum callus induction from endosperm used as an explant. Endosperms are triploid in nature, formed during double fertilization, where one of the male nucleus fussed with two nuclei of the female gamete resulting triploid nucleus formation. Although triploid plant produced sterile seeds, but if chromosome complements can be doubled through chemical treatment, polyploidy plants can be produced. These polyploids can be utilized in breeding programs. During in vitro culture,

the embryo (diploid in nature) was removed completely and only the endosperm parts were inoculated in the culture into the medium. Murashige and Skoog (MS) medium supplemented with growth regulators, such as 2,4-Dichlorophenoxyacetic acid (2,4-D) alone or in combination with Naphthalene acetic acid (NAA) was used to induce callus. More than 30 % cultures showed calluses from endosperms cultured at 15 µM 2,4-D concentration alone. The combined presence of growth regulators, NAA and 2,4-D, at different concentrations, induced calluses only in 25 % cultures. When NAA was used alone in the medium, the cultures showed no callus development. MS basal medium devoid of any growth regulator was used as control and no callus development was observed. The induced callus was further subjected to different growth regulator treatments for callus proliferation. The MS media fortified with 6-Benzylaminopurine (BAP), 2,4-D and IBA (Indole-3-butyric acid) combination gives best result. The experiments on regeneration from these calli is in process.

References

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