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European Journal of Experimental Biology, 2015, 5(10):15-17

ISSN: 2248 –9215
CODEN (USA): EJEBAU

An easy and reliable technique for the extraction of genomic DNA from the young leaves of black scented rice(chakhao)

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ABSTRACT

The present study deals with the extraction of genomic DNA from the black scented rice of Manipur. Here, an easy and reliable technique introduced by Whatman (a part of GE Healthcare) using FTA Cards is used for genomic DNA extraction from the young leaves of the black scented rice. The samples were collected from different parts of the state during the growing season. The result shows clear DNA band during Gel Electrophoresis. Further research for the qualification and quantification of the DNA extracted is going on for the development of the black scented rice using different molecular techniques.

Key words: genomic DNA, black scented rice, FTA Cards, molecular techniques.

INTRODUCTION

More than 50% of the world population rely on rice as their staple food as compared to 85% of Indian population which have rice as their staple food. Black scented rice or purple rice is a range of rice types of the species *Oryza sativa L.*, some of which are also called as glutinous rice. The varieties of black scented rice include Indonesian black rice and Thai jasmine black rice. Black rice is high in nutritional value and is a good source of iron, vitamin E and antioxidants (more than in blueberries) [10]. It also contains essential amino acid like lysine, tryptophan; vitamins such as vitamin B1, vitamin B2, folic acid; and is a good source of minerals including iron, zinc, calcium and phosphorus[12].The bran hull (outermost layer) of black rice contains one of the highest levels of anthocyanin, an antioxidant found in food[13]. The grain has a similar amount of fiber to brown rice and, like brown rice, has a mild, nutty taste[2, 11]. Black rice has a deep black color and usually turns deep purple when cooked. Its dark purple color is primarily due to its anthocyanin content, which is higher by weight than that of other colored grains[1, 8]. It is suitable for making porridge, dessert, traditional Chinese black rice cake or bread. Noodles have also been produced from black rice. Thai black jasmine rice, while not as prevalent as the white and brown varieties, adds more vibrant color to meals, as well as providing additional health benefits[5]. Black rice is popular in asian countries where it mixed with white rice prior to cooking enhanced the flavor, color and nutritional value. The black scented rice of Manipur, India is scented with dark purple color pericarp. Anthocyanins have been recognized as health promoting food ingredients due to their antioxidant activity[6, 7] and anticancer[4], hypoglycemic, and anti-inflammatory effects[9].

DNA discrimination techniques have been applied to various aspects of plant breeding such as marker-assisted selection using DNA markers, cultivar identifications of the cultivars for the protection of the breeding rights and for

the prevention from the contamination of undesirable cultivars and the examination of the relationships between closely-related cultivar lines[3].

So far there is no systematic work on the improvement of black scented rice of Manipur has been taken up. However, germplasm collection, characterization and evaluation of these aromatic rice cultivars of have been made. The recent advancement in the field of molecular biotechnology, genomic research, transgenic breeding and molecular marker applications with conventional plant breeding techniques has created the foundation for molecular plant breeding which will be very useful for the improvement of indigenous rice varieties including the black scented rice in Manipur. Here, a reliable and easy technique designed by Whatman (Part of GE HealthCare) for extraction of genomic DNA is reported.

MATERIALS AND METHODS

Sample Collection

More than 50 samples of black scented rice were collected from different parts of the state during the growing season (June-July 2015). Sample bags were used for the collection so that maximum handling is avoided. The samples were preserved at 4ºC before the processing starts.

Plant Homogenate Preparation

A minimum of 10 gm of plant tissue and using a ratio of 1 part plant tissue to 5 parts PBS is taken, groud leaf material to a smooth homogenate using a mortar and pestle. The 1:5 ratio is based on using young plant leaf tissue. Using a cut pipette tip apply the homogenate to the FTA Classic Card matrix inside the marked circle , and allow the FTA Classic Card to air dry for two hrs(minimum) at room temperature.

Sample Purification Protocol

1. The FTA Matrix card is placed on the FTA sample mat
2. Using the 2.0mm Harris Micro Punch^{TM} Tool, a disc is remove from the centre of the dried sample area. Another punch of a comparable diameter can be used or matrix could be cut with a sharp scalpel.
3. The punch is transferred to an appropriate PCR amplification tube or 1.5ml centrifuge tube.
4. 200 µl of FTA Purification Reagent is added to each tube, capped, inverted the tube twice and incubated for 4-5 mins at room temperature.
5. The FTA reagent is pipette up and down twice.
6. Now most of the reagent is discarded from the tube leaving the punch in the tube.
7. Step no. 4 to 6 is repeated twice.
8. 200 µl of $TE_{0.1}$(10mM Tris, 0.1 mM EDTA) is added to each tube, capped, inverted twice and incubated for 4-5 mins at room temperature.
9. The reagent is pipetted up and down twice.
10. Now much of the reagent is removed using a pipette.
11. Step no. 8-10 is repeated twice.
12. The punch is allow to air dry completely at room temperature or 20mins at 56ºC.
13. The PCR amplification is conducted within three hours of punch drying or else it should be stored at -20ºC.

PCR Amplification

25-50µl of the complete PCR amplification mix is added directly to the PCR tube containing the dried punch. Thermocycling is performed assuming the DNA volume used as zero.

RESULTS AND DISCUSSION

Electrophoresis was performed at constant power of 100 Watt for around 2 hrs. DNA ladder used in the electrophoresis was 1 KB ladder (Figure-1). And when run on 1% agarose, a clear bright band (Figure 1) which shows that the DNA quantity and quality is good for further usage.

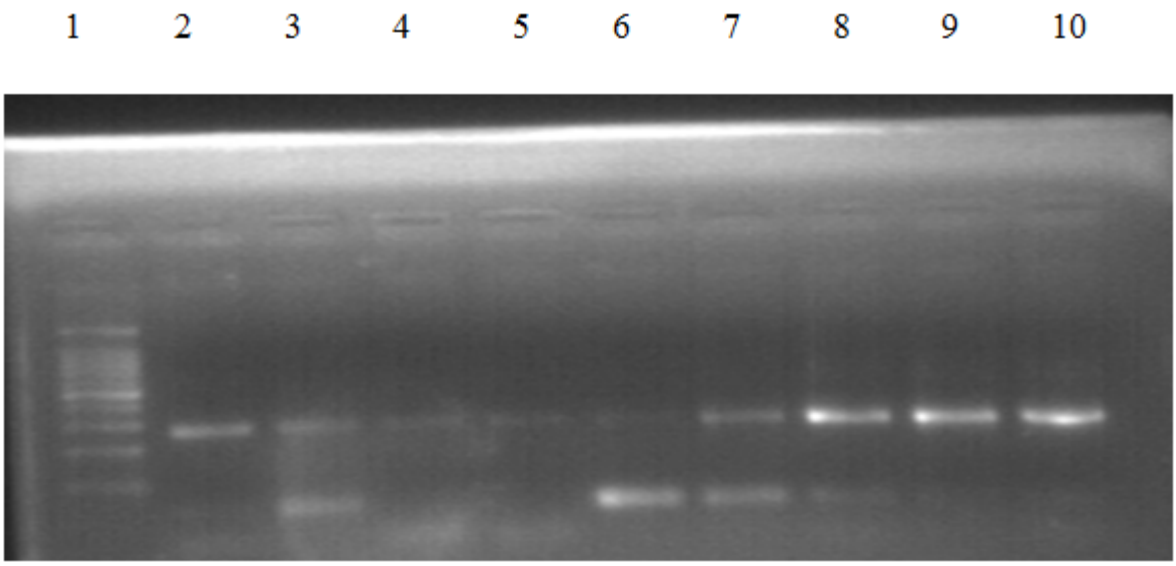

Figure 1. Extracted DNA on Gel Electrophoresis. Lane no. 1 - 1 KB ladder, Lane no. 2-10 -DNA Sample of the black scented rice

Since the technique is new, we need more exposure so that a standard protocol is maintain. Comparing with the CTAB method, this method is easy and reliable and it is less time consuming. Here thermocycling or PCR is done directly before checking the quality and quantity of the DNA. So, care should be taken while extracting the DNA following very strict protocol of the manual make sure that the DNA is present in the punch. Quantification and qualification of the DNA extracted with this technique is also in process using other techniques like UV Spectrophotometer. Further studies for the development of black scented rice in molecular level is going on.

Acknowledgement
We are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India, New Delhi, for providing official support and laboratory facilities at DBT-Biotech Hub, S. Kula Women's College, Nambol, Manipur, Kamakhya Pemton College, Hiyangthang, Manipur and Pettigrew College, Ukhrul, Manipur.

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