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Biotechnology

AROMATIC RICES OF BHAGALPUR AND BANKA REGION (BIHAR).

KEY WORDS: *Oryza sativa*, Aromatic, katarni, monocot, aromatic, Bihar, pest.

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ABSTRACT
 Rice is the seed of the grass species *Oryza sativa* (Asian rice). Rice, a monocot is normally grown as an annual plant although in tropical areas it can survive as a perennial crop. Aromatic rices constitute a small but special group of rices which are considered best in quality. In Bihar, Bhagalpur and Banka has been a traditional aromatic rice growing area, where the varieties, such as : (i) Katarni, (ii) Tulsi Manjari, (iii) Badshahbhog, (iv) Br-9, and; (v) Br – 10 are mostly common.. However, over the period there has occurred a large variation, which has resulted into various types, such as (i) Bhauri katarni, (ii) Deshla katarni, and; (iii) Sabour katarni, (iv) Ghorayiya katarni.. Most of these varieties have either already lost, or are at the verge of extinction. Local varieties have yield potential ranging from 15 to 30 qtls/ha, and are tall possessing short grains. Many of them are highly susceptible to various insect pests and diseases, like: (i) stem borer and (ii) bacterial blight. Lower productivity of these aromatic rices, these varieties being short grained, and consequently of low export values could be contended to be some of the remarkable factors for large decline of areas under katarni paddy in this region.

INTRODUCTION

Rice (*Oryza sativa* L.), a member of the Poaceae family and the Oryzoidea subfamily, is one of the main staple foods eaten by 70% of the world's population. One fifth of the total land covered by cereal crops is occupied (Chakravarthi and Naravaneni, 2006). For the world population, rice, which is primarily eaten as a whole grain, provides 20% of the daily calories. The adaptation of rice cultivars to broad climatic conditions has led to the production of thousands of varieties of rice of various qualities Characteristics in terms of physical characteristics, cooking, eating and product creation (Bhattacharya, 2005). In addition, rice as a model crop with a completely sequenced genome, relatively small size of the genome, and significant polymorphism levels (McCouch et al., 1988; Wang et al., 1992)

In many developing countries, rice is by far the most economically important food crop, accounting for two-thirds of the calorie intake of over 3 billion people in Asia and one-third of the calorie intake of almost 1.5 billion people in Africa and Latin America (FAO, 1995a). Recently, due to food diversification and immigration, rice consumption has increased in many developed countries such as North America and the European Union (EU). The per capita intake of rice has risen at different rates in the last two decades (1970-90), ranging from 2.4 percent per year in Italy to 8.2 percent per year in the UK (Faure and Mazaud, 1996). The current high population pressure, high production costs and demands for an improvement in income have encouraged rice growers to increase yields and crop intensity on limited land, such as high land productivity, in order to generate adequate world food.

Due to its favorable hot and humid climate, Asia accounts for 90 percent of the world's production and consumption of rice, but suitable land for growing rice production is almost exhausted. In Africa, over the last two decades, the economic value of rice has gradually increased. Following the gradual evolution in the nutritional habits of many Africans, particularly those living in urban areas, the amount imported has more than doubled, moving from eating only traditional foods such as cassava, millet and sorghum to rice and wheat. For the period 1980-1992, the per capita consumption of rice increased from 14.8 kg/year to 16.3 kg/year. In the last decade (1983-93), rice production in Latin America and the Caribbean has increased by 32 percent and per capita rice

consumption has also increased from 25.8 to 26.3 kg per year (FAO, 1995a).

Three key factors typically define fragrant rice: appearance, aroma and taste (Chaudhary, Tran and Duffy 2003). With a pleasant and subtle fragrance, it is characterized as a superfine grain. It has a soft texture with a breadth-wise swelling that happens with cooking, and extreme grain elongation. Basmati and jasmine are long-grain, quality rice. Fragrant rice grows best and under wet, humid, and valley-like conditions, produces the best quality grains. Some stakeholders are likely to supply fragrant rice, not derived from its genuine regions, due to the attractive price premiums, and thus give consumers less aroma.

It is possible to detect aroma by tasting the corresponding flavour in rice grains or leaf tissue aroma by either heating in water or reacting with a KOH solution (Tragoonrung et al., 1996).

The most important quality attribute of aromatic rice is the aroma. Which commands a higher price than rice which is not aromatic. Aromatic or perfumed rice therefore plays a crucial role in the global Trading in rice. Different chemical components, including distinct volatile compounds are the main causes of flavor in cooked rices. In addition, Bradbury et al. have stated that a recessive gene (*igr*) on chromosome 8, 8 bp deletions and 3 single nucleotide polymorphism results in nonfunctional Betaine Aldehyde Dehydrogenase 2 (BADH2) enzyme that results in rice flavouring.

In Bihar, while aromatic Rices are grown throughout the state, they are primarily concentrated in the divisions of Bhagalpur and Magadh. Bhagalpur was a traditional region of aromatic rice cultivation, where most varieties are common, such as: (I) Katarni, (II) Tulsi Manjari, (III) Badshahbhog, (IV) Br-9, and; (v) Br-10. These are sensitive to photoperiods, big, and therefore susceptible to lodging and many diseases and pests. Their performance ranges from 2.0 to 2.5 t/ha. However, a wide variation has occurred over the time, resulting in different forms, such as (i) Bhauri katarni, (ii) Deshla katarni, and; (iii) Sabour katarni. Farmers develop Karibank, Marueya, Mehijawain, Shyamjira, Tulsiphool, Sonachur and Shah Pasand in the Magadh area, which is the main rice-growing tract of Bihar. Over time, the areas under these varieties have been drastically reduced, while Karibank and Marueya

farmers still expand, but only on a small scale. At one time, the tarai area of West Champaran was renowned for its high-quality aromatic rice varieties, including I Lal champaran basmati, (ii) Bhuri champaran basmati, (iii) Kali champaran basmati, (iv) Baharni, (v) Badshahbhog, (vi) Chenaar, (vii) Dewtabhog, (viii) Kesar, (ix) Kamod, (x) Kanakjeera, (xi) Marcha, (xii) Ram Janwain, (xiii) Sonalari, and (xiv) Tulsi Pasand. Most of these types have either been lost or are on the brink of extinction (Singh et al. 2000). Local varieties have a yield potential of between 15 and 30 qtls/ha and are tall with small grains. Many of them like: I stem borer and (ii) bacterial blight, are highly susceptible to different insect pests and diseases.

GENERAL DESCRIPTION OF THE STUDY AREA BHAGALPUR

The district of Bhagalpur is located at a height of 141 feet above sea level between 25° 07' to 25° 30' N latitude and 86° 37' to 87° 30' E longitude in the basin. The district of Bhagalpur is unique in that it forms part of two separate agro-climatic zones. Although its main location is located in the Agro-climatic Zone-III A (South Alluvial Plane), but its area falls north of the Ganges River, the Agro-climatic Zone-II A is precisely its Naugachia Sub-division (North-East Alluvial plane). The pH varies between 6.8 and 8. The climate of the district of Bhagalpur is sub-humid and sub-tropical monsoon with an average annual precipitation of approximately 1167.16 mm. In the region, the agricultural scenarios were extremely varied. The main field was the old alluvial soil south of the Ganges River. These are common soils that grow rice with a texture that ranges from silt loam in its upper peripheries to clay loam in low lying regions. Rice is the only crop grown during the kharif season in these lands, followed during the rabi season by wheat, grams, and a number of para crops. There was a wide area of the district under 'Diara lands' which during the rainy season remains under flood water. However in the post-flood Kharif season, rabi season, summer season and pre-Kharif seasons, these lands are intensively cultivated. The region's most important crops are maize, wheat, green gram, while banana is the cash crop covering a wide area in the Naugachia sub-division falling north of the Ganges River. With sand deposits, the soil here was highly permeable. The alluvium added during flooding acts as a good source of soil fertility replenishment. There was some area referred to as 'Tal lands' on the southern flank of the River Ganges. There are bowl-shaped depressions where during the rainy season, water accumulates. The land is available for cultivation many times in the month of October when this accumulated water percolates or evaporates. These were heavy clays of the form montmorillonite that develops large and deep cracks during the summer season, which also created means of accumulated water rapidly percolating. These lands were best suited to the pulse and oilseed during rabi season. A part of the Bhagalpur region was also situated in the foothills of the Kharagpur, Mandar and Rajmahal mountain ranges. Sloppy and highly permeable, these lands were. The main crops during the Kharif season were Arhar, groundnut and maize, and pulses and oilseeds with low water requirements were favored during the Rabi season. Rice was also grown in low-lying plane areas known as finished ground. There were hot summers in the district and mild winter seasons. The district's mean temperature in the month of May/June was 44 ° to 45 ° Celsius, and the minimum temperature dropped to 8 ° Celsius in the month of December/January. The minimum and maximum humidity percentages were 38 and 95, respectively. Precipitation, minimum and maximum temperature and humidity were included in the weather. However there was a shift in the weather season wise. Rainfall in Bhagalpur, mainly affected by the south-western monsoon, begins in the second week of June and continues until the end of September. It is revealed that from June to September every year the overall rainfall was 93.36 percent of the total rainfall. As far as the maximum and minimum temperature and humidity are concerned, 44.05 °C,

8.0 °C and maximum humidity were 95.00 percent, while the minimum was 38.00 percent, while the average rainfall was 38.00 percent while the average rainfall in the district is 1167.16 mm.

BANKA

The district of Banka is distinctive because it forms part of the Agro-climatic Zone-III A (South Alluvial Plane). The pH ranged from 6.8 and 8. Sub-humid and sub-tropical monsoon farms with average annual rainfall of about 1123.29 mm have been found in the climate of the Banka district. In the region, the agricultural scenarios were extremely varied. The district's soil is medium to high in texture. In summer, it is grey to red in colour, moderately alkaline in answer and cracks (5 to 8 cm wide and 16 to 120 cm deep). During summer, soil becomes boundary and stays inundated during rains. Banka can be split into two regions: the northern plains and the hilly southern regions. There were hot summers in the district and mild winter seasons. The district's maximum temperature was 43 ° Celsius in the month of May/June and the minimum temperature dropped to 8.8 ° Celsius in the month of December/January, as per the available normal. The minimum and maximum humidity ratios were 28.8 and 77.6, respectively. The state is in truth, richly endowed with water supplies and very strong rainfall. Nevertheless, neither the distribution of water supplies nor the rainfall in the state was found to be uniform, creating unequal potential/coverage of irrigation throughout the state/district.

MATERIAL AND METHODS

Survey of Katarni rice in different blocks of Bhagalpur and Banka.

The study entitled **AROMATIC RICES OF BHAGALPUR AND BANKA REGION (BIHAR)** is mainly based on primary data collected from 30 katarni paddy growing cultivators each from Bhagalpur and Banka districts. 'Multi stage simple random sampling method' was followed to select respondents.

At the first stage of sampling, the two districts, namely: Bhagalpur and Banka were purposively chosen, as the specific variety of Katarni, to which this study is devoted, is grown only in particular areas of these two districts.

At the second stage of sampling, one block from each district was selected on the basis of area under Katarni paddy and potential. Jagdishpur and Rajoun blocks were selected from Bhagalpur and Banka Districts, respectively.

At the third stage of sampling, maintaining the harmonious basis of choosing potential villages, in regard to cultivation of Katarni paddy, two villages each from the two selected blocks of the concerned districts were identified. Thus, two villages, namely: Bhawanipur-Deshari and Jagdishpur cluster of villages under Jagdishpur block were selected of Bhagalpur district. Similarly, (i) Singhnan and; (ii) Rupsa villages were selected from Rajoun block of Banka district.

At the fourth stage of sampling, enlistment of Katarni paddy growers in the selected villages was made. In Bhawanipur-Deshari and Jagdishpur cluster of villages under Jagdishpur block of Bhagalpur district, the number of marginal, small, medium and large farmers growing katarni paddy also, were 40, 50, 55 and 21 respectively. Number of katarni paddy growers, who belonged to marginal, small, medium and large farm size classes of 'Sighnan' and 'Rupsa' villages in Rajoun Block of Banka district were 45, 40, 60 and 18 respectively.

At the fifth stage of sampling, indispensable classification of farmers from out of the enlisted growers was done based on farm size owned by them. All the enlisted growers were broadly kept in four categories: (I) Marginal --- owning land up to 1 hectare, (II) Small --- 1.01 to 2 hectare, (III) Medium ---

2.01 to 4 hectare, and; (IV) Large --- > 4 hectare.

At the sixth stage of sampling, 15 farmers from each of the selected villages (if required number of Katarni paddy growers was not found in a particular village, then cluster of adjoining villages was also considered) were selected for detail study. The selection of farmers was done on probability proportion method. Further, with the view to maintain discreet selection of respondents, due emphasis was given on social composition of the enlisted growers. In this way, the selection of sample can be illustrated as below: 2 districts x 1 block each (=02) x 2 villages each (04) x 15 farmers = 60 Katarni paddy growers.

Collection of plant sample.

A total of 60 plant samples were collected in the form of paddy seeds, leaves and roots from the different katarni farmers of different surveyed villages of two different blocks of Bhagalpur and Banka districts. Samples were collected in sterilized polyethylene zipper bags to avoid any cross contaminations.

Table 1.0: District Wise Katarni Seed Sample Collection.

Blocks	District			
	Bhagalpur		Banka	
	Block 1	Block 2	Block 1	Block 2
Villages	Village 1	Village 2	Village 1	Village 2
Size of farmers	1	2	1	2
Marginal	04	03	04	04
Small	05	04	04	03
Medium	06	04	06	06
Large	02	02	02	02
Total	30		30	
Total farmers surveyed	60			

Sensory test

The sensory test of aroma were performed by using a 1.7% KOH solution (Sood and Siddiq 1978). Five gram of green leaf blade at the heading stage was cut into small pieces and put into Petri dishes with 5 ml of a 1.7% KOH solution at room temperature. After 30 minutes, the dishes were opened and smelled immediately. Twenty samples were evaluated at a time and only two batches were tested in a single day with 5-7 hour intervals. The presence (+) or absence (-) of aroma was then scored. Every sample was evaluated by 15 persons.

DNA extraction from leaf sample.

Total DNA was isolated from approximately 100 mg of leaf tissue per sample based on the cetyl

Tri-methyl ammonium bromide (CTAB) method (Doyle, 1991).

Determination of fragrance related gene Rice genome restriction digestion

The gene responsible for the production of fragrance were isolated from whole genome through restriction digestion with the help of restriction enzyme Acc11, AluB1 & BstFNI, identified through naive cutter software of NCBI.

PCR based amplification of *frg* gene

The primer of isolated DNA fragment were used for extension of *frg* gene.

PCR process

Prepare the following 50 µL reaction in a 0.5 mL PCR tube on ice:

Table 2.0: Amplification parameters of PCR

S. No	Component	Stock	Concentration required	Volume to be taken
1	PCR Buffer	10X	1X	5 µL

2	dNTPs	10 mM (each)	50 µM (each)	1 µL (0.25 µL each)
3	Primer (Forward)	10 mM	20 pmoles	2.5 µL
4	Primer (Reverse)	10 mM	20 pmoles	2.5 µL
5	Template DNA	Variable	1ng-1µg	5.0 µL
6	DNA polymerase	3 units/µL		0.5 µL
7	MiliQ water			33.5 µL

Mix the reaction gently and spin it down in a micro centrifuge. Put a drop of mineral oil to avoid the evaporation. Set the cycle conditions in the thermo cycler for PCR. For comparison, routine cycling conditions are given for a PCR using Taq Polymerase.

Table 3.0: PCR Cycle temperature and time.

Cycle step	Temperature	Time	Cycles
Initial Denaturation	95 °C	30 seconds	1
Denaturation	95 °C	15-30 seconds	32
Annealing	59.79 °C	45 seconds	
Extension	72 °C	1 min per kb amplified	
Final Extension	72 °C	5 minutes	
Hold	4 °C	∞	1

After the PCR cycle is over, load the products on to an agarose gel and document it.

Agarose gel electrophoresis

Prepare 50 X TAE solutions by dissolving 242 gm Tris, 100 mL 0.5 M EDTA (pH 8.0) and 57.1 mL Glacial Acetic acid, and adjust the volume to 1000 mL by adding double distilled water. A working solution is prepared by 1:49 dilution (20 mL stock solution of 50X TAE + 980 mL double distilled water).

0.5% Agarose gel is prepared by dissolving 500 mg Agarose in 100 mL 1X TAE buffer. Boil the

Solution to dissolve Agarose. When the solution cools down a bit, 2 µL ethidium bromide is added to it and casted in an electrophoretic casting plate and an electrophoretic comb is placed at an end of the gel in a way that the legs of the comb remain inside the liquefied gel. It is then allowed to solidify.

12 µL DNA sample is mixed with 2 µL (approx.) 6X Gel Loading dye and loaded in the gel.

Placing the gel and loading the sample:

Remove the comb slowly, after the gel gets solidified, leaving behind fine wells in the gel. Place the solidified Agarose gel, along with the casting tray, place inside the electrophoresis chamber keeping the wells towards the cathode.

Fill the electrophoretic tank with 1X TAE buffer to such an extent that the gel remains submerged.

Load the DNA samples along with molecular size markers into the wells with the help of T-20 micropipette at the cathode end.

Run the gel till the time the tracking dye covers more than ¾ distance in the gel.

A DNA-ethidium bromide complex is seen as a dark orange coloured band separated on agarose gel according to confirmation and size of DNA fragments.

RESULTS AND DISCUSSION

TABLE 4.0: Aroma testing of different rice varieties.

S.NO	RICE VARIETIES	AROMA
1	R1	++
2	R2	++
3	R3	++
4	R4	+

5	J1	++
6	J2	++
7	J3	++
8	J4	--

Table 4.0 shows the result of 8 different randomly selected rice varieties from two different districts of Bihar viz. Banka and Bhagalpur. Each district was surveyed for katarni rice samples and for aroma test four rice varieties were selected randomly from each district. The presence of Aroma was physically validated by Sood and Siddiq (1978) method and it was revealed that, out of 4 different varieties randomly selected from Rajoun Block (R1 R2 R3 and R4) showed positive aroma test. The R1, R2 and R3 varieties had strong and distinct aroma while R4 varieties was found to have a faint aroma in relation to other varieties. The katarni rice varieties (J1, J2 and J3) of Jagdishpur block was found to have strong aroma except variety J4.

Isolation of DNA sample and validation

The leaf samples were used for the isolation of genomic DNA for the estimation of *fgr* gene in different selected varieties of katarni rice. The validation of DNA extraction was done through spectrophotometric method for each rice varieties leaf samples (prince et al., 2013). It was quantified that 100 mg of dried leaf sample contains about 115 µg/ml.

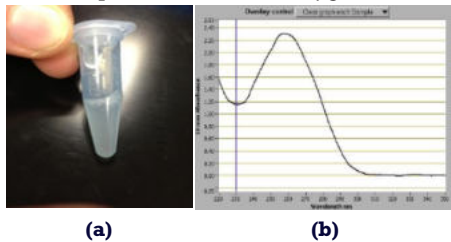


Fig 1.0: Isolation (a) and validation (b) of DNA isolated from leaf samples.

Details of fragrance gene sequence

The details of the *fgr* gene nucleotide sequence is isolated from the NCBI nucleotide database of fragrant rice genome. The different exonic and intronic databases are available for fragrant rice. For the identification and designing of forward and reverse primer of *fgr* gene 7737 truncated betaine aldehyde dehydrogenase (BADH2) gene, Exon 13 and 14 were considered.

Oryza sativa isolate 7737 truncated betaine aldehyde dehydrogenase (BADH2) gene, exons 13 and 14

GenBank: FJ704974.1
 GenBank Graphics PopSet
 >FJ704974.1 Oryza sativa isolate 7737 truncated betaine aldehyde dehydrogenase (BADH2) gene, exons 13 and 14

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TCATCTTGCAGCATATGTATATACTCTGTGGCATATGAACT
TATTCTGCTACTACTACTTTTGTATAGTTA
TGGTCTGGCTGGTGCTGTGCTTTCCGGTGACCGGAGC
GATGCCAGAGATTAAGTATATCCAACT
GAAGGGGGTTGGCATTGTTGATTCATATGACATGGTTG
CATCAAGCTGATATTCAAGAATCTCATTTAT
TACTTGCATTCTATGCATCTCCAGTTCTTCCCTGGACTCC
GGTCAATGTTAATATAGTTTGTGTTGCTAGT
AGTATGCTACTCCAATTAAGTTGCTTTCACCTCCACATC
ATCTGATCCATGACTTTATATTTGACCCCT
TTTTTTTGCAAAAGAAAGGGAAATACTTAACGAAAATTTCT
CTACTGCAGGAAATCGATGCCGGAATTATC
TGGGTGAACTGCTCGCAACCCTGCTTCTGCCAAGCTCC
ATGGGGCGGGAACAAGCCGACGGCTTTGGAC
CGGAGCTCGGAAAAGGCT
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Details of fragrance primers

Table 5.0: Different primer design against *fgr* gene through NEB cutter (V2.0)

Primer pair	Sequence (F+R)	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self T complementarity
Primer pair 1	Forward primer	CCTGACCTCCGTCATGTT	Plus	20	241	260	59.68	55.00	2.00
	Reverse primer	CATGGACCTGGCAGAGCA	Minus	20	461	442	60.96	65.00	2.00
	Product length			221					
Primer pair 2	Forward primer	ATCCAGTGAAGGGGTTGG	Plus	20	133	152	59.59	55.00	0.00
	Reverse primer	CGGAGCTCAGGGAAGACT	Minus	20	252	233	60.91	60.00	2.00
	Product length			120					
Primer pair 3	Forward primer	TATGGTCGGCTGGCTGCT	Plus	20	69	88	61.20	55.00	0.00
	Reverse primer	CCAACCCCTTCACTGGAT	Minus	20	152	133	59.59	55.00	2.00
	Product length			84					
Primer pair 4	Forward primer	TTCCCTGGACCTCCGTCAT	Plus	20	238	257	60.55	55.00	2.00
	Reverse primer	GGAGCACTTCCACCATG	Minus	19	436	418	60.00	57.89	2.00
	Product length			199					
Primer pair 5	Forward primer	TAGTATGGCTGGCTGGTGC	Plus	21	65	85	59.79	52.38	2.00
	Reverse primer	ATGCCAACCCCTTCACTTG	Minus	20	155	136	60.54	55.00	2.00
	Product length			91					

The primer designing of 7737 truncated betaine aldehyde dehydrogenase (BADH2) gene, Exon 13 and 14 was done with the help of restriction site finding NEB cutter (V2.0), which suggest about a set of 15 different primer pairs. Out of which best five primers pairs is depicted in table no 5.0. The primer pair 5 (table 6.0) was taken for the amplification of isolated *fgr* gene in the leaf sample of rice.

Table 6.0: Primer for fragrance gene (*fgr*)

Primer name	5' Primer sequence 3'
External Sense Primer (ESP)	TTGTTTGGAGCTTGCTGATG
Internal Fragrant Antisense Primer (IFAP)	CATAGGAGCAGCTGAAATATA TACC
Internal Non-fragrant Sense Primer (INSP)	C'TGG'TAAAAAGATTATGGCTT CA
External Antisense Primer (EAP)	AGTGC'TTTACAAAGTCCCGC
Forward Primer	TAGTTATGGTCTGGCTGGTGC
Reverse Primer	ATGCCAACCCCTTCACTTG

Agarose gel electrophoresis of Amplified *fgr* gene of different rice varieties

The amplified DNA samples were electrophoresed within Agarose gel (0.5%) using ETBr staining dye and documented through GENIE Gel Doc imaging system under UV light (200nm). Four different varieties were amplified and electrophoresed along with a negative control as pure water. The L1, L2, L3 and L4 shows the electrophoretic pattern of one non-fragrant and three fragrant katarni rice. The absence of DNA fragments in L2, L3 and L4 suggest the presence of fragrance due to the inactivation of BADH2 gene in fragrant varieties of katarni rice. The L1 shows different exonic bands of BADH2 gene and therefore responsible for the loss of fragrance.

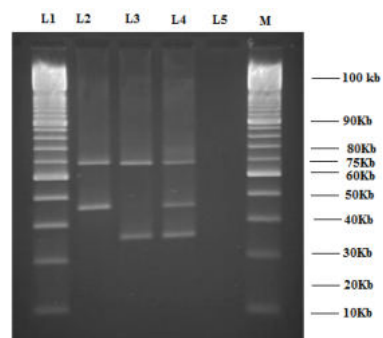


Fig 2.0: Agarose gel showing *fgr* gene amplification of fragrant and non-fragrant katarni rice varieties.

DISCUSSION

Katarni is a fragrant variety of rice confined to Bhagalpur and nearby Banka district. The distinct aroma within grains makes it a unique kind of fragrant rice comparable to basmati (Arpit

et al., 2016). The Bhagalpur and Banka district are well suited for the cultivation of this unique variety of rice. The variety under present investigation are shows confirm biochemical testing for the presence of aroma due to the presence of volatile compound (Sood and Siddiq 1978). Out of eight varieties under study had revealed with the presence of aroma except J4 variety of Bhagalpur region. This maybe due to the presence of very low content of aromatic compounds or due to the presence of a different array of aromatic compounds that needs a more precise method of aroma detection. The different selected varieties were tested for the presence of fragrancd gene (fgr) and in connection of this their genomic DNA were isolated from the leaf samples. The considerable amount of DNA was extracted from leaf samples and further tested for the presence of BADH2 gene. The presence of functional BADH2 gene results in a non-fragrant variety of rice (Saihua et al., 2008). The primer were designed through Insilco method using NEB cutter V2.0 and use for the amplification of BADH2 gene. The randomly selected four variety of rice revealed that the absence of BADH2 gene responsible for the development of aroma in selected rice varieties. This is the molecular validation of BADH2 gene inhibitory effect on aromatic compound biosynthesis (Michael et al., 2009).

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