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RESEARCH ARTICLE

Detection of Lgc1 Gene Low Glutelin Content in Rice Cultivars Iraq using Indel Markers

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Abstract

Low glutelin content rice is a good diet for patients with renal failure. Delete about a 3.5kb of a Low glutelin content Lgc1 gene sequence located in the second chromosome on the short arm between highly similar glutelin genes GluB5 andGluB4. To increase the significant, we proposed to select the adequacy in Lgc1 rice breeding, 2 molecular markers we developed to reveal the gene Lgc1, which were specified as InDel-Lgc1-2 & InDel-Lgc1-1, respectively. Our results such as The PCR results showed that band 509bp in all varieties of rice, we conclude varieties of rice in Iraq have high glutelin content. Therefore, we cannot use it for the patients with kidney failure and diabetes.

Keywords: *Oryza sativa L, Lgc1 Gene, Indel Markers.*

Introduction

Glutelin is the main storage of protein in the rice grain and it forms 80% of the total endosperm [1] and it can be readily digested and absorbed. However, people are having diabetes and kidney disease, absorption of glutelin may be lead to higher side effects for patients so, we must provide patients with a specific plan of treatment depends on a reduced-protein diet [2].

Because these patients cannot eat rice with soluble protein content higher than 4%.Therefore, we need to meet the particular necessity of these patients of low protein foods, improve low glutelin-content cultivar that has become a significant direction in present rice breeding. The mutation is the important genetic resource of low glutelin-content rice [3].

That obtained the mutant cultivar under his name NM67 by processing the rice seeds of "Nihonmasari" that reported by giving a higher percent of prolamin content and reduced glutelin content in crop [4]. Then, a low-glutelin cultivar developed by utilizing the Lgc1 mutation and used as a good source of diet for patients with chronic kidney failure. As evidenced by modern studies on diabetic patients, low-glutelin content rice is working as an effective complementary food, particularly for People who use rice as their main food [5]. Genetic analysis indicated the dominant gene Lgc1 control on low glutelin content trait which located on chromosome two of the short arm as a result, between 39.1 cM to 8.5 cM [6].

With the great evolution in molecular biology techniques and the mechanism for mutation in low glutelin diversity, LGC-1 had been explained by [7]. In the LGC-1,deletion of a 3.5-kb among the GluB5 and GluB4 genes, which was determined with the inverted orientation and shared 99.8% nucleotide sequence similarity, indicated in the production of a tail-to-tail inverted repeat of the two GluB genes. Transcription of the merge GluB4-GluB5 gene (lgc1accession number AB093593) produced a mRNA with sense GluB4 and antisense GluB5 sequences, that formed a hairpin frame structure with the intramolecular double-stranded RNA in the complementary region [8].

In this research we use Indel marker to detect the Lgc1 gene in rice cultivars in Iraq, according to deletion 3.5kb nucleotide

sequence form Oryza sativa cultivars related low glutelin content.

Materials and Methods

The rice cultivars were collected from Mashkhab Research Center in AL-Najaf IraqTab.1

Table 1: List of Iraq rice cultivars analyzed in this study

No.	Name of Varieties	Source	Pedigree
1	Aba 1	Mashkhab Research Center in AL-Najaf	Aba Research Center
2	Eanberalbaraka	Mashkhab Research Center in AL-Najaf	Indian
3	Forat 1	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
4	Ghadeer	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
5	Barnamaj4	Mashkhab Research Center in AL-Najaf	Research International Rice Institute
6	Yasmine	Mashkhab Research Center in AL-Najaf	Vietnam
7	Buhooth1	Mashkhab Research Center in AL-Najaf	Turkey
8	Eanber 33	Mashkhab Research Center in AL-Najaf	Local
9	Dijlah	Mashkhab Research Center in AL-Najaf	Chinese
10	Meshkhab 2	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
11	Meshkhab1	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
12	First genotype (T85)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
13	Second genotype(HT-1)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
14	Third genotype (LT-2)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
15	Fourth genotype (BT-7)	Mashkhab Research Center in AL-Najaf	Research Intemational Rice Institute
16	Fifth genotype (aromatic 64)	Mashkhab Research Center in AL-Najaf	Research International Rice Institute

Genomic DNA Extraction

DNA was extracted from fresh leaves after 25 days from agriculture, according to [9] Quality of DNA was verified by Gel electrophoresis and quantification was achieved by using the bio drop technique.

The sequences of primer for In Del-Lgc1-1 were as follows: 5'-TTC TACAATG AAGGCGATGC-3'&5'-CTGGGCTTTAACGGGACT-3' amplified fragment lengths 881 bp; Either sequences primer InDel-Lgc1-2 were as follows: 5'-ACCGTGTTATGGCAGTTT-3' and 5'-ATTCAAGGGCTAT CGTCT-3'. Amplified fragment lengths 509 bp according to this reference [8].

PCR Amplification

The volume of the PCR reaction mixture is 20µL that included 2.0 of DNA(10ng/µL), 10×Buffer 2.0 µL, 1.2µL of $MgCl_2$ 25 mmol/L, 1µM each of forward and reverse primers (4 pmol/µL), 12.2 µL of ddH2O, 0.4µL of d NTPs 2.5mmol/L , 0.2µL of Taq 5 U/µL .

The Program Amplification PCR

It is as follows: 35 cycles, each cycle Includes the denaturation of 30 s at 95℃, the annealing step of 30 s at 56℃ and 1min extension at 72℃, afterward specific timing which is the extension at 72℃ of 5 min. Amplification outcomes were electrophores on 2%agarose gel and dyed with ethidium bromide.

Results

Total sixteen rice cultivars were utilized for PCR detection by the marker InDel-Lgc1; the data from electrophoresis detection appear there is no a DNA fragment was observed in all cultivars and genotypes.

Because of that, there wasn't 3.5kb deletion between GluB5 gene andGluB4 genes in standard glutelin content rice. Consequently, it should be amplified DNA fragment to 4381 bp in these varieties. That's why the band was not presented in this particular experiment.

In additional off, the Taq DNA polymerase limited capability to amplify a big fragment of DNA sequences in order to achieve a good way for distinguished varieties of Lgc1, As for To another pair of primer (InDel-Lgc1-2) was manufacturing in the 3.5kb deletion between the GluB5 gene and GluB4gene, The PCR results of detection pronounced that a DNA fragment is about 509bp could be more stable by amplified in all varieties. According to our data, all varieties can be considered as natural glutelin-content as is showing in (Fig. 1).

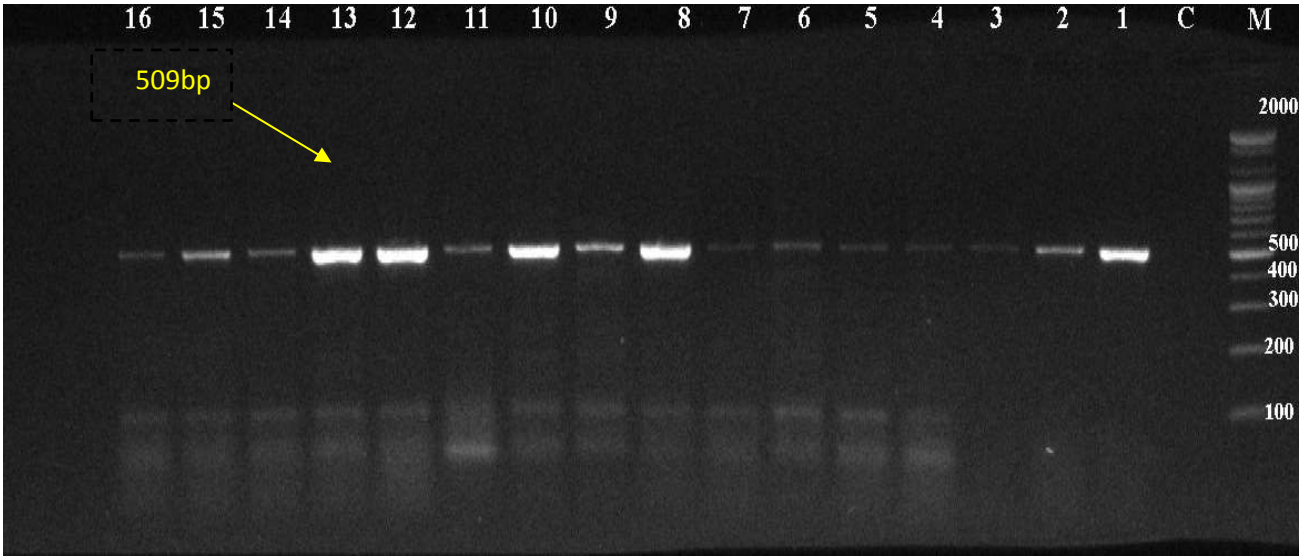

Fig. 1: Molecular detection in sixteen cultivars rice with marker InDel-Lgc1-2 . Lanes 2control, Lanes3 Aba 1. Lanes4 Eanberalbaraka, Lanes5 Forat 1, Lanes6 Ghadeer, Lanes7 Barnamaj4, Lanes8 Yasmine, Lanes 9 Buhooth1, Lanes10 Eanber 33, Lanes11 Dijlah, Lanes12 Meshkhab 2, Lanes13 Meshkhab1, Lanes14 First genotype (T85), Lanes15 Second genotype(HT-1) , Lanes16 Third genotype (LT-2), Lanes17 Fourth genotype (BT-7) , Lanes18 Fifth genotype (aromatic 64) . DNA marker, Lane 1 (100–2,000 bp)

Discussion

According to many studies that indicated the renal fiasco is a clinically common disease and it has converted the worldwide public health trouble with the change of dietary structure and lifestyle in recent years [10]. As a result of rise-protein foods lead to the dangerous disorder of protein metabolism, so rise with low protein content is regarded as an effective tool for diet therapy in patients with renal failure without making any major changes in dietary habits [11] .

Cultivation of the cultivars rice low glutelin is having more significant in the future. On the one part, it is a good chance for the breeders to extend the breeding direction .Also; it is bringing a tremendous benefit to renal patients the Lgc1 gene that is still highly popular to breeders of plant consequently how we can distinguish Lgc1 gene more carefully by the simple and quick method. It has become a dangerous trouble for breeding low glutelin rice.

With the expansion of biotechnology, molecular marker-aided selection has come to be important supplementary resources for breeding and to detect the variation in genomic DNA. Recently, many gene markers related were proved, but they exhibited low polymorphism in breeding inhabitance and couldn't be used for rice improvement because of what we mentioned previously [12]. The better method for determining this problem is to advance functional marker, it depends on the sequence discrepancy of the target gene that can be 100% guaranteed of molecular marker-aided selection [13]. In this study we use two marker (InDel-Lgc1-1 & InDel-Lgc1-2) to identify gene Lgc1 depends on 3.5-Kb deletion in the rice genome.Theoretically,InDel-Lgc1-1 which amplified 881 bp in mutant containing Lgc1, on the other hand, for amplifying a large fragment of DNA sequences must use special Taq DNA polymerase in the experiment and this is increasing the cost of the test and decrease the likelihood in practice applying to differentiate genotypes of additional marker designated as In Del-Lgc1-2 which amplified 575 bpin normal plants was synthesized as the complement for InDel-Lgc1-1Inbreeding experience practice we adopted the PCR method of detection and got results for all varieties of rice in Iraq to have high glutelin content. Therefore, it cannot be used by patients with kidney failure and diabetes and this the main aspect of our research

Conclusion

In conclusion, we suggest that the interest in planting of low-glutelin content rice in Iraq to help patients with diabetes and renal failure in the healthy diet, which ensures the breadth of genetic diversity among the varieties of rice through the use of hybridization programs between these cultivars in order to produce new varieties and better specifications, on the one hand, the use of mutagenic substances to ensure a mutation in high- glutelin Iraqi varieties achieves the same purpose above.

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