# IDENTIFICATION OF FRAGRANCE GENE *fgr* IN THE EGYPTIAN JASMINE RICE (EJR) AND ITS CROSSES WITH THREE CHINESE CYTOPLASMIC MALE STERILE LINES

**AMIRA EL-KEREDY[1] AND EL-DEGWY, I., S.[2]**

***1 Genetics Department, Faculty of Agriculture, Tanta University, Egypt***

***2 Crops Department, Faculty of Agriculture, Kafr El Sheikh University, Egypt***

Jasmine or Aromatic rice *(Oryza sativa* L.*)* has aroma components, which are cumulation of volatile contents (Widjaja *et al*., 1996; Yoshihashin 2002 and Bourgis *et al.,* 2008). It has important food (Wang and Li, 2005) which composes of carbohydrate sources to world populations. Rice cultivation began in the north of the Delta in Egypt (El-Keredy *et al*., 2003 and Shehata *et al.,* 2009). Genetics of fragrance gene (*fgr*) adapted lines was studied in new rice breeding program (Bhattacharjee.*et al.,* 2002 and Bradbury *et al.,* 2005). Jasmine type of rice is widdly cultivated in Middle East. There are a possibility of producing aromatic hybrids when crossed kind of aroma rice like Egyptian Jasmine rice with CMS lines (El-Keredy *et al.,* 2008 and Choi *et al*., 2018 ). This type of rice has quality of grain size, tast, point of milling, shape, volatile odor and cooking characteristics (Sakthivel *et al.,* 2009 and Civáň *et al.,* 2019), and shows a response to genetic and environmental improvement (Juliano and Duff, 1991 and Kishor *et al.,* 2020). More studies noted that a single recessive gene control aroma rice trait (Sood *et al.,* 1978 and Jin *et al.,* 2003). The fragrance type including Jasmine and basmati rice loss some enzyme function according to the development of *fragrance gene* in recessive nature of the trait (Kottearachehi *et al.,* 2010 and Liu *et al.,* 2017). The region of *fgr* sequence is placed between Japonica and Indica rice subspecies (Chen *et al*., 2006; Jimenez *et al.,* 2013 and Serba *et al.,* 2019.). These types of rice with using more technologies could be adapted to be more tolerant under some environmental stresses (Hashemi *et al.,* 2018), and showed superior grain quality (Kishor *et al.,* 2020).

## MATERIALS AND METHODS

### Plant materials

Experiments of this study were undertaken at Genetics Dept., Faculty of Agriculture, Tanta University, and Crops Dept., Faculty of Agriculture, Kafr El Sheikh University in 2018 and 2019 seasons. The study including Egyptian Jasmine rice obtained from Crops Department, Faculty of Agriculture, Kafr El Sheikh University, Egypt, and three

*Egypt. J. Genet. Cytol., 50:193-202, July, 2021*
Web Site (*www.esg.net.eg*)

Chines cytoplasmic male sterile lines (CMS) are shown in Table (1).

**DNA extraction for gene detection**

Total genomic DNA was extracted from fresh-frozen leaf tissues for the four samples using liquid nitrogen and Plant Kit-Bio Basic. Plants were harvested after 3 to 4 weeks from planting according to line, all samples were collected on ice to the Lab.

**PCR amplification**

Mixture of the polymerase chain reaction (PCR) included 1.2 µl from DNA template, 1.5 µl of 10X buffer, 1.2 µl of both forward and reverse primers as 0.5 µl (Table 2), 0.5 µl of Taq DNA polymerase, 0.75 µl of dNTPs and 9.25 of double distilled water (ddH$_2$O). The initial denaturation tempreture of DNA was at 94ºC for 5 min followed by 35 cycles of 1 min at 94ºC, 35 cycles at 67.3ºC of 1 min for annealing primers and 1 min to primer extention at 72ºC and final cycle for 10 min at 72ºC to complete total volume which was 15 µl in an ependorf tube. Electrophoresis of amplified PCR products were carried out in a 1.5 % agarose gel included a 0.5 µl of ethidium bromide. Restriction enzymes (RE) used in this study, were *EcoR1*, *BamH1* and *HindIII.*

**Determination of crude protein (CP) in rice grains**

The protein content of grains were determined according to Randhir and Pradhan (1981). Kjeldhal method was used an attempt to find a relation between the effect of hybridization and the grain crude protein (CP) content.

**RESULTS AND DISCUSSION**

Table (3) showed the phenotype characters evaluation. According to days to 50% heading trait, it ranged from 91.67 (D297) to 108.3 (E. Jasmine) days. Regarding to plant height trait, it ranged from D297 (84.67) to Hexi 41B (112.67 cm) while panicle length trait ranged between 17.83 ( Hexi 41B and Yimi 15B) and 21.83 cm for E. Jasmine. Number of filled grains per panicle trait ranged from 112.67 for D297 to 139.00 E. Jasmine and as for 1000 grain weight trait ranged from 27.50 g Yimi 15B to 23.03 gm. D297 as well as grain yield per plant trait ranged from 38.00 (Yimi 15B ) to 25.67 gm (D297). Table (3) showed the mean of morphological traits of crosses between E.Jasmine and the three CMS lines.

The heterosis clearly appered in traits such as days to 50% heading, number of unfilled grains/ panicle, number of filled grains/ panicle, number of pancicles/ plant, and 1000 grain weight. The result of the hybrid between EJR and D297 showed zero in the second generation.

Table (4) illustrates the total protein in grains for all parents and the resulting hybrids, where in EJR (6.91) was higher than both Hexi 41B (6.48) and Yimi15 B (6.15), while was superior in D297 (7.35 ). On the other hand, a marked result was found in the total protein for two hybrids 7.22 (Hexi41B X (EJR) and

7.70 Yimi15B X (EJR), while the top of them was 8.30 in D297 X EJR hybrid.

There is a strong relationship between nuclear genes and cytoplasmic genes, and their effect on traits together, they affect both phenotypic characters like plant hight, days to 50% heading, number of unfilled grains/ panicle, number of filled grains/ panicle, number of pancicles/ plant, and 1000 grain weight, where it can be seen, counted or estimated or their effect on the cell like protein appreciation. These characters were estimated for the CMS lines, hybrids, and Egyptian Giza 178 (El-Keredy *et al*., 2003). Rice under two levels of nitrogene grew and changed character in the similar Chinese lines of rice (Chu *et al*., 2004). The effect of crosses appeared in the number of unfiled grains/panicle compared to parents due to the influence of the hybrid. Aroma gene was placed on chromosome 8 of *Oryza sativa* L. (Ahuja *et al*., 2012) and it is special trait of rice, and a good quality including special amino acid (Sekhar *et al.*, 1982). The ratio of segregation of $F_2$ non to aromatic rice 3 :1 included the inheritance of gene in aromatic rice variety (Sun *et al*., 2008; Salgotra *et al*., 2011). The results indicate that the divergence presence between japonica and indica rice (Matsuoka *et al*., 2002; Garris *et al*., 2005; Bourgio *et al*., 2008 and Choi *et al*., 2018 ) in most morphological and field characteristics as in Table (4), the lowest appears with D297 x Jasmine but the highest in each Hexi41B x Jasmine and Yimi15B x Jasmine lins. Perhaps this is because both the Egyptian Jasmine rice (EJR) and D297 x Jasmine (DJR), have the same Indica origin (Kishor *et al*., 2020). Aromatic trait has been transmitted during the crosses process between Egyptian Jasmine rice (EJR)- aromatic and three Chinese cytoplasmic mail sterile (CMS) lines, so, expressing that the *fgr* gene transmission as in the gel (Fig. 1). Phenotypic and genotypic variation were found, and high number of *fgr* locus were detected in aromatic rice (Jewel *et al*., 2011; Aliyu *et al*,. 2013 and Civáň *et al.*, 2015). At molecular breeding level, fragrance trait inheritance in rice varieties, and using biotechnology has become the future of increasing aromatic rice productivity in Egypt (Civáň *et al*., 2019).

As Conclusion, Through breeding and crossbreeding programs between Egyptian or foreign aromatic and other non-aromatic rice, it is possible to produce local aromatic strains of rice that can bear Egyptian environmental conditions at lower costs. By interacting between nuclear and cytoplasmic inheritance, and the effect of one on the other, hybrids are bearing the ever-changing environmental and climatic conditions.

## SUMMARY

Aroma Egyptian Jasmine rice (EJR) is one of the most important types, It is one of the high quality rice varieties all over the world, have the fragrance gene *fgr*. Three cytoplasmic male sterile lines, and EJR were hybridized. The three cytoplasmic male sterility Chinese lines were:

*Hexi41B*, *Yimi15B* and *D297* and their crosses were *Hexi41B* x E.Jasmine (*HJR*), Yimi15B x E. Jasmine (*YJR*) and *D297* x E. Jasmine (*DJR*). The results showed that the male sterile lines outperform the Egyptian Jasmine rice in most of morphological trail such as; 1000 grain weight, plant height, number of panicles plant, and grain yield for plant traits. For identification of *fgr* gene primers were used in PCR reaction, the *fgr* gene was identified in the Egyptian Jasmine rice (EJR) and its hybrid lines. The gene was also digested using three different restriction enzymes (RE) such as; *EcoR1, BamH1 and Hind III*. By estimating the total amount of protein in the grains, the result noticed that it reached 6.91 in the Egyptian Jasmine rice (EJR) and about 6.91(grain with cover), while it reached the highest amount in DJR hybrid as crosse between the Egyptian Jasmin rice (EJR) and male sterility lines leads to *fgr* gene transfer enhancement and increase of protein content in rice grains which reached up to 8.3 in *D297* x Egyptian Jasmine rice hybrid (EJR). The main target of this study was to determine the fragrance gene (*fgr*) from EJR in cytoplasmic male strile (CMS) rice after crosses between them and the EJR, that resulting in changes of charachterstics after transferring the gene to these CMS lines.

## ACKNOWLEDGMENTS

We would like to thank you to Prof. El-Keredy, M. S., Professor of Plant Breeding, Department of Crops, Faculty of Agriculture, Kafr El Sheikh University, Egypt. To supply plant of Chinese lines of rice from China. Also thanks to Research Fund, Tanta University for the financial facilities required to develop the research work. Special thanks to the Animal Production Department for using Biotechnology Lab. to perform the Laboratory part.

## REFERENCES

Ahuja K. P., Jain U. and Jain .R. K., (2012). Fragrance analysis using molecular and biochemical methods in recombinant inbred lines of rice. African Journal of Biotechnology, 11 (91): 15784-15789.

Aliyu R. E., Wasiu A. A., Dangora D. B., Ademola J. O. and Adamu A. K., (2013). Comparative study of genomic DNA extraction protocols in rice species. International Journal of Advancements in Research & Technology, Vol.2, (2):ISSN: 2278-7763.

Bhat A. J., Ali. S., Salgotra R. K., Mir Z. A., Dutta S., JadonV., Tyagl A., Mushtaq M., Jain N., Singh P. K., Singh G. P. and Prabhu K. V., (2016). Genomic Selection in the Era of Next Generation Sequencing for Complex Traits in Plant Breeding. Frontiers in Genetics, Vol. 7: 221.

Bhattacharjee P., Singhal R. S. and Kulkarni P. K., (2002). Basmati Rice: A Review, Int. J. Food Sci., Technol., 37: 1.

Bourgis F., Guyot R.,Gherbi H., Talliez E., Amabile I., Salse J., Lorieux M., Delserry M. and Ghesquiere A., (2008). Characterization of the major fragance gene from an aromatic japonica rice and analysis of its diversity in Asian cultivated rice. Theor. Appl. Genet., 117: 353-368.

Bradbury L. M., Fitzgerald T. L., Henry R. J., Jin Q. and Waters D. L. E., (2005). The gene for fragrance in rice. Plant Biotechnology Journal, 3: 363-370.

Chen S., Wu. J., Yang Y., Shi W. and Xu M., (2006). The fgr gene responsible for rice fragrance was restricted within 69kb. Plant Sci., 171(4):505-14.

Choi J. Y., Groen S. C., Zaaijer S. and Purugganan M. D., (2018). Nanopore sequence-based genome assembly of the basmati rice. bioRxiv, 396515. doi: 10.1101/396515.

Chu S. C., Lee H. H., Ryu S. N. and Koh H. J., (2004). Grain characteristics and inheritance of green-kerneled rice (*Oryza sativa* L.). Korean J. Breed, 36(4) : 222-228 .

Civáň P., Craig H., Cox C. J. and Brown T. A., (2015). Three geographically separate domestications of Asian rice. Nat. Plants 1: 15164-15164. doi: 10.1038/nplants.2015.164.

Civáň P., Ali S., Batista-Navarro R., Drosou K., Ihejieto C., Chakraborty D., (2019). Origin of the aromatic group of cultivated rice (*Oryza sativa* L.) traced to the Indian Subcontinent. Genome Biol. Evol., 11 (3): 832-843. doi:10.1093/gbe/evz039.

El-Keredy M. S., El-Weheishy M. M., El-Degwy I. S., Tan X. L. and Lijuan C., (2008). Combining ability of some hybrid rice varieties with Chinese bred cytoplasmic male sterile lines. Journal of Agricultural Research Kafr Elsheikh University Vol.34 No (1): 11-22.

El-Keredy M. S., Abdel Hafez A. G., El-Weihishy M. M. and El-Degwy I. S., (2003). Potential of hybrid rice production under Egyptian conditions, Egypt. J. Plant Breeding, 7(1): 471-486.

Garris J. A., Tai H., Coburn T., J., Kresovich S. and McCouch S., (2005). Genetic and structure and diversity in *Oryza sativa* L. Genetics society of America, 169: 1631-1638.

Hashemi F. S. G., Ismail M. R., Rafii M. Y., Aslani F., Miah G. and Muharam F. M., (2018). Critical multifunctional role of the betaine aldehyde dehydrogenase gene in plants.

Jewel Z. A., Patwary A. K., Maniruzzaman S., Barua R. and Begum S. A., (2011). Physico-chemical and genetic analysis of aromatic rice

(*Oryza sativa* L.) germplasm. The Agriculurists 9 (1& 2): 82-88

Jimenez, F. R., Maughan. P. J., Alvarez.,A., Kietlinski K. D., Smith. S. M., Pratt D. B., et al (2013). Assessment of genetic diversity in Peruvian Amaranth (Amaranthus caudatus and A. hybridus) germplasm using single nucleotide polymorphism markers. Crop Sci., 53: 532-541. doi: 10.2135/crop sci.2012. 07.0413.

Jin Q. S., Waters D., Cordeiro G. M., Henry R. J. and Reinke R. F., (2003). A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.) plant Sci., 165: 359-364.

Juliano B. O. and Duff B., (1991). Rice grain quality as an emerging priaity in national rice. Rice Grain Quality Research, :55-64.

Kishor D. S. J., Chin J. H. and Jong K. H. (2020). Evaluation of whole-genome sequence, genetic diversity, and agronomic traits of Basmati rice (*Oryza sativa* L.). Frontiers in Genetics, 11: Article 86.

Kottearachehi N., S., Priyangani E. G. D. and Attanayaka D. P. S. T., (2010). Identification of fragrent gene, *fgr*, in traditional rice varieties of Srilanka. J. Natn. Sci. Foundation Srilanka, 38 (2): 139-143.

Liu W., Ghouri F., Yu H., Li X., Yu S., Shahid M. Q., (2017). Genome wide re-sequencing of newly developed Rice Lines from common wild rice (*Oryza rufipogon* Griff.) for the identification of NBS-LRR genes. PloS One 12 (7), e0180662. doi: 10.1371/Journal.Pone.0180662.

Matsuoka Y., Vigouroux Y., Goodman M. M, Sanchez G. J.. and Buckler E., (2002). A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci., USA 99: 6080-6084.

Randhir S. and Pradhan K., (1981). Forage evaluation. First Published, Printox, New Dslhi, Dhawan Printing works.

Sakthivel K., Rani N. Shobha, Pandey M. K., Sivaranjani A. K. P., Neeraja C. N. and Balachandran S. M., (2009). Development of a simple functional marker for fragrance in rice and its validationin IndianBasmati and non-Basmati fragrant rice varieties. Mol. Breed., 24 (2): 185-190. doi: 10.1007/s11032-009-9283.

Salgotra R. K, Millwood R. J., Agarwal S. and Stewart Jr.C. N., (2011). High-throughput functional marker assay for detection of Xa/xa and *fgr* genes in rice (*Oryza sativa* L.). Electrophoresis, 32: 2216-2222.

Sekhar B. P. S and Reddy G. M., (1982). Amino acid profiles in some scented rice varieties. Theoretical and Applied Gentics, 62: 35-37.

Serba D., Muleta K., Amand P., Bernardo A., Perumal R. and Bashir E., (2019). Genetic diversity, population structure, and linkage disequilibrium of pearl millet. Plant Genome. 12, 180091. doi: 10.3835/plantgenome2018.11.0091

Shehata S. M, Ammar M. H., Abdelkalik A. F. and Zayed B. A., (2009). Morphological, molecular and biochemical evaluation of Egyptian Jasmine rice variety and its M 5 derived mutations. Africa Journal of Biotechnology, 8 (22): 6110-6116.

Shobha Rani N., Pandey M. K. and Prasad I., (2006). Historical significance, grain quality features and precision breedind for improvement of export quality basmati varieties in India. J. crop Sci., :29-41

Sood B. C. and Siddiq E. A., (1978). A rapid technique for scent determination in rice. Indian J. Genet. Plant Breed., 38: 268-271.

Sun S. X., Gao F.Y., Lu X. J., Wu X. J., Wang X. D. and Ren G. J., (2008). Genetic analysis and gene fine mapping of aroma in rice (*Oryza sativa*.L. Cyperales, poaceae). Genetics and Molecular Biology 31 2: 532-538.

Wang Y. and Li J., (2005). The plant architecture of rice (*Oryza sativa*). Plant Mol. Biol., 59 (1): 75-84. doi: 10.1007/s11103-004-4038-x.

Widjaja R., Craske D. J. and Wootton M., (1996). Comparative studies on volatile components on-fragrant and fragrant Rices. J.S.c.i. Food Agric., 70: 151-161.

Yoshihashin T., (2002). Quantitative analysis on 2-Acetyl-1-pyrroline of an aromatic rice by stable isotope dilution method and models studies on its formation during cooking. Food Chemistry and Toxicology, 67: 619-622.

Table (1): The name of CMS rice lines, cytoplasmic source and their variety types.

| Name of line | Cytoplasmic source | Variety type |
|---|---|---|
| Hexi41B | Dian type | Japonica |
| Yimi15B | Dian type | Japonica |
| D297 | Dissi | Indica |

Table ( 2): The DNA primer sequences which were used for *fgr* gene detection.

| Primer No.# | sequence |
|---|---|
| 1 | **F-** 5′- TGC TCC TTT GTC ATC ACA CC- 3′ |
| | **R-** 5′- TTT CCA CCA AGT TCC AGT GA-3′ |
| 2 | **F-** 5′- GAG TGA GCT TGG GCT GAA AC-3′ |
| | **R-** 5′- GAA GGC AAG TCT TGG CAC TG-3′ |
| 3 | **F-** 5′- GACGGTGAACATTCAATTAAAAAG-3′ |
| | **R-** 5′- AGTGGGATTTCATTAATTTCCTG-3′ |
| 4 | **F-** 5′- CGGCCACTCTCCTGGGTTGCAGAATC-3′ |
| | **R-** 5′- CATTCCCCACCATTCATCCCATGG-3′ |

Table (3): The mean of the different morphological traits of the four parents.

| parents | Days to 50% heading | Plant height (cm) | Panicle length (cm) | No. of un filled grains/ panicle | No. of filled grains/ panicle | No. of panicles/ plant | 1000-grain weight | Grain yield (g/ plant |
|---|---|---|---|---|---|---|---|---|
| E.Jasmine | 108.3 | 96.33 | 21.83 | 21.67 | 139.00 | 14.30 | 24.50 | 33.06 |
| Hexi 41B | 98.67 | 112.67 | 17.83 | 13.00 | 132.67 | 16.33 | 26.13 | 34.67 |
| Yimi 15B | 97.67 | 96.33 | 17.83 | 18.00 | 119.00 | 47.50 | 27.50 | 38.00 |
| D297 | 91.67 | 84.67 | 20.67 | 16.67 | 112.67 | 21.33 | 23.03 | 25.67 |
| **Crosses** | | | | | | | | |
| Hexi 41B x Jasmine | 119.50 | 103.89 | 23.55 | 95.22 | 87.89 | 19.44 | 24.04 | 26.44 |
| Yimi15B x Jasmine | 129.80 | 103.00 | 25.72 | 58.10 | 113.20 | 26.6 | 22.25 | 37.80 |
| D297 x Jasmine | 122.66 | 79.00 | 26 | 198.66 | 14.00 | 28.67 | 23.67 | - |

Table (4): Total protein in different rice lines.

| Parent/cross | Name | Total protein (grain with cover) |
|---|---|---|
| Parent | 1-Egyptian Jasmine | 6.91 |
| | 2- Hexi 41 B | 6.48 |
| | 3- Yimi 15 B | 6.13 |
| | 4- D 297 | 7.35 |
| Cross | 1- Hexi 41 B X Jasmine (HJR) | 7.22 |
| | 2- Yimi 15 B X Jasmine (YJR) | 7.70 |
| | 3- D 297 X Jasmine (DJR) | 8.30 |

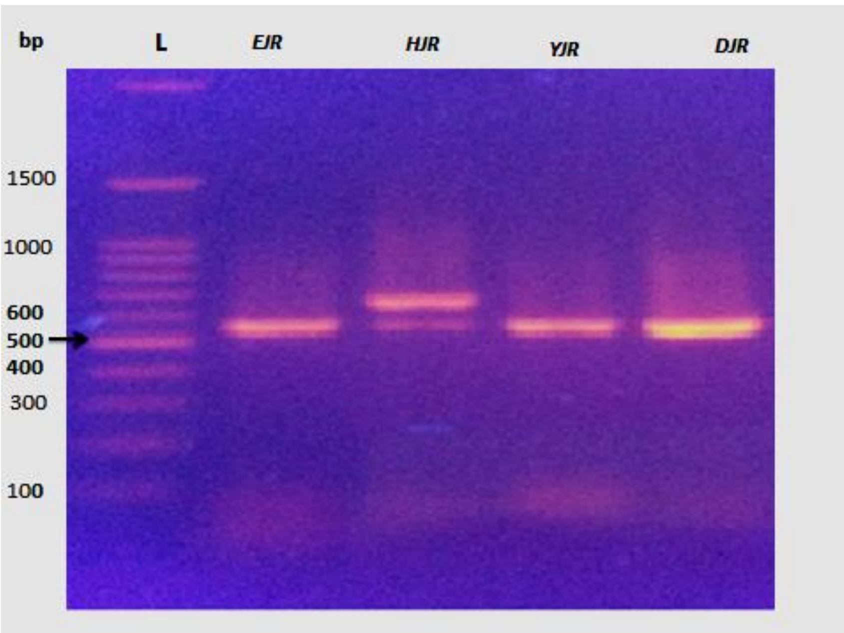


Fig. (1): PCR product of *fgr* gene in EJR and its crosses with the three Chinese CMS lines.