QTL VALIDATION FOR GRAIN YIELD AND NITROGEN USE EFFICIENCY UNDER DIFFERENT NITROGEN LEVELS IN RICE

M. A. ABDEL-RAHMAN[1], M. E. EL-DENARY[2], M. H. AMMAR[1], A. F. ABDELKHALIK[1], A. E. DRAZ[1], S. A. DORA[3]

1. *Rice Biotechnology Lab., Rice Research and Training Center, Egypt*
2. *Department of Genetics, Faculty of Agriculture, Tanta University, Egypt*
3. *Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Egypt*

Rice, *Oryza sativa* L. is one of the most important and strategic cereal crops for the majority of world countries especially the over populated areas in Asia, Africa and South America (IRRI, 1994). It is a stable food in many countries and constitutes a major part of the diet of many others and provides over 25% of the global caloric intake (Mbabaali, 1998). It is also considered as the main food for all human ages because of its grain's richness in proteins, minerals, vitamins and fibers (Alam *et al*., 1998).

Nitrogen (N) is a crucial macronutrient and is needed in the greatest amount of all mineral elements required by plants. It comprises 1.5-2% of plant dry matter and approximately 16% of total plant protein (Frink *et al*., 1999). Plants consume much less than half of the applied fertilizers applied (Frink *et al*., 1999; Socolow, 1999), while the majority of N fertilizers is lost to the atmosphere or leached into groundwater, lakes and rivers, which increasingly causes increasingly severe pollutions to the environment. United Nations Environment Programme reported that, worldwide, N pollution along with water shortage and global warming poses the main threats to human survival and the environment (UNEP, 1999). Moreover, fertilizer application has now become the major cost in crop production, which greatly affects the income of the farmers. Thus, developing crops that are less dependent on the heavy application of N fertilizers is essential for the sustainability of agriculture. In most of the rice producing regions of the world, nitrogen is one of the most yield limiting nutrients for rice production.

Demand for low-input sustainable crop cultivation is increasing to meet the need for environment-friendly agriculture. Technically, this could be achieved through the development of crop varieties that can withstand soils of low N concentration by managing sufficient uptake (high uptake efficiency), and making best use of the N nutrient that the plant has absorbed from the soil for producing the products (high utilization efficiency).

N-fertilizer is not used efficiently because rice grows in an environment that is conducive to N losses i.e., through nitri-

Egypt. J. Genet. Cytol., 44:235-251, July, 2015
Web Site (*www.esg.net.eg*)

fication-denitrification, ammonia volatilization, runoff, and leaching. Genetic selection and plant breeding techniques to improve the rice crop's nitrogen use efficiency (NUE) has not yet been done (Singh *et al*., 1998). One of the critical steps limiting the efficient use of nitrogen is the ability of plants to acquire it from applied fertilizer. Therefore, the development of crop plants that absorb and use nitrogen more efficiently has been a long-term goal of agricultural research (Shrawat *et al*., 2008). Field evaluation is an important step in the evaluation of crop genotypes for mineral stresses and their subsequent uses in breeding programs.

Genetic improvement of rice for NUE would reduce N inputs and maintain high yield, which would be an effective way to reduce the environmental pollution and the cost of rice production (Shan *et al*., 2005; Cho *et al*., 2007). In recent years, molecular markers attracted breeders to use them in different purposes in crop improvement. Genetic mapping of simple traits, quantitative trait loci (QTL) mapping, utilization of markers linked to the traits of interest (marker assisted selection MAS), could result in saving time for selection and accuracy. These markers have many advantages in plant breeding since the genetic material could be handled without the effect of the environment. Moreover, they save a lot of money needed for long and very expensive field evaluation.

QTL validation is a prerequisite for MAS establishment for a particular quantitative trait. QTL validation is generally known as the verification of QTL effectiveness in different genetic backgrounds or environment (Langridge *et al.*, 2001; Swamy *et al.*, 2012).

The main objective of this study was to validate QTLs for grain yield and nitrogen use efficiency traits under four nitrogen treatments using association analysis as well as single marker analysis.

MATERIAL AND METHODS

Plant material

A set of 55 rice genotypes were selected including local traditional and exotic accessions representing rice subspecies (japonica, indica, indica japonica and tropical japonica). The list, type, pedigree and origin of selected genotypes are presented in Table (1).

Field evaluation

Experiment was laid out at the farm of Rice Research and Training Center (RRTC), Sakha, Kafrelsheikh, Egypt, in a split plot design with three replicates. Main plots were occupied by four nitrogen treatments (Urea form); no nitrogen, low nitrogen, medium nitrogen, and recommended dose of nitrogen (i.e. 0, 20, 40, and 60 Kg N/Fed, respectively), while genotypes were assigned to the subplots. Nitrogen was applied in two splits; 2/3 of nitrogen amount was applied and ported in the dry soil before flooding as basal application, and the rest was added after 30 days from transplanting. Other agronomic

practices were followed according to RRTC.

Based on the obtained data, plant height (PH), days to heading (DAH), panicle length (PL), panicle weight (PW), number of filled grain panicle^{-1} (NFG) and weight of filled grains panicle^{-1}(WFG), 1000-Grain weight (1000-GW), biomass (biological) yield (BY), grain yield (GY), straw yield (SY), and harvest Index (HI) traits were recorded according to the standard evaluation system for rice crop (IRRI-SES., 1996). Total nitrogen content was determined in grains as well as straw at harvest using orange-G dye method according to Hafez and Mikkelson (1981).

Then nitrogen uptake in grains, apparent recovery efficiency of applied nitrogen (ARE), N utilization efficiency for grain production or physiological nitrogen use efficiency (PNUE), fertilizer NUE (FNUE), NUE for biomass production (NUEb), Partial factor productivity (PFP) of N fertilizer, N Harvest Index (NHI) and N productivity ratio (NPR) were estimated according to Peng and Bouman (2007).

Molecular analysis

Molecular analysis was conducted at Rice Biotechnology Lab. (RBL), at RRTC. Leaves were collected from individual plants for each genotype. Genomic DNA was extracted following Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980). DNA quantity and quality were assessed using spectrophotometry and agarose gel electrophoresis with known concentrations of Lambda un-cut genomic DNA. The samples were diluted in T10E1 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to a final DNA concentration of 20 ng/µl for amplification.

The PCR amplification was carried out according to the manufacturer (ROVALAB 2x Red PCR Master Mix, Kantstr, Germany). The PCR analysis was performed in a thermal cycler (PerkinElmer, Geneamp PCR system 2400) as per following cycling traits: initial denaturation at 94°C for 5 min., followed by 35 cycles of (denaturation at 94°C for 1 min., annealing at 55°C for 30 sec. and extension at 72°C for 30 sec.) and final extension at 72°C for 7 min. PCR products were resolved on 2% agarose gels (CBS Scientific, USA) with ethidium bromide (0.5 µg/ml). The gels were visualized and photographed using a gel documentation system (Biometra, Biodoc Analyze) to detect polymorphism. Size of the amplified DNA fragments was determined based on the migration relative to a molecular size of marker (50 bp DNA ladder, MBI Fermentas).

The fifty five selected genotypes used in this study were subjected to DNA polymorphism screening and assessment using four simple sequences repeats (SSR) pair of markers. SSR markers used in this study are linked to QTLs for GY and NUE according to Senthilvel *et al.* (2004) and Cho *et al.* (2007). The markers sequences as well as other information were directly downloaded from gramene website

(www.gramene.org), and were as presented in Table (2).

Statistical analysis

For molecular statistical analysis, PCR fragments were scored as 1 for present and 0 for absent. Fragments that could not be reliably scored were declared as missing values. PCR fragments were tested for association with phenotypic data using Mann-Whitney-U test, as well as, linear regression analysis to estimate the coefficient of determination using SPSS® software package.

In regard to Mann-Whitney-U test, probability was estimated according to Wheater and Cook (2000), since the two values of U were calculated as follow:

$$U1= n1n2+\{n1(n1+1)/2\}-\sum R1$$

$$\text{and } U2= n1n2+\{n2(n2+1)/2\}-\sum R2$$

Where: n1 and n2 are the numbers of data in the samples 1 and 2, $\sum R1$ and $\sum R2$ are the sums of the values of samples 1 and 2. Then U1 and U2 values were compared to the table of U values.

Concerning regression analysis, coefficient of determination was calculated according to Gomez and Gomez (1984) with the following formula: $R^2= SSR/\sum y^2$. Where R^2 is the coefficient of determination, SSR is the sum of squares due to regression, and $\sum y^2$ is the Sum of squares of deviation of the values of dependent variable from their means. To test the significance of R^2, the F values were computed as follow; $F = \{SSR/k\}/\{SSE/(n-k-1)\}$, where k and n are numbers of the independent and dependent variables, and SSE is the residual sum of squares.

RESULTS AND DISCUSSION

PCR procedure was conducted using four simple sequence repeats markers, namely; RM223, RM246, RM242, and RM72. These markers are known to be linked to grain yield as reported by Senthilvel *et al.* (2004) for RM223 and RM246 markers and by Cho *et al.* (2007) for RM242 and RM72 markers, in addition, RM246 marker is also linked to QTL for NUE trait. The amplified DNA fragments generated using these markers are shown in Figs (1, 2, 3 and 4). High polymorphism was revealed by SSR markers insuring the existence of significant genetic variations among the tested genotypes. The generated alleles, their abbreviations, expected and observed sizes, number of bearing genotypes and their frequencies are presented in Table (3). The four SSR primers generated 13 alleles and number of generated alleles ranged from two and five for RM223 and RM72, respectively.

Two different statistical analyses were used in this study. The first one was Mann-Whiteny-U test, which was used for marker-trait association test. This statistical analysis had been used before by Gebhardt *et al.* (2004), when they studied the association between five markers with the phenotypic data for a germplasm of potatoes. The second one was the linear regression based single-marker analysis to measure the coefficient of determination (R^2), which was used to estimate the de-

gree of relation between the marker fragment and the associated trait, according to Acquaah (2007). This statistical analysis was also used by Chaitra *et al.* (2006) to conduct a QTL validation experiment for two markers linked to QTLs associated with root length in rice.

RM223 marker

The RM223 marker revealed two DNA fragments with fragment sizes of 167 and 158 bp (Table 3 and Fig. 1), which were abbreviated by a1 and a2, respectively. These two fragments sizes were in the range of the expected fragment size of tis loci (165 bp). On the other hand, as shown in Fig. (1), some other fragments with sizes between 305 and 315 bp were appeared on the gel, these fragments were not discussed, because they were not in the range of the expected fragment size and they did not have reproducible patterns. The results showed that the presence of the two fragments of this marker was associated with nine of the studied traits, these traits were GY, 1000-G, SY, TDW, NUEB, NPR, DAH, PL and PFP, the remained traits under study were not associated with this marker as listed in Table (4).

There were different degrees of association between the two fragments and the different traits under the different N-treatments, since it is well known that most of the QTLs are not stable under different environments. The first fragment (a1) showed non-significant association with NUEb and DAH traits, similar results were obtained by the regression analysis. At the same time the first fragment (a1) showed significant association with SY trait under all treatments, except under the lowest N treatment, which was highly significant.

Highly significant associations with low R^2 values were obtained for the association between a1 and a2 fragments and the PFP trait (0.12 for both), under only the low-N treatment. Similar associations were obtained between the two fragments and grain yield (GY), since the two fragments showed significant and highly significant associations (with low R^2 values) with (GY) under only zero and low-N treatments.

The two fragments (a1 and a2) showed significant and highly significant association with total dry weight (TDW) and (PL) traits. Regarding the a1 fragment, R^2 values were ranged between 0.098 and 0.16 for the association with TDW and between 0.11 and 0.13 for the association with PL trait. While in respect to the a2 fragment, R^2 values were ranged between 0.084 and 0.15 for the association with TDW trait and were ranged between 0.12 and 0.17 for the association with PL trait. Also, significant and highly significant associations were recorded between the two fragments and the (1000-GY) and (SY) traits under most of the N-treatments.

RM246 marker

Regarding RM246 marker, three fragments with sizes of 130, 121 and 110 bp which were abbreviated by b1, b2 and

b3, respectively were obtained as shown in Fig. (2) and Table (3).

The results showed that this marker was associated with only six traits. The first fragment (b1) showed non-significant association with all the studied traits, except for 1000-GW trait which was significant with low R^2 values under zero and medium-N treatments and highly significant under lowest N treatments as shown in Table (5).

The other two fragments (b2 and b3) showed highly significant associations with high R^2 values with the NPR and the PL traits under all N-treatments, while it was highly significant in most of the N-treatments for PNUE trait.

The second fragment (b2), showed highly significant association with GY, HI and 1000-GW traits. In contrast, the third fragment (b3) showed no significant association with 1000-GW trait, but it was significant with GY only under the lowest N-treatment. At the same time, it showed non-significant associations in most of the N-treatments in regard to HI, except under the medium-N treatment which was significant with very low R^2 value (0.076). On the other hand, there were no associations between these fragments and the other studied traits. Also some other fragments with different sizes were appeared on the gel, but were not discussed, where their sizes were out of the range of the expected fragment size (116 bp).

RM242 marker

Using the RM242 marker, three different DNA fragments with sizes of 237, 225 and 215 bp which were abbreviated by c1, c2 and c3, respectively were noticed as shown in Fig. (3) and Table (3). Only the first (c1) and the third (c3) fragments were used to conduct the association and the regression analysis, since the frequency of the second fragment (c2) was low to be used in the statistical analysis (10.91%) as shown in Table (3). This marker revealed homozygous fragments for all the tested genotypes, except genotype No. 29, which was heterozygous for this locus and contained the two fragments c1 and c3.

These two fragments were associated with only five of the studied traits as listed in Table (6), while there was no association between this marker and the other studied traits. The two fragments (c1 and c3), showed highly significant associations with 1000-GW and PL with high R^2 values across the different N-treatments. Regarding the first fragment (c1), R^2 values were ranged between 0.13 and 0.23 for 1000-G trait and between 0.13 and 0.18 for PL trait, while with c3 fragment, these values were ranged between 0.108 and 0.19 for 1000-G and between 0.26 and 0.36 for PL.

RM72 marker

For RM72 marker, five fragments with sizes of 180, 172, 165, 160 and 152 bp and abbreviated by d1, d2, d3, d4 and d5, respectively, were obtained as shown

in Table (3) and Fig. (4). The d4 and d5 fragments were excluded when conducting the statistical analysis, because of their low observed frequency among the tested genotypes (7.27 and 3.64%, respectively) as shown in Table (3).

Nine traits were associated with this marker as listed in Table (7). There were no associations between RM72 marker and the other studied traits. The largest fragment (d1) showed highly significant association with 1000-G and PNUE traits in the different N-treatments. The R^2 value was highly significant in both traits, which ranged between 0.21 and 0.31for 1000-G trait, and between 0.15 and 0.19 for PNUE trait. On the other hand, there were significant associations between this fragment and NPR trait in the different N- treatments with highly significant R^2 values which ranged between 0.12 and 0.16.

In contrast to the other fragments, the second fragment (d2) showed significant association with DAH trait, while different significant and highly significant associations had been obtained between this fragment and the PL trait. On the other hand, there were no significant associations between this fragment and all other traits, except for FGNP, which was highly significant under the medium and high-N treatments only.

The third fragment (d3) showed highly significant association with GY, FGNP, PNUE (except under the highest-N treatment, it was significant), NPR, PL, and PFP traits, under most of the N-treatments, while it was significant and highly significant in regard of 1000-GW trait. On the other hand the highest R^2 value (0.26) was recorded for PL.

From these results, it could be concluded that for all the QTL validated there were association between one or more of the markers fragments with GY and with one or more of the grain yield related traits and the NUE components.

Also, it could be concluded that the a2, b2, b3, c1 and c3 fragments were superior in their association with PL, but b3 and c3 were the best in this regard. Moreover, b2, b3, d1 and d3 fragments were superior also in their association with NPR trait. Regarding GY trait, the b2 and d3 fragments showed highly significant association and b2 was better than d3. Also, fragments c1 and d1 were highly associated with 1000-g, but d1 was better than c1. On the other hand, d3 expressed the best association with FGNP.

These superior fragments could be utilized in the marker assisted selections MAS programs for different GY and NUE under different nitrogen treatments. The b2 fragment obtained by RM246 is very promising allele for its association with GY and PNUE under no nitrogen treatment as well as the highest N treatment. This DNA fragment should be isolated for DNA sequencing and further studies to detect more information about the importance of this allele.

Therefore, the genotypes which hold marker fragments that exhibited su-

perior association with important GY and/or NUE related traits could be utilized in the breeding programs for low N inputs and high yielding as well as high NUE traits.

SUMMARY

High yielding rice genotypes with high N use efficiency is very important for agriculture sustainability. The study aimed to validate QTLs linked to grain yield (GY) and Nitrogen Use Efficiency (NUE) related-traits using association analysis and single marker analysis in order to identify marker alleles that are associated with target trait under different nitrogen environments. Fifty five rice genotypes were phenotyped for GY and NUE related traits under four different nitrogen treatments (i.e. 0, 20, 40, and 60 Kg N/Fed, respectively). The fifty five rice genotypes were genotyped using four SSR (RM223, RM246, RM242 and RM72) marker linked to QTLs for GY and NUE.

Results showed that different degrees of association between the markers fragments and the different traits under the different N-treatments were found. For RM 223, the obtained two fragments (a1 and a2) showed significant and highly significant association with total dry weight (TDW) and (PL) under all N environments. Regarding RM246 marker, b2 and b3 fragments showed highly significant associations with high R^2 values with PNUE, NPR and PL traits under all N-treatments. The b2 fragment showed highly significant association with high R^2 values for GY, HI and 1000-GW traits.

The RM242 fragments (c1 and c2), showed highly significant association with 1000-G and PL traits with high R^2 values across the different N-treatments. Regarding the first fragment (c1), R^2 values were ranged between 0.13 and 0.23 for 1000-G trait as well as between 0.13 and 0.18 for PL trait, while with c3 fragment, these values were ranged between 0.108 and 0.19 for 1000-G trait and between 0.26 and 0.36 for PL trait. For RM72 marker d1 fragment showed highly significant associations with 1000-GW, PNUE and NPR traits in the different N-treatments, and the R^2 values were ranged between (0.21- 0.31), (0.15- 0.19) and (012-016), respectively. On the other hand there were highly significant association between RM72 d3 fragment and GY, NPR and PL with R^2 values were ranged between (0.12-0.18), (0.16-0.19) and (0.15-0.26), respectively. These results will assessed in initiating marker-assisted breeding program for NUE and GY traits under low treatments of nitrogen fertilizers.

ACKNOWLEDGMENT

This work had been funded by National Rice Program of Rice Research and Training Center (RRTC), Field Crop Research Institute (FCRI), Agricultural Research Center, Giza, Egypt. The authors would like to thank Agronomy department of RRTC for their support in chemical analysis.

REFERENCES

Alam, M. F., K. Datta, E. Abrigo, A. Vasquex, D. Senadhira and S. K.

Datta (1998). Production of transgenic deepwater indica rice plants expressing a synthetic *Bacillus thuringiensis cryIA*(b) gene with enhanced resistance to yellow stem borer. Plant Science, 135: 25-30.

Acquaah, G. (2007). The principles of plant genetics and breeding. Blackwell Publishing.

Chaitra, J., M. S. Vinod, N. Sharma, S. Hittalmani and H. E. Shashidhar (2006). Validation of markers linked to maximum root length in rice (*Oryza sativa* L.). Current Science, 90: 835-838.

Cho, Y., J. WenZhu, C. JoongHyoun, P. ZhongZe, C. YongGu, S. R. McCouch and K. HeeJong (2007). Identification of QTLs associated with physiological nitrogen use efficiency in rice. Molecules and Cells, 23: 72-79.

Frink, C. R., P. E. Waggoner and J. H. Ausubel (1999). Nitrogen fertilizer: retrospect and prospect. Proc. Natl. Acad. Sci. USA, 96: 1175-1180.

Gebhardt, C., A. Ballvora, B. Walkemeier, P. Oberhagemann and K. Schüler (2004). Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. Molecular Breeding, 13: 93-102.

Gomez, K. A. and A. A. Gomez (1984). Statistical procedures for agricultural research (2nd ed.). John Wiley and sons, New York

Hafez, A. A. R and D. S. Mikkelson (1981). Colorimetric determination of Nitrogen for evaluating the nutrition status of rice. Soil Sci. and Plant Analy., 12: 61-69.

IRRI (1994). Program Report for 1993. IRRI, Manila, Philippines.

Langridge, P., E. Lagudah, T. Holton, R. Appels, P. Sharp and K. Chalmers (2001). Trends in genetic and genome analyses in wheat: a review. Aust. J. Agric. Res., 52: 1043-1077.

Mbabaali, S. (1998). Supply and demand for rice: a medium- and longer-term perspective, In: Proceedings of the 19th Session of the International Rice Commission, FAO.

Murray, A. A. and W. F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res., 8: 4321-4325.

Peng, S. and B. A. M. Bouman (2007). Prospects for genetic improvements to increase lowland rice yields with less water and nitrogen. Scale and Complexity in Plant Systems Research: Gene-Plant Crop Relations, 249-264.

Senthilvel, S., P. Govindaraj, S. Arumugachamy, R. Latha, P. Malarvizhi, A. Gopalan and M. Maheswaran (2004). Mapping genetic loci associated with nitrogen use efficiency in rice (*Oryza sativa* L.). New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress.

Shrawat, A. K., R. T. Carrol, M. DePaum, G. J. Taylor and A. G. Good (2008). Genetic engineering of improved nitrogen use efficiency in rice by the tissue-specific expression of alanine aminotransferase. Plant Biotechnology Journal, 6: 722-732.

Swamy, B. M. and A. Kumar (2012). Sustainable rice yield in water short drought prone environments: conventional and molecular approaches. In Lee T. S. (ed.). Irrigation systems and practices in challenging environments. INTECH Publishers, Croatia, p. 149-168.

Shan, Y. H., Y. L. Wang and X. B. Pan (2005). Mapping of QTLs for nitrogen use efficiency and related traits in rice (*Oryza sativa* L.). Sci. Agric. Sin., 4: 721-727.

Singh, U., J. K. Ladha, E. G. Castillo, G. Punzalan, A. Tirol-Padre and M. Duqueza (1998). Genotypic variation in nitrogen use efficiency in medium- and long-duration rice. Field Crops Research, 58: 35-53.

Socolow, R. H. (1999). Nitrogen management and the future of food: lessons from the management of energy and carbon. Proc. Natl. Acad. Sci. USA, 96: 6001-6008.

UNEP (1999). Global Environment Outlook 2000. United Nations Environment Programme and London Earthscan, Nairobi, Kenya.

Wheater, C. P. and P. A. Cook (2000). Using statistics to understand the environment. London, New York: Routledge

Table (1): Name, type, parentage and origin of the selected rice genotypes.

Origin	Parentage	Type	Genotype	No..
EGYPT	Selection from Introductions	J	Sabieny	1
EGYPT	Selection from Agami M1	J	Nabatat Asmar	2
EGYPT	Giza14/Agami M.1	J	Giza 159	3
EGYPT	Selection from Introductions	J	Yabani LuLu	4
EGYPT	GZ4120/Suweon349	J	GZ 5830-59-10-2	5
EGYPT	Yabani Pearl/Iraki16	J	Giza 14	6
EGYPT	Sakha101/HR4856-1-1-2	J	GZ 7718-13-3-1-3	7
EGYPT	Selection from Introductions	J	Nahda	8
EGYPT	GZ4122-23-4-2/IRI396	J	GZ 6214-4-1-1-1	9
EGYPT	Giza176/Milyang79	J	Sakha 101	10
IRRI	JINMIBYEO/YR14987-91	T.J.	IR 68373-R-R-B-22-2-2	11
EGYPT	Giza181/IR39422//Giza181	I	Giza 182	12
EGYPT	Giza177/IDSA	J	GZ 7922-B-44-1	13
INDIA	India selection	I	Pusa Basmati 1	14
EGYPT	GZ4096/GZ4100	J	Sakha 104	15
IRRI	IR8333-6-2-1///IR1561-149-1//IR24*4/O. NIVARA	I	IR 28	16
EGYPT	GZ5581/GZ4316	J	GZ 6522-15-1-1-3	17
IRRI	IR5657-33-2-1/IR2061-4665-1-5-5	I	IR 64	18
EGYPT	Sakha101/HR4856-1-1-2	J	GZ 7718-13-3-2-2	19
EGYPT	Giza171/Yamji No.1//PI NO.4	J	Giza 177	20
IRRI	IR13240-108-2-2-3/IR9129-209-2-2-2-1	I	IR 66	21
EGYPT	Sakha101/GZ24316$_{(MUT)}$	J	GZ 6910-28-1-3-1	22
IRRI	IR19660-73-4/IR54//IR9828-36-3	I	IR 70	23
EGYPT	Selection from cultivates varieties	J	Agami M.1	24
EGYPT	GZ4120/Suweon349	J	Sakha 103	25
EGYPT	Java3/Yabani Montkhab 3	I/J	Arabi	26
KOREA	TONGIL/IR946-33-2-2-2//YR675-131-2	I/J	Milyang 63	27
CHINA	Chinese selection	J	Yen Geng 135	28
IRRI	SR18977-TB-4/JINMIBYEO	T.J.	IR 73689-31-1	29
EGYPT	Giza175/Milyang49	I/J	Giza 178	30
Africa Rice	---	I	WAB 450-1-B-P-91-HB	31
SRILANKA	---	I	BG304	32
INDIA	---	I	MTU 1010	33
IRRI	CHEOLWEON49/KYWHA9	T.J.	IR 68353-35-3-3-2-2-1-2	34
EGYPT	(IR28/IR1541)/(Giza180/Giza14)	I/J	Giza 175	35

Table (1): Cont'

Africa Rice	---	I	WAB 880 SG 73	36
CHINA	EWAN NO.5/857	J	E 7034	37
IRRI	98-Y-116/Sakha102	I/J	SKC 23808-28-5-2-1-1	38
INDIA	TN1/CO.29	I	IET 1444	39
China	Jingo9601	J	Black Rice	40
IRRI	IR2035-290-2-1-1/MASINO	T.J.	IR 7421-35-1-1-2	41
EGYPT	Sakha101/Suweon313	J	GZ 6903-3-4-2-1	42
IRRI	M202/Giza177	I/J	SKC 23822-304-3-1-1-1	43
TAIWAN	C253///J692130/BL6//TAINUNG67/IR4547-2-1-2	J	Taikeng Yu 1420	44
EGYPT	IR262-43-8-11/KDML105	I	Egyptian Yas-mine	45
IRRI	IR10198-66-2//GZ2175/CSR1	I	IR 67075-2B-5-2	46
IRRI	IR19661-131-1-2/IR15795-199-3-3	I	IR 74	47
JAPAN	HOYOKU/AYANISHKI	J	Reiho	48
IRRI	TJRERMAS/BPI76//PALAWAN/AZUCENA	I	C 22	49
CHINA	LUYIN NO.7/YUNANJINGDAO-38	J	Yun Lu No. 48	50
IRRI	PETA/DEE GEO WOO GEN//TADUKAN	I	IR 22	51
EGYPT	IR69625A/Giza178R	I	SK2034	52
EGYPT	IR69625A/Giza181R	I	SK2046	53
EGYPT	IR70368A/Giza178R	I	SK2035	54
EGYPT	IR69625A/Giza182R	I	SK2058	55

J, Japonica; I, Indica; T.J., Tropical japonica; I/J, Indica japonica and IRRI, International Rice Research Institute.

Table (2): List and some features of SSR markers used for QTL validation.

References	Expected size	Chromosome		Sequences		Marker
		cM*	No			
Senthilvel *et al*. (2004)	165	80.5	8	Reverse Forward	GAAGGCAAGTCTTGGCACTG GAGTGAGCTTGGGCTGAAAC	RM223
	116	115.2	1	Reverse Forward	CTGAGTGCTGCTGCGACT GAGCTCCATCAGCCATTCAG	RM246
Cho *et al.* (2007)	225	73.3	9	Reverse Forward	TATATGCCAAGACGGATGGG GGCCAACGTGTGTATGTCTC	RM242
	166	60.9	8	Reverse Forward	GCATCGGTCCAACTAAGGG CCGGCGATAAAACAATGAG	RM72

* Cornell SSR 2001genetic map.

Table (3): Markers, observed PCR fragments and number of holder genotypes.

Frequency	No. of holder genotypes	Observed fragments		Marker expected size (bp)	Markers
		Abb.	size (bp)		
49.09%	27	a1	167	165	RM223
50.91%	28	a2	158		
45.46%	25	b1	130	116	RM246
36.36%	20	b2	121		
18.18%	10	b3	110		
32.73%	18	c1	237	225	RM242
10.91%	6	c2	225		
58.18%	32	c3	215		
41.82%	23	d1	180	166	RM72
18.18%	10	d 2	172		
29.09%	16	d 3	165		
07.27%	4	d 4	160		
03.64%	2	d 5	152		

Table (4): The probability and the R^2 values of the association between RM223 marker fragments and different studied traits across the different N-treatments.

Marker fragments	N treatment	P	GY	1000-G	SY	TDW	NUEB	NPR	DAH	PL	PFP[a]
a1	0	P	0.014	0.006	0.028	0.012	ns	0.019	ns	0.006	----
		R^2	0.100**	0.120**	0.087*	0.110**	ns	0.091*	ns	0.111*	----
	20	P	0.001	0.011	0.009	0.002	ns	0.013	ns	0.003	0.001
		R^2	0.120**	0.120**	0.120**	0.160**	ns	0.107*	ns	0.130**	0.120**
	40	P	0.018	0.040	0.040	0.007	ns	0.027	ns	0.004	0.018
		R^2	ns	0.081*	0.091*	0.100*	ns	ns	ns	0.115*	ns
	60	P	ns	ns	0.014	0.010	ns	0.037	ns	0.002	ns
		R^2	ns	ns	0.101*	0.098*	ns	0.089*	ns	0.110**	ns
a2	0	P	0.011	0.010	0.025	0.010	ns	0.008	ns	0.003	----
		R^2	0.110**	0.120**	0.086*	0.120**	ns	0.110**	ns	0.120**	----
	20	P	0.001	0.014	0.015	0.003	0.040	0.006	0.048	0.001	0.001
		R^2	0.120**	0.120**	0.104*	0.150**	0.074*	0.120**	0.090*	0.170**	0.120**
	40	P	0.022	ns	ns	0.083	ns	0.013	0.033	0.002	0.023
		R^2	ns	ns	ns	0.084*	ns	0.084*	0.097*	0.130**	ns
	60	P	ns	0.043	0.031	0.019	ns	0.022	0.029	0.000	ns
		R^2	ns	0.084*	0.082*	0.085*	ns	ns	0.110*	0.150**	ns

[a] no data for zero nitrogen treatment, * significant at 0.05 and ** significant at 0.01

Table (5): The probability and the R^2 values of the association between RM246 marker fragments and different studied traits across the different N-treatments.

Marker fragments	N treatment	P	GY	HI	1000 g	PNUE	NPR	PL
b1	0	P	ns	ns	0.037	ns	ns	ns
		R^2	ns	ns	0.086*	ns	ns	ns
	20	P	ns	ns	0.040	ns	ns	ns
		R^2	ns	ns	0.13**	ns	ns	ns
	40	P	ns	ns	0.043	ns	ns	ns
		R^2	ns	ns	0.113*	ns	ns	ns
	60	P	ns	ns	ns	ns	ns	ns
		R^2	ns	ns	ns	ns	ns	ns
b2	0	P	0.015	0.013	0.010	0.001	0.000	0.000
		R^2	0.140**	0.360**	0.160**	0.200**	0.220**	0.280**
	20	P	0.008	0.012	0.015	0.002	0.001	0.000
		R^2	0.160**	0.190**	0.082*	0.190**	0.200**	0.260**
	40	P	0.005	0.017	0.049	0.004	0.002	0.000
		R^2	0.170**	0.120**	0.130**	0.170**	0.200**	0.250**
	60	P	0.024	ns	0.008	0.027	0.003	0.000
		R^2	0.130**	ns	0.170**	0.111*	0.180**	0.290**
b3	0	P	0.014	ns	ns	0.006	0.002	0.000
		R^2	0.110*	ns	ns	0.140**	0.200**	0.230**
	20	P	0.042	ns	ns	0.008	0.002	0.000
		R^2	0.074*	ns	ns	0.130**	0.190**	0.360**
	40	P	ns	0.041	ns	0.011	0.001	0.000
		R^2	ns	0.076*	ns	0.170**	0.220**	0.370**
	60	P	ns	ns	ns	0.034	0.001	0.000
		R^2	ns	ns	ns	0.111*	0.190**	0.380**

* Significant at 0.05 and ** significant at 0.01

Table (6): The probability and the R^2 values of the association between RM242 marker fragments and different studied traits across the different N-treatments.

Marker fragments	N treatment	P	GY	1000-G	NPR	PL	PFP[a]
c1	0	P	0.047	0.000	0.037	0.001	----
		R^2	0.073*	0.230**	0.081*	0.180**	----
	20	P	0.019	0.004	0.020	0.002	0.018
		R^2	0.096*	0.190**	0.083*	0.150**	0.095*
	40	P	0.005	0.005	0.030	0.002	0.005
		R^2	0.120**	0.170**	0.085*	0.160**	0.120**
	60	P	0.038	0.016	0.024	0.003	0.038
		R^2	ns	0.130**	0.091*	0.130**	ns
c3	0	P	0.036	0.004	0.022	0.000	---
		R^2	0.090*	0.108*	0.950*	0.360**	---
	20	P	0.007	0.013	0.019	0.000	0.007
		R^2	0.130**	0.160**	0.088*	0.290**	0.130**
	40	P	0.002	0.011	0.022	0.000	0.002
		R^2	0.150**	0.150**	0.096*	0.290**	0.150**
	60	P	0.031	0.002	0.014	0.000	0.031
		R^2	ns	0.190**	0.108*	0.260**	ns

[a], no data for zero nitrogen treatment, * significant at 0.05 and ** significant at 0.01.

Table (7): The probability and the R^2 values of the association between RM72 fragments and different studied traits across the different N-treatments.

Marker fragments	N treatment	P	GY	1000-G	FGNP	NUEB	PNUE	NPR	DAH	PL	PFP[a]
d1	0	P	ns	0.000	ns	0.030	0.003	0.021	ns	ns	----
		R^2	ns	0.270**	ns	0.095*	0.150**	0.150**	ns	ns	----
	20	P	ns	0.000	ns	ns	0.004	0.016	ns	0.048	ns
		R^2	ns	0.310**	ns	ns	0.160**	0.150**	ns	ns	ns
	40	P	0.050	0.001	ns	ns	0.006	0.021	ns	ns	0.049
		R^2	0.089*	0.21**	ns	ns	0.170**	0.120**	ns	ns	0.072*
	60	P	0.013	0.000	ns	0.029	0.008	0.004	ns	ns	0.013
		R^2	0.130**	0.250**	ns	0.084*	0.190**	0.160**	ns	ns	0.130**
d2	0	P	ns	ns	ns	ns	ns	ns	0.044	0.042	----
		R^2	ns	ns	ns	ns	ns	ns	0.070*	0.071*	----
	20	P	0.037	ns	ns	ns	ns	ns	0.033	0.049	0.036
		R^2	ns	ns	ns	ns	ns	ns	0.075*	ns	ns
	40	P	ns	ns	0.001	ns	ns	ns	0.020	0.001	ns
		R^2	ns	ns	0.160**	ns	ns	ns	0.093*	0.180**	ns
	60	P	ns	ns	0.010	ns	ns	ns	0.014	0.001	ns
		R^2	ns	ns	0.120**	ns	ns	ns	0.107*	0.170**	ns
d3	0	P	0.009	0.010	0.001	0.029	0.004	0.001	ns	0.001	----
		R^2	0.140**	0.140**	0.230**	0.083*	0.14**	0.19**	ns	0.200**	----
	20	P	0.003	0.009	0.001	0.029	0.003	0.001	ns	0.000	0.004
		R^2	0.140**	0.160**	0.230**	0.076*	0.15**	0.190**	ns	0.230**	0.140**
	40	P	0.009	0.048	0.004	ns	0.003	0.001	ns	0.006	0.009
		R^2	0.120**	0.102*	0.16**	ns	0.12**	0.17**	ns	0.150**	0.130**
	60	P	0.000	0.041	0.011	ns	0.023	0.001	ns	0.000	0.000
		R^2	0.180**	0.115*	0.160**	ns	0.087*	0.160**	ns	0.260**	0.180**

[a], no data for zero nitrogen treatment, * significant at 0.05 and ** significant at 0.01.

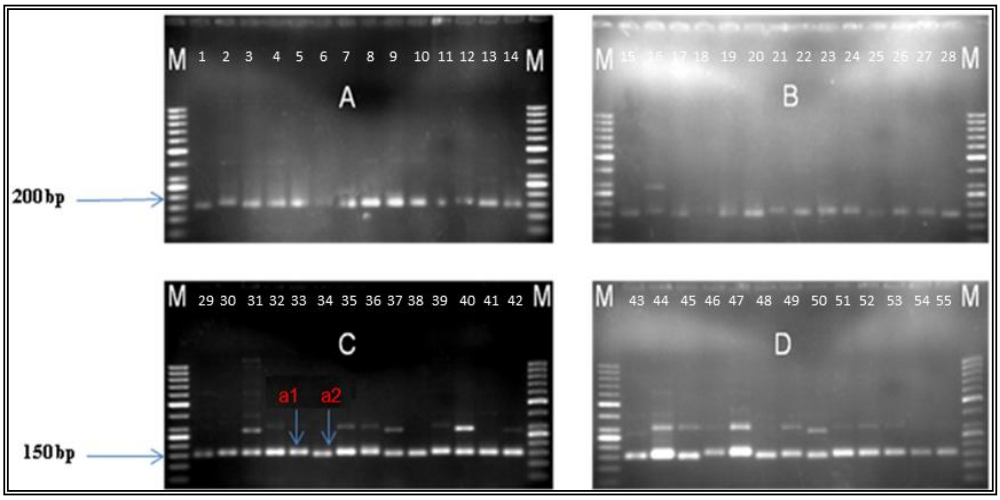

Fig. (1): Banding pattern of RM223 microsatellite marker. (A): genotypes1-14, (B): genotypes15-28, (C) genotypes 29-42, (D) genotypes 43-55 and (M) 50 bp ladder.

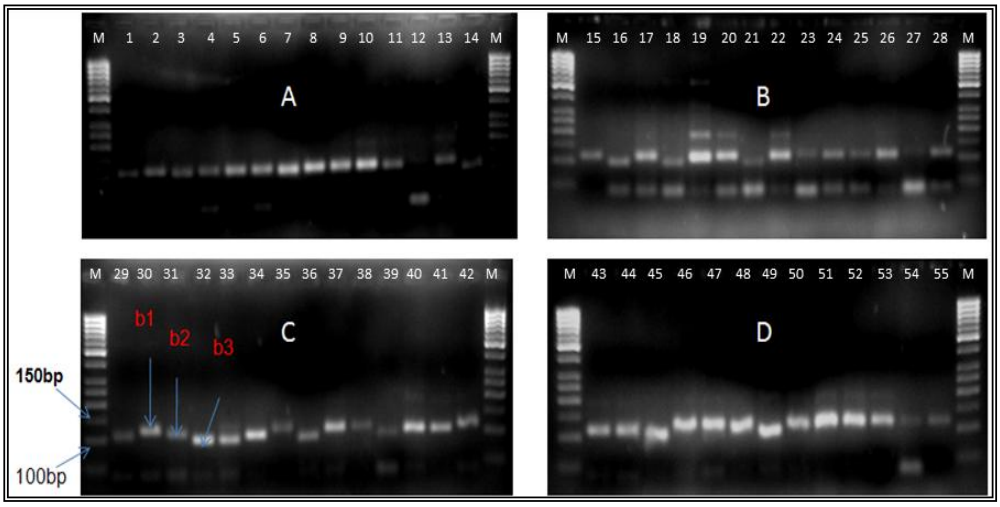

Fig. (2): Banding pattern of RM246 microsatellite marker. (A): genotypes1-14, (B): genotypes15-28, (C) genotypes 29-42, (D) genotypes 43-55 and (M) 50 bp ladder.

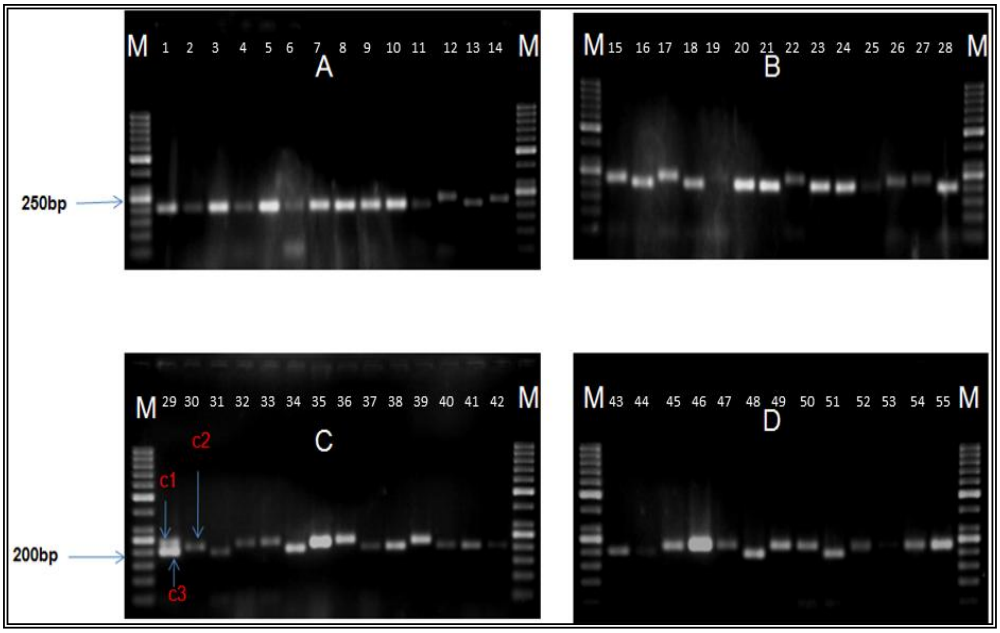

Fig. (3): Banding pattern of RM242 microsatellite marker. (A): genotypes1-14, (B): genotypes15-28, (C) genotypes 29-42, (D) genotypes 43-55 and (M) 50 bp ladder.

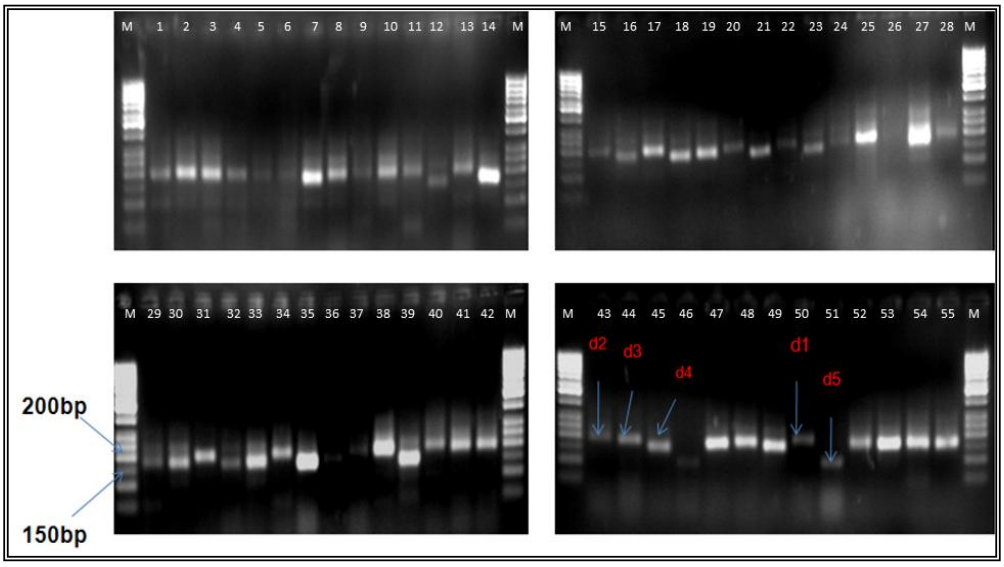

Fig. (4): Banding pattern of RM72 microsatellite marker. (A): genotypes1-14, (B): genotypes15-28, (C) genotypes 29-42, (D) genotypes 43-55 and (M) 50 bp ladder.