

To Analyze Sequence against the Rice Repeat Database VIA Known Repeats & Transposes DNA

Zuhair Hasnain, Naila Rozi

Abstract—Rice ESTs and FL-cDNAs and transcript assemblies (PUTs) from the Plant GDB were aligned to the pseudomolecules using *gmap*. Only the FL-cDNA and PUTs alignments are shown in the browser. Only the EST and FL-cDNA alignments were used for gene model improvement by PASA. We search sequence against a rice repeat database to identify known repeats and transpose's (DNA transposes, retroelements, MITEs, etc). To analyze the growth and yield response of rice under different sowing dates and split nitrogen application, a field study was conducted at farmer's field Punjab Pakistan during Kharif season 2011. The study revealed that N in split form had no significant effect on yield the yield components of rice while early-sowing is considered suitable for farmers under agro-ecological conditions of Punjab Pakistan.

Index Terms—Rice repeat DNA sequences, transplanting time, Nitrogen, Yield and yield components

I. INTRODUCTION

Rice crop is of vital importance and is the 2nd major food grain crop of Pakistan. In Pakistan, rice is grown on an area of 2365 thousands hectare, with an annual production of 4823 thousand tons having an average yield of 2039 kg ha⁻¹ (Economic Survey of Pakistan 2011). This average yield is very discouraging since, there is a big gap between the actual and potential yield per unit area of the crop. The reasons for stagnating yields are thought to be mismanagement in the use of inputs such as fertilizer, unfit underground water, injudicious use of irrigation water, soil nutrient depletion, poor quality seed, and low plant population per unit area (Anonymous, 2001). Sowing time and nitrogen application are the most important variables among the major agronomic and environmental factors that influence the phenology, growth and yield of rice affecting growth and yield.

Sowing date is one of most important factor influencing crop growth and development. Xie *et al.* (1996) concluded that final crop dry weight and grain yield decreased as sowing was delayed. Similarly a study on the effect of sowing date on quality of rice shows that delay in sowing results in reduction of growth cycle and plant height (Andrade *et al.* 1995). It has been reported that early sowing resulted in higher yield attributes Rao and Jackson (1997).

Nitrogen is the key factor in achieving high yield. It increases leaf area that positively effects interception of PAR and its use efficiency, crop growth rate and consequently contributes towards higher yield.

Manuscript received on April, 2013.

Zuhair Hasnain, Department of Agronomy, University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan.

Naila Rozi, Sir Syed University of engineering &Technology, Karachi, Pakistan.

It is the most deficient element in our soils being an integral part of structural and functional proteins, chlorophyll and nucleic acid that plays a vital role in crop development (Tisdale *et al.* 1990). From the results of experiments conducted at International Rice Research Institute (1976), it was found that incorporation of N fertilizer in the soil before transplanting resulted in increased yield.

Temperature and radiant energy are the major climate factors affecting phenology, growth and yield of rice. According to Gallagher and Biscoe (1978) Total Dry Matter (TDM) production of a cereal crop is directly proportional to intercepted photosynthetically active radiation (PAR) and its utilization efficiency by well husbanded crop.

The high yielding varieties are a group of crop created internationally during the Green Revolution to increase global food production .This project enabled labor market in Asia to shift away from agriculture, and into industrial sectors. The first "Rice Car", IR8 was produced in 1966 at the International Rice Research Institute which is based in the Philippines at the University of the Philippines Los Banos site. IR8 was created through a cross between an Indonesian variety named "peta" and a Chinese variety named "Dee Geo Woo Gen". Scientist has identifying and cloned many genes involved in gibberellins signaling pathway, including GAI1 (Gibberellin Insensitive) and SLRI (Slender Rice). Disruption of gibberellins signaling can lead to significantly reduced stem growth leading to a dwarf phenotype. Photosynthetic investment in the stem is reduced dramatically as the shorter plants are inherently more stable mechanically. Assimilates become redirected to grain production, amplifying in particular the effect of chemical fertilizers, and intensive crop management these varieties increase their yield two to three times.

II. MATERIAL AND METHODS

A field experiment to determine the effect of sowing date and split nitrogen application on growth, development and yield of rice was carried out at the farmer's field Punjab during Kharif season 2011. Experiment was laid out in a randomized complete block design with split plot arrangement, keeping the sowing date in main plots and split N application in sub plots with three replications. Plot size was 5.62m x 1.35 m along with plant to plant and row to row spacing of 22.5 cm. The experiment comprised of two sowing date S1 (09-07-11), S2 (23-07-11) and three split nitrogen application timings i.e., N₁= full N at transplanting, N₂= 1/2 N at transplanting+ 1/2 N at 30 DAT and N₃=1/3 N at transplanting + 1/3 N at 30 DAT +1/3 N at 50 DAT, respectively.

The nursery for raising seedlings was shown on 22.05.2011 and 19.06.2011, first and second date respectively. Thereafter crop was transplanted manually on respective dates on puddled soil.

The plots were measured with measuring tape and boundaries were marked. Then the plant to plant and row to row distance was maintained at 22.5 cm apart. Recommended doses of P and K were applied @ 79.07 kg ha⁻¹ and 61.78 kg ha⁻¹ at time of transplanting while nitrogen fertilizer was applied as per treatments mentioned earlier. All other agronomic practices such as hoeing, weeding, irrigation, plant protection measures etc. were kept normal for all the treatments.

On final harvest at maturity, data for number of fertile tillers m⁻², plant height, no. of grains per panicle, 1000-grain weight, paddy yield, TDM and harvest index were recorded by following the standard procedures. Analysis of variance technique was employed to analyze the data. Differences among the treatment means were compared using least significant difference (LSD) at 5% probability level (Steel and Torrie, 1984).

All rice BAC/PAC was downloaded from HTGS division of GenBank as well as the PLANT division of GenBank. BAC/PAC sequences were assembled into 12 pseudomolecules. Each of the pseudomolecule sequences then were processed by our annotation pipeline as described below. Please note that all data is from automated processes and is NOT manually curated.

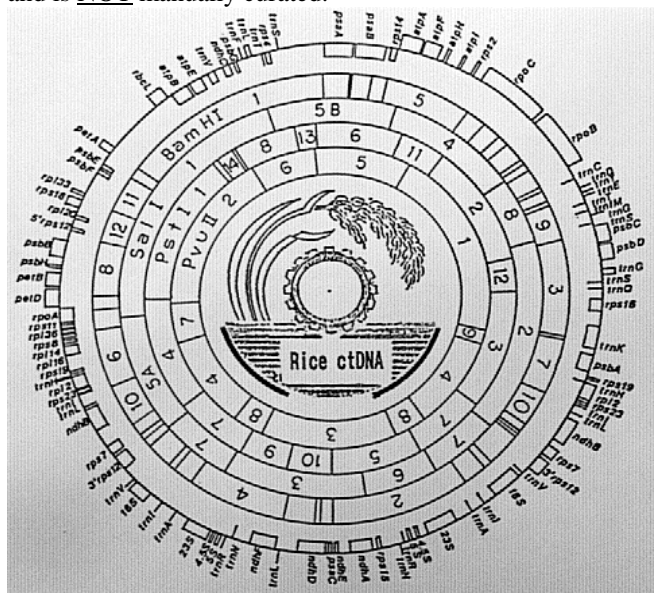


Fig-I: Rice of CtDNA

Osg340-0	1	GCGCAGGTTT	GPTTCAGCTT	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	50
Osg340-1		GCGCAGGTTT	GPTTCAGCTT	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	
Osg340-31	TT	GPTTCAGCTT	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	
Osg340-21	TT	GPTTCAGCTT	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	
Osg340-12	CTGTTT	GPTTCAGCTT	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	
Osg340-0	51	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	100
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-0	101	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	150
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-0	151	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	200
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-0	201	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	250
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-0	251	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	300
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-0	301	AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	327
Osg340-1		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-31		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-21		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	
Osg340-12		AAGATATPPTG	TAAATPAGAT	TATTTAAATC	CTGATATATA	ATAAGCTTACA	

Fig-II: Sequences of Rice DNA

The genome is a sequence of chemicals represented by symbols rice looking like this in Fig1 ATTGTGTAGCTTCTT that goes on for 389 million letters, so computers are the only practical way to store and analyze it. In each nucleotides of rice (A,C,G,T) in position k in 25-mer probe is modeled by a triple in the indicator function a;

(a_{3k-2},a_{3k-13},ak) i.e., an A at position K has corresponding coefficient a_i (a_{3k-2},a_{3k-13},ak)=(1,0,0), a C has corresponding coefficient (0,1,0) a G has corresponding coefficient (0,0,1) and T has corresponding coefficient (-1,-1,-1). A least square solution to this system of equations for y gives estimates of the expected contribution to the expression for any nucleotide at position k. That is the estimated contribution to the expression level over different replicates the mean log likelihood are computed where each nucleotide type (A,C,G or, T) in position K in a 25 mer probe is modeled by a triple in the indicator function. The estimated contribution to the expression for any nucleotide at position given by y_{3k-2} a C at position K is given by y_{3k-3}, G at position k is given by y_k and T as position K is given by addition of these three. To obtain a representative expression level over different replicates the mean log likelihood are computed

$$LL = \frac{1}{3} \sum_{i=1}^3 \log \frac{PM}{estimate \ PM} \quad PM = \text{Probe mutation}$$

Where LL is log likelihood model obtain by applying least square solution for nucleotides (A, C, G or, T).

III. RESULTS AND DISCUSSION

Data regarding number of fertile tillers m⁻² is presented in Table-I. Both sowing dates and split nitrogen application did not affect the number of fertile tillers m⁻² significantly. However the interaction between sowing date and split nitrogen application enhanced the number of fertile tillers m⁻² significantly. The results showed that 9 July sowing and N₃ (3rd level of split nitrogen application) perform better compared to other treatments. The mean value of the number of fertile tillers m⁻² was 445.91 obtained at the final harvest. In 2005 Scientist completed the genetic map of Rice plant; a scientific milestone that we hope will accelerate efforts to feed the hungry by improving the world most basic food. Rice is the first crop plant whose complete genetic sequence or genome ascertained and placed in computer data banks around the world .It will be an input tool for researchers working on improved strains rice as they struggle to stay ahead of human population growth. In this way we could equate this to being as important as the Human Genome Project .Rice coding project can lead to important discoveries and finding so that can help the condition of the poverty. The poorest of the poor are the ones that depend on the rice most hit number of people in this world is expected to increase 50% to 9billionby the middle of century. Much of that growth will come in Asian countries where rice is the dietary staple. The International Rice Genome Sequencing Project began in 1988.

Table-I shows the effect of treatments on plant height at maturity. Sowing date had significant effect on plant height. The average plant height was 108.98 cm in 9 July sowing and 98.53 cm in 23 July sowing. The mean value of plant height was 103.76 cm obtained at the final harvest. Andrade *et al.* (1995) concluded any delay in sowing significantly reduced the plant height. Split nitrogen application did not affect the plant height significantly. According to Oh *et al.* (1990) N as a basal dose (no splits) significantly enhanced the plant height.

Table-I shows the effect of treatments on the Number of grains per panicle at the final harvest. The number of grains panicle⁻¹ was significantly affected by sowing date. The average number of grains panicle⁻¹ was 100.22 and 87.89 in 9 July and 23 July sowing respectively. Split nitrogen

application had no significant effect on number of grains panicle⁻¹. The mean value of number of filled grains panicle⁻¹ was 94.06 obtained at the final harvest.

Table-I shows the effect of treatment on the 1000-grain weight at the final harvest. Both sowing date and split nitrogen application did not affect the 1000-grain weight. Similarly the interaction between sowing date and split N application did not show any difference. The mean value of 1000-grain weight was 23.02 g obtained at the final harvest. Table-I shows the effect of treatments on the paddy yield at the final harvest. Sowing date had significant effect on paddy yield. The average paddy yield was 5942 kg ha⁻¹ and 3900 kg ha⁻¹ for 9 July and 23 July sowing respectively. Xie *et al.* (1996) concluded that grain yield decreased as sowing was delayed. Similarly results also reported by Jha *et al.* (1991), William *et al.* (1997), Rao and Jackson (1997) and Singh and Singh (2000). The effects of split N application were non-significant on paddy yield. Similarly interaction between sowing date and split N application was also non-significant. The mean value of grain yield was 4920.59 kg ha⁻¹ at the final harvest. Nossa and Vergas (1980) indicated that split N application had no effect on grain yield. Similar results also reported by other workers including Budhar and Palaniappan (1993), Voss and Zini (1993) and Murali and Reddy (1995). Table-I shows the effect of treatments on final total dry matter (TDM). Total dry matter production was significantly affected by sowing date. The average total dry matter production was 26621 kg ha⁻¹ and 20558 kg ha⁻¹ for 9 July and 23 July sowing respectively.

The effects of split N application were non-significant on the TDM. Similarly the effect of interaction between sowing date and split N application was also non-significant. The mean value of TDM was 23589.32 kg ha⁻¹ obtained at final harvest. Budhar and Palaniappan (1993) concluded same results that N in split form had no significant effects on yield and yield components. Similar results were also reported by others including IRRI (1976), Khan and Vargara (1981) and Voss and Zini (1993).

Data in table shows the effect of treatments on harvest index (HI) at the final harvest. The ability of a crop to convert the dry matter into economic yield is indicated by its harvest index that is the measure of proportion dry matter distributed between grains and rest of plant parts. Harvest index was significantly affected by sowing date. The average value of the HI was 22.38% for 9 July sowing and 20.87% for 23 July sowing. The effects of split N application were non-significant on harvest index. Similarly the interaction between sowing data and split N application did not showed any difference. The mean value for harvest index was 20.72 at final harvest of the crop.

IV. CONCLUSION

The general conclusion for this study is that the paddy yield among various treatments was related to their photosynthetic activity. The higher yield in 9 July sowing was mainly due to higher LAI, LAD and greater light interception. The data suggested that there is no significant effect of split nitrogen application. The proposed research is very applicable as rice DNA has significant effect on number of grains panicle.

So from this study, recommendations for the semi-arid conditions of Punjab Pakistan are that 9 July sowing (early sowing) is the better as compared to 23 July sowing and there is no alternative of early sowing for obtaining all best results.

Nitrogen application did not matter whether one use it in split form or use as a complete dose.

REFERENCES

- [1] Andrade, W.D.B., G.M.B. Fernandes and N.S. Amorim. 1995. Effect of sowing date on quality of rice grains. Comunicadotecnicoempresa de pesquisaagropecuaria do estado do Rio de Janeiro, No. 231. 5pp.
- [2] Anonymous. 2001. Rice in Pakistan. Website: <http://www.riceweb.org/countries/pakis.htm>.
- [3] Budhar, M.N. and S.P. Palaniappan. 1993. Nitrogen management in aged rice seedlings. Madras Agric. J. 80(2): 24-25 (Rice Absts., 18(1): 230; 1995).
- [4] Economic Survey of Pakistan. 2011. Ministry of Finance, Government of Pakistan.
- [5] Gallagher, N.J. and P.V. Biscoe. 1978. Radiation absorption, growth and yield of cereals. J. Agric., Sci. Camb., 19: 47-60.
- [6] Hiria.A.T.Ishibashi.A.Morikami.N.Iwatsuki.Shinozakiand M.Sugiura 1985, Rice chloroplast DNA Appl Genet.70:117-122.
- [7] IRRI. 1976. Root zone placement of nitrogen. Int.Rice Res. Inst., Manila Reporter.
- [8] Jha, K.P., C.Gangadharan and G.B.Manna. 1991. On-farm evaluation of promising lowland rice varieties & their response to date of sowing & nitrogen level under flood prone rainfedlowland.Ind. J. Agri. Sci., 61:4,237-242.
- [9] Khan, M.R. and B.S. Vargara. 1981. Response of traditional deep water to nitrogen application. IRRI. Newsletter 6(3): 10-11 (Field Crop Absts., 35(8): 6554; 1983).
- [10] Murali, B. and T.M.M. Reddy. 1995. Response of rice cultivars to levels and times of nitrogen application. J. Res. APAU, 23(2): 43-44 (Rice Absts., 20(4): 2488; 1997).
- [11] Nossa, I.E. and Z.J.P. Vergas. 1980. Response of rice (*Oryza sativa* L.) cv. Cica-8 to split application of nitrogen under field conditions. ActaAgronomica Colombia, 30(1/4): 49-60 (Field Crop Absts., 36(4): 2938; 1983).
- [12] Oh, Y.B., J.K. Ahn, R.K. Park, and S.H. Park. 1990. Effect of nitrogen fertilizer application method on the growth and grain yield of rice plant. Research reports of the Rural Development. Ad., Rice, 32(1): 16-20 (CAB Absts., 1990-1991).
- [13] Rao, N.K. and M.T. Jackson. 1997. Effect of sowing dates and harvest time on longevity of rice seeds. Seed Sci. Res., 70: 1, 13-20.
- [14] Singh V.P. and V.K. Singh. 2000. Effect of sowing date and nitrogen level on the productivity of spring sown rice. Ind. J. Agron., 45: 3, 560-563.
- [15] Steel, R.G.D. and J.H. Torrie. 1984. Principles and Procedures of Statistics. 2nd ed. McGraw Hill, Int. Book Co. Inc. Singapore.
- [16] Tisdale, S.L., W.L.Nelson and J.D. Beaton.1990.Soil fertility and fertilizers.Mc-Milan Pub.Co., NY, USA: 60-62.
- [17] Voss, M.A. and E. Zini. 1993. Forms and dates of application of urea to irrigated rice, 1992-93. Pelotas, Brazil; Centro de PesquisaAgropecuaria de ClimaTemperado, EMBRAPA 163-164 (Field Crop Absts., 48(1): 181; 1995).
- [18] Williams, R.L., S. Fukai, M. Cooper and J. Salisbury. 1997. Effect of growth, nitrogen and sowing date on grain yield of Australian Rice, a modeling approach. Breeding Strategies for rainfed low land rice in drought prone environments: Proc. Int. Workshop, UbonRatchathani, Thailand, 5-8 Nov. 1996, 239-244, ACIAR Proc. No. 77.
- [19] Xie, G.H., B.L. Su, L. Shi and A.Y. Tian. 1996. Study on growth and dry matter production of rice. J. China Agric. Univ., 1:1, 89-94.

To Analyze Sequence against the Rice Repeat Database VIA Known Repeats & Transposes DNA

Table-I: Effect of sowing date and split nitrogen application on yield & yield components of rice.

	Fertile tillers (m ²)	Plant height (cm)	Grains Panicle ⁻¹	1000-grain weight (g)	Grain yield (kg ha ⁻¹)	TDM (kg ha ⁻¹)	HI (%)
S ₁ = 9 July	450.34	108.98a	100.22 a	23.46	5942 a	26621 a	22.38 a
S ₂ = 23 July	441.48	98.53 b	87.89 b	22.59	3900 b	20558 b	20.87 b
LSD 5%	35.68	6.32	6.73	0.87	695	5827	2.06
Significance	NS	*	*	NS	*	*	*
N ₁ = full N at transplanting	440.12	102.19	90.57	23.00	4776	23321	20.51
N ₂ = 1/2 N at transplanting+1/2 N at 30 DAT	458.76	105.38	94.84	23.20	5113	24587	20.75
N ₃ =1/3 N at transplanting + 1/3 N at 30 DAT +1/3 N at 50 DAT	438.76	103.69	96.76	22.87	4873	22860	20.90
LSD 5%	19.25	4.94	6.23	0.63	544	3125	1.82
Significance	NS	NS	NS	NS	NS	NS	NS

Means having different letters differ significantly from each other by LSD (P= 0.05)

*, **= Significant and highly significant
NS= Non significant

First Author Zuhair Hasnain is PhD research student in Agriculture Agronomy. He is recently backed from The International Rice Research Institute (IRRI), Philippines, and worked with C4 Rice Project there. He is also the active member of the Editorial Advisory Board of the Journal of Natural Resources and Development, Chile. While working with rice, his expertise includes nutrient and water management, Rice Agronomy, and major field crop production.

Zuhair is also Dignity Advisor at IRRI-Philippines and a brief overlook about him can have from the following URL:

http://irri.org/index.php?option=com_k2&view=item&id=11718:hasnain-zuhair&lang=en

<http://scholar.google.com.pk/citations?user=Y8kz0xkAAAAJ>

Second Author Naila Rozi PhD Assistant Professor Department of Mathematics10 Research Publication member of ISOSS.