



**Genetic variability for nitrogen use efficiency and yield related traits in rice under low nitrogen and Available soil nitrogen conditions.**

**Naveen Kumar V.M<sup>1</sup> and Dr. M. S. Uma<sup>2</sup>**

<sup>1</sup>SRF, Rice Breeding division, ZARS, V.C. Farm, Mandya

<sup>2</sup>Professor and Head, Department of Genetics and Plant Breeding, College of Agriculture, V C Farm, Mandya

**Abstract**

*Rice is the most important food crop in Asian countries and feeding more than half of world population. The local rice genotypes are reservoirs of valuable traits. Hence an attempt was made to study the genetic variability for nitrogen use efficiency, yield related traits and quality related traits in a set of 55 local rice genotypes during 2014-15 at the College of Agriculture, V.C. Farm, Mandya. Field screening of 55 local rice genotypes under low and no nitrogen conditions revealed highly significant genotypic differences for all the traits studied. High genotypic coefficient of variability and phenotypic coefficient of variability values with less difference observed for nitrogen use efficiency, yield and quality related traits indicating less influence of environment factors on their expression. High heritability coupled with high genetic advance was recorded for plant height, number of productive tillers, panicle length, panicle weight, number of spikelets per panicle, test weight, grain and straw yield per plant, nitrogen use efficiency, grain quality traits and protein content at both low and no nitrogen conditions. This indicated that these traits were predominantly controlled by additive gene action and more amenable for selection. Significant positive correlation of nitrogen use efficiency with grain and straw yield in both low and no nitrogen conditions was observed. The genotypes Parimalakalavi, Mosaleputtabatta, Mundoni, Bilihasudi, and Bolumallige were found to be nitrogen use efficient.*

*Key words: Local rice genotypes, Nitrogen use efficiency, Genetic variability, Heritability and Genetic advance*

**I. INTRODUCTION**

Rice is second most important staple food crop and feeding more than one half of the world population, Over 90% of rice is produced and consumed in Asia(1). Rice production accounts for about 20 per cent of global nitrogen consumption and it must be increased drastically to meet the demand projected for 2025. If the technologies that affect nutrient utilization by rice crop remain unchanged, the production increase will require almost 300% more than the present nitrogen application rate and it will have undesirable consequences both in economically and environment wise (2). Nitrogen Use Efficiency is relatively low in irrigated lowland rice because of rapid losses of nitrogen from ammonia volatilization, denitrification, surface run-off and leaching in the soil floodwater system (3). These nitrogen losses are much greater under excessive nitrogen application and cause severe environmental consequences, such as groundwater contamination, eutrophication of lakes and rivers and acid rain. The nitrogen application stimulates growth and activity of methanogens, thus increase effectiveness of CH<sub>4</sub>. The increase nitrogen application directly and indirectly affects emission of methane gas (CH<sub>4</sub>), this leads finally to global warming and environmental change. Continuous increase in rice production has to be achieved with less nitrogen fertilizer by improving Nitrogen Use Efficiency (NUE) through better nitrogen fertilizer management and development of new nitrogen use efficient rice varieties. Some

efforts were made to improve germplasm for NUE. The genotypic variation in NUE has been realized and however, plant traits that are associated with high grain yield and high NUE should be identified so that breeders are able to use these traits easily as selection criteria in the breeding programme to develop nitrogen efficient varieties without the scare of playing with rice yield potential.

The improvement of trait in a population over base population is a function of variability present in the original population. The main objective of plant breeders to formulate breeding programs accompanied with exploitation of existing variability. The variability is mainly altered by both genotypes and genotype x environment interaction.

The variability is mainly altered by both genotypes and genotype x environment interaction. Since all the variations are not heritable due to high environmental effect. Therefore, it is appropriate to partition the phenotypic variation into heritable and non- heritable components. Hence breeding value of the genotypes can be accurately estimated by separating genetic variance from the environmental variance by genetic variability parameters.

## II. MATERIAL AND METHODS

About 55 local rice genotypes were evaluated for studying of genetic variability for nitrogen use efficiency and yield related traits. The study was conducted at Department and Genetics and Plant Breeding, College of Agriculture, V.C. Farm, Mandya during *kharif*2014. For the field screening of 55 local rice genotypes were grown in simple lattice design with two replications under low nitrogen and no nitrogen (Available soil nitrogen) conditions. This experiment design was laid out in puddled field by following 8 X 8 simple lattice design with two replications and in two treatment conditions i e., Low nitrogen condition and available soil nitrogen condition(No nitrogen condition) as separate plots. The spacing maintained was 30 cm between rows and 10 cm between plants with in the row. Before experiment was laid out, soil sample were collected from the experiment sites and analyzed for nutrient status including soil chemical properties. Based on results properties of nitrogen application at low nitrogen condition was decided. About 23 days old seedlings were transplanted to main field at the rate of one seedling/hill. The soil analysis results depict low soil available nitrogen in experimental plots. Five randomly selected plants in each genotype for 17 characters viz., plant height(cm) at 35 days, number of tillers at 35 days, chlorophyll meter reading(SPAD) at 45 days, days to 50 percent flowering, days to maturity, plant height(cm) at maturity, number of tillers at harvest, number of productive tillers per plant, panicle length(cm), panicle weight(g), number of spikelets per panicle, panicle fertility(%), grain yield per plant(g), straw yield per plant(g), test weight(g), biomass per plant(g) and nitrogen use efficiency. Nitrogen use efficiency calculated by formula total grain yield divided by unit quantity of nitrogen applied.

### A. STATISTICAL ANALYSIS

The data of mean value for all the characters were analyzed for their variance followings implelattice design out lined by Cochranand Cox (4). Analysis was done using SAS 9.2 statistical software.

#### *Analysis of variance (ANOVA) for yield related traits and nitrogen use efficiency*

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F ratio
Replication	(r-1)	A	P	
Genotype (un adjusted)	(k <sup>2</sup> -1)	B	Q	Q/S
Blocks within replications (adjusted)	r(k-1)	C	R	
Intra –block error	(k-1) (rk-k-1)	D	S	
Total		E		

Where,

r= number of replications  
 k<sup>2</sup>=total number of genotypes  
 k= number of genotypes in a row or column  
 A= replication sum of squares

B= treatment (unadjusted) sum of squares  
 C= sum of squares for blocks within replication (adjusted)  
 D = intra – block error sum of squares  
 E= total sum of square

**Phenotypic coefficient of variation (PCV)**

$$PCV = \frac{\sigma_p}{\bar{X}} \times 100$$

**Genotypic coefficient of variation (GCV)**

$$GCV = \frac{\sigma_g}{\bar{X}} \times 100$$

Where,

$\bar{X}$  = grand mean of the character  
 $\sigma_p$  = phenotypic standard deviation  
 $\sigma_g$  = genotypic standard deviation

PCV and GCV were classified as suggested by Shivasubramanian and Menon (5) into low (0 – 10 per cent), Moderate (10 – 20 per cent) and High (>20 per cent).

**Heritability (h<sup>2</sup>) broad sense**

Heritability in broad sense for all the characters was computed by the formula suggested by Lush (6).

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where,

$\sigma_g^2$  = genotypic variance  
 $\sigma_p^2$  = phenotypic variance

Heritability was classified as suggested by Robinson and Comstock (7) in to low (0 – 30 per cent), moderate (30 – 60 per cent) and high (> 60 per cent).

**Genetic Advance (GA)**

**Predicted Genetic Advance**

The predicted genetic advance was estimated according to the formula given by Johnson (8).

$$GA = h^2 \cdot K \cdot \sigma_p$$

Where,

h<sup>2</sup> = heritability  
 K = selection differential at given intensity  
 $\sigma_p$  = phenotypic standard deviation

**Genetic advance as per cent mean (GAM)**

The expected GA as per cent of mean was estimated as given below

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where,

GA = Genetic advance

$\bar{X}$  = General mean

The genetic advance as per cent of mean was categorized as as low (0 – 10 per cent), moderate (10 – 20 per cent) and high (>20 per cent) suggested by Johnson (8).

### III. RESULTS AND DISCUSSION

Genetic variability studies gives basic information regarding the genetic architecture of the population based on which breeding methods are formulated for further improvement of the crop. These studies are also helpful to gain knowledge about the nature and extent of variability that can be attributed to different causes, sensitivity of crop to environment, heritability of the character and genetic advance. The analysis of variance for differences for all the characters under study showed a wide range of variation and significant in both nitrogen treatments (Table.1 and 3), indicating the presence of adequate variability for further improvement crop with high nitrogen use efficiency. The genotypic mean square values were highly significant for all traits, implying that the genotypes tested under both low nitrogen and no nitrogen conditions were highly variable. The estimates of mean, range, phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as percent of mean under both low nitrogen and no nitrogen conditions are presented in Table. 2 and 4.

**Table 1: Analysis of variance for yield related characters and Nitrogen use efficiency in rice genotypes under low nitrogen condition.**

Source	D F	Plant height at 35 days (cm)	Number of tillers at 35 days	SPAD value	Days to 50 % flowering	Days to maturity	Plant height at maturity	Number of tillers at harvest	Number of productive tillers/plant	Panicle length (cm)
Replications	1	1.350	5.360	0.018	2.258	0.281	0.081	0.08	0.998	0.106
Blocks within Replications (Adjusted)	14	0.370	4.940	2.81	0.865	0.121	8.05	0.05	2.989	0.17
Genotypes (Unadjusted)	63	74.78**	35.68**	18.25*	234.71**	74.805**	653.82**	36.14**	15.08**	14.18**
Intra Block Error	49	0.40	2.750	1.423	0.656	0.184	8.801	0.04	1.808	0.352
LSD @ 5%		1.180	4.650	2.52	1.618	0.858	5.928	0.433	2.818	1.185
S.Em		0.530	0.380	0.278	0.955	0.539	1.602	0.37	0.2579	0.237

Source	DF	Panicle weight (g)	Number of spikelets / panicle	Panicle Fertility (%)	Grain yield / plant (g)	Straw yield / plant (g)	Test weight (g)	Biomass / plant (g)	Nitrogen use efficiency
Replications	1	0.072	76.076	0.290	0.41	0.001	0.272	0.123	0.858
Blocks within Replications (Adjusted)	14	0.034	79.076	0.059	0.362	0.512	0.078	0.968	2.764
Genotypes (Unadjusted)	63	1.978**	3177.74**	31.231**	100.03**	54.465**	50.23**	173.88**	165.92**
Intra Block Error	49	0.015	75.945	0.084	0.221	0.933	0.103	1.169	3.752
LSD @ 5%		0.264	17.415	0.58	0.985	1.93	0.641	2.161	3.871
S.Em		0.08	3.552	0.3483	0.6234	0.4629	0.441	0.8235	0.8104

\* and \*\*- Significant at 5% and 1% levels.

Under low nitrogen condition, high phenotypic coefficient of variability and genotypic coefficient of variability was obtained for number of tillers at 35 days, number of tillers at harvest, number of productive tillers, panicle weight, number of spikelets per panicle, grain yield, straw yield, test weight, harvest index and nitrogen use efficiency. The findings were in accordance with Vaishali (9), Rita Binseet *al.* (10), Selverajet *al.* (11). Moderate phenotypic coefficients of variability and genotypic coefficients of variability was shown by days to 50% flowering, plant height at maturity, panicle length and biomass. Number of spikelets per panicle, days to maturity showed low phenotypic coefficient of variability and genotypic coefficient of variability values. While, low phenotypic coefficient of variability values for number of spikelets per panicle was agree with the observations of Paikombaet *al.* (12). Sreenatha (13) reported low genotypic coefficient of variability values for days to maturity. Those Characters having high phenotypic coefficient of variability and genotypic coefficient of variability indicate large scope for selection and improvement in the present set of local rice genotypes and less difference between phenotypic coefficient of variability and genotypic coefficient of variability indicating that less influence of environment on expression of traits.

**Table 2: Estimation of genetic variability parameters for yield related traits and nitrogen use efficiency in local rice genotypes under low nitrogen condition.**

Characters	Mean	Range		Coefficients of Variability		Heritability % (Broad Sense)	GAM (%)
		Minimum	Maximum	PCV %	GCV %		
Plant height at 35 days (cm)	51.00	41.40	67.00	12.98	11.92	98.90	26.62
Number of tillers at 35 days	14.67	6.40	36.40	29.88	27.65	85.70	67.39
SPAD value	39.28	32.90	46.60	7.98	7.39	85.60	18.63
Days to 50% flowering	97.24	73.00	126.00	11.16	11.13	99.40	24.01
Days to maturity	134.95	120.00	154.00	4.54	4.53	99.50	10.08
Plant height at maturity (cm)	104.56	65.90	144.30	17.41	17.17	97.30	36.79
Number of tillers at harvest	14.74	6.00	35.50	5.65	5.48	94.20	18.03
Number of productive tillers	11.02	5.20	18.90	25.2	24.63	95.50	60.58
Panicle length (cm)	21.70	14.20	24.80	12.79	12.47	95.20	30.86
Panicle weight (g)	3.15	0.90	5.52	29.66	29.4	98.30	92.32
Number of Spikelet's/panicle	118.38	40.67	262.50	34.07	33.27	95.30	70.99
Panicle fertility (%)	92.82	79.40	98.80	4.26	4.25	99.50	9.85
Grain yield /plant (g)	25.85	13.30	46.10	27.39	27.33	99.60	60.27
Straw yield /plant (g)	22.69	14.30	35.80	23.19	22.8	96.60	52.04
Test weight (g)	22.61	10.50	31.70	22.19	22.14	99.60	50.11
Biomass /plant (g)	48.52	28.40	69.60	19.28	19.15	98.70	41.75
Nitrogen use efficiency	34.42	18.40	60.70	26.76	26.16	95.60	57.90

High heritability coupled with genetic advance as per cent mean for the traits were plant height at 35 days (98.90%), number of tillers at 35 days (85.70%), days to 50% flowering (99.40%), plant height at maturity (97.30%), number of tillers at harvest (94.20%), number of productive tillers (95.50%), panicle length (95.20%), panicle weight (98.30%), number of spikelets per panicle (95.30%), grain yield per plant (99.60%), straw yield per plant (96.6%), test weight (99.60%), biomass per plant (98.70%), and nitrogen use efficiency (95.60%). These results agrees with the findings of Vikas Kumar *et al.* (14), Selverajet *al.* (11), Thirumala Rao *et al.* (15), Afzal *et al.* (16). But high heritability with moderate and

low genetic advance as percent mean observed for SPAD value, number of tillers at harvest and panicle fertility respectively. High heritability and genetic advance as percent mean of these traits depicting that variation obtained is mainly due to genetic factors and also moderate role of environmental factors. It clearly indicates that the improvement of the above traits can be obtained by simple selection in the present set of material. Moderate and low heritability indicates simple selection not sufficient to improvement of traits like SPAD value, number of tillers at harvest and panicle fertility.

*Table 3: Analysis of variance for yield related characters and Nitrogen use efficiency in rice genotypes under no nitrogen (available soil nitrogen) condition.*

Source	DF	Plant height at 35 days (cm)	Number of tillers at 35 days	SPAD value	Days to 50 % flowering	Days to maturity	Plant height at maturity	Number of tillers at harvest	Number of productive tillers	Panicle length (cm)
Replications	1	0.649	0.195	2.140	0.383	1.125	44.888	0.861	0.475	0.366
Blocks within Replications (Adjusted)	14	0.092	0.400	4.539	2.481	0.795	13.028	0.29	1.935	0.102
Genotypes (Unadjusted)	63	74.48**	10.138**	16.22**	170.44**	67.841**	600.03**	17.210**	11.736**	12.027**
Intra Block Error	49	0.389	0.374	3.433	1.947	0.730	14.675	0.271	1.17	0.162
LSD @ 5%		1.240	1.222	3.703	2.788	1.707	7.655	1.04	2.267	0.803
S.Em		0.538	0.202	0.278	0.818	0.516	1.543	0.26	0.225	0.217

Source	DF	Panicle weight (g)	Number of spikelets/plant	Panicle fertility (%)	Grain yield/ plant (g)	Straw yield / plant (g)	Test weight (g)	Biomass/ plant (g)	Nitrogen use efficiency
Replications	1	0.013	77.346	0.002	0.13	3.618	3.781	0.245	1.721
Blocks within Replications (Adjusted)	14	0.032	46.614	0.778	2.476	1.438	2.018	2.438	13.844
Genotypes (Unadjusted)	63	1.733**	2914.49**	105.900**	56.195**	34.084**	56.264**	113.980**	171.050**
Intra Block Error	49	0.030	41.611	0.675	1.908	1.039	1.886	1.575	7.335
LSD@ 5%		0.345	12.891	1.642	2.760	2.037	2.744	2.508	5.720
S.Em		0.083	3.386	0.643	0.475	0.37	0.475	0.67	0.835

\* and \*\*-Significant at 5% and 1% levels

In no nitrogen condition, High values of genotypic coefficient of variability and phenotypic coefficient of variability was observed for number of tillers at 35 days, number of tillers at harvest, number of productive tillers, panicle weight, number of spikelets per panicle, grain yield per plant, straw yield per plant, test weight and nitrogen use efficiency. A moderate value of phenotypic coefficient of variability and genotypic coefficient of variability was observed for panicle length and biomass per plant. A low value of phenotypic coefficient of variability and genotypic coefficient of variability obtained for SPAD value, days to 50% flowering, days to maturity and spikelets fertility. High values of phenotypic coefficient of variability and genotypic coefficient of variability with less difference for those traits revealed that less influence of environmental factors and simple selection is sufficient for improvement.

Plant height at 35days (89.01%), number of tillers at 35 days (92.88%), days to 50 % flowering (97.74%), plant height at maturity (95.22%), number of productive tillers (81.97%), panicle length (97.54%), panicle weight (96.60%), grain(93.43%) and straw(94.08%) yield per plant, test weight

(93.50%), biomass per plant(97.27%) and nitrogen use efficiency (91.78%) had high heritability and genetic advance as per cent mean. Similar findings reported by Prasad *et al.* (17). Rita Binseet *al.* (10) reported similar results for the traits like total number of chaffy spikelets per panicle, grain yield per plant, total number of filled spikelets per panicle and spikelet fertility. A high heritability with moderate genetic advance as per cent mean observed for SPAD value and panicle fertility. Low genetic advance for days to maturity was observed. Presence of high heritability with high genetic advance indicate simple selection is sufficient to improve the traits like plant height at 35 days, number of tillers at 35 days, days to 50% flowering, plant height at maturity, number of productive tillers, panicle length, panicle weight, grain yield, straw yield per plant, test weight, biomass per plant and nitrogen use efficiency

**Table 4: Estimation of genetic variability parameters for yield related traits and nitrogen use efficiency in local rice genotypes under no nitrogen condition.**

Characters	Mean	Range		Coefficients of variability		Heritability % (Broad sense)	GAM (%)
		Minimum	Maximum	PCV %	GCV %		
Plant height at 35 days (cm)	47.62	35.20	64.40	13.33	12.58	89.01	29.34
Number of tillers at 35 days	8.13	4.50	15.45	28.18	27.15	92.88	69.45
SPAD value	37.42	33.20	44.49	8.38	6.76	65.07	19.00
Days to 50% flowering	93.80	82.00	122.00	9.90	9.79	97.74	21.43
Days to maturity	131.38	122.00	151.00	4.46	4.41	97.87	9.93
Plant height at maturity (cm)	100.25	66.30	135.50	17.49	17.07	95.22	36.98
Number of tillers at harvest	10.98	5.40	22.10	26.93	26.51	96.91	64.29
Number of productive tillers	9.23	3.35	15.18	27.51	24.89	81.87	65.55
Panicle length (cm)	21.48	14.90	27.80	11.49	11.35	97.54	28.20
Panicle weight (g)	2.96	1.44	6.84	31.68	31.13	96.60	97.84
Number of Spikelets /panicle	117.08	33.85	209.00	32.84	32.37	97.19	68.47
Panicle fertility (%)	92.04	48.07	97.84	7.93	7.88	98.72	17.41
Grain yield /plant (g)	18.55	9.48	36.56	29.05	28.08	93.43	64.88
Straw yield/plant (g)	16.41	7.06	32.22	25.54	24.78	94.08	58.35
Test weight (g)	22.76	11.45	31.65	23.70	22.91	93.50	52.92
Biomass /plant (g)	41.29	24.26	61.54	18.41	18.16	97.27	40.28
Nitrogen use efficiency	33.74	17.27	59.92	27.99	26.81	91.78	60.37

#### IV. CONCLUSION

The present investigation to be concluded as, it has identified some of the promising local genotypes of rice which had higher yield with superior nutritional quality under low nitrogen conditions than check varieties and the yield attributing characters were also found superior to check variety. Genetic parameters studied were inferred that selection can be made on those characters for their utilization in crop improvement. Majority of the local genotypes evaluated in present experiment, had revealed negative correlation of plant height with yield and nitrogen use efficiency, hence, it may required to reduce height to achieve maximum yield with high nitrogen use efficiency .

## BIBLIOGRAPHY

1. Fageria, N. K. and Barbosa Filho, M. P. 2001. Nitrogen use efficiency in lowland rice genotypes. *Communications in Soil Science and Plant Analysis*, **32**: 2079–2089.
2. Fischer, K. 1998, Towards increasing nitrogen use efficiency in rice cropping system: the next generation technology. *Field Crop Res.*, **56**: 1-6.
3. De Datta, S. K. and Buresh, R.J. 1989. Integrated nitrogen management in irrigated rice. *Adv. Soil Sci.*, **10**: 143-169.
4. Cochran, W. G. and Cox, G. M. 1957. *Experimental Designs*. John Wiley and Sons, Inc., New York, 611.
5. Shivasubramanian, S. and Menon, N., 1973, Heterosis and inbreeding depression in rice. *Madras Agri. J.*, **60**:1134-1144.
6. Lush, J. L. 1945. Intra- sire correlation on regression of off- spring on dams as a method of estimating heritability of characters. *Proc. American. Soc. Animal Prod.*, **33**: 292-301.
7. Robinson, H. E. and Comstock, R. S. 1949. Biometrical studies of yield in segregating population of Korean lespedeza. *Agron. J.*, **48**: 268-272.
8. Johnson, H. W.; Robinson, H. F. and Comstock, R. E. 1955. Estimates of genetic and environmental variability in soybean. *Agron.J.*, **7(7)**: 314-318.
9. Vaishali,M.G.2003.DNAmarker assisted mapping of blast and yield loci, graphical genotyping and candidate gene analysis in Rice(*OryzasativaL.*).*Ph.DThesis*, Univ.Agric. Sci., Bangalore, India.
10. Rita Binse, Sarawgi, A. K. and Verulkar, S. B. 2009. Study of heritability, genetic advance and variability for yield contributing characters in rice. *Bangladesh J. Agril. res.*, **34** (2): 175-179.
11. Selvaraj, I.C.; Nagarajan, P.; Thiyaagarajan, K.; Bharathi, M. and Rbindran,R. 2011. Genetic parameters of variability , correlation and path co-efficient studies for grain yield and other yield attributes among rice blast disease resistant genotypes of rice (*Oryza sativa L.*). *African J. Biotechnol.*, **10**(17): 3322-3334.
12. Paikhomba, N.; Arvind Kumar; Chaurasia, A. K. and Prashant Kumar Rai. 2014. Assessment of Genetic Parameters for Yield and Yield Components in Hybrid Rice and Parents.*J. Rice res.*,**2**(1): 1-3.
13. Sreenatha, K.S. and Shivashankar.G. 1987. Genetic analysis and evaluation of Basumathi derivatives for yield and yield components. *M.Sc., Thesis, Univ. Agric. Sci.*, Bangalore.
14. Vikas Kumar, Nidhi Koshta, Neha Sohgaure and Kouta, G.K. 2014. Genetic evaluation of RIL's Population for yield and quality Attributing Traits in Rice. *J. Agric. Technol.*,**1**(1): 43-51.
15. Thirumala Rao, V.; Mohan V. C.; Bhadru, D.; Bharathi, D. and Venkanna, V. 2014. Genetic Variability and Association Analysis In Rice. *Int. J. Appl. Biol. & Pharmaceutical Technol.*,**5**(2): 63-65.
16. Afzal,Z.M .;Akhter,M.;Zaheen,S. M. andTahir,A.2006. Correlation and Path Analysis Studie of Yield and Economic Traitsin Basmati Rice (*OryzasativaL.*).*Asian J.Plant Sci.*, **5**:643-645.
17. Prasad, B, A.; Patwary K. and Biswas, P. S. 2001. Genetic variability and selection criteria in fine rice (*Oryza sativa L.*). *Pakistan J. Biol. Sci.*,**4**: 1188-1190.