### **Scholars Journal of Agriculture and Veterinary Sciences**

Sch J Agric Vet Sci 2017; 4(1):13-17 ©Scholars Academic and Scientific Publishers (SAS Publishers) (An International Publisher for Academic and Scientific Resources) e-ISSN 2348–1854 p-ISSN 2348–8883

DOI: 10.36347/sjavs.2017.v04i01.003

# Effect of Drought Stress on Grain Dry Weight, Photosynthesis and Chlorophyll in Six Rice Genotypes

S. Moonmoon<sup>1</sup>, M.S.A. Fakir<sup>2</sup>, M.T. Islam<sup>3</sup>

<sup>1</sup>Department of Crop Botany and Tea Production Technnology, Sylhet Agricultural University, Bangladesh <sup>2</sup>Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh <sup>3</sup>Crop Physiology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

## \*Corresponding Author

Name: S. Moonmoon

Email: moondj311@yahoo.com

Abstract: Drought is an alarming issue limiting crop production worldwide and is becoming increasingly severe with the passage of time in Bangladesh. Rice production in Bangladesh is thus, being adversely hampered by drought. Therefore a pot experiment was carried out with six rice genotypes to assess the effect of drought stress on grain dry weight, photosynthesis and chlorophyll content during July to December 2013-2014 at Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh. Drought stress (control and 40% FC) was imposed on Binadhan-13, Kalizira, BRRI dhan-34, Ukunimodhu, RM-100-16 and NERICA mutant rice genotypes at grain filling stage (anthesis to maturity) and discontinued when the specific stage was over. The experiment was laid out in a Complete Randomized Design with three replications. Grain dry weights of 10 grains of selected panicles, chlorophyll content (SPAD reading) and photosynthetic rate of flag leaf were measured at 3 days interval from anthesis to maturity. Data was recorded at anthesis to maturity. Drought stress decreased grain dry weight, photosynthetic rate and chlorophyll content of flag leaf significantly. The highest grain dry weight, chlorophyll content and photosynthetic rate were found in Binadhan-13 and NERICA mutant. Grain dry weight increased with age till 24 days after anthesis followed by a Plateau whereas SPAD reading and photosynthesis rate decreased with grain age until physiological maturity. From the current research, it appeared that increased drought tolerant mechanisms were observed in the genotypes NERICA mutant and BINAdhan-13.

Keywords: Rice, drought stress, grain dry weight, photosynthesis, chlorophyll content

#### INTRODUCTION

Rice (Oryza sativa L.) is a globally important cereal crop, and as a primary source of food it accounts for 35-75% of the calorie intake of more than 3 billion humans. Rice, cultivated as food for direct consumption more so than any other crop, is the second largest consumed cereal after wheat and provides about 80% of the food calorie requirements of more than half of the world's population [1]. As the world's population continues to grow toward 10 billion by 2050, the demand for rice will grow faster than for other crops because population growth is greatest in the riceconsuming and rice-producing regions of Asia, Africa, and the America [2-4]. In addition, the shortage of land and water for rice cultivation [5], accompanied by increases in food demand, has also forced cultivation to extend beyond normal monsoon periods, where temperatures are optimal for growth to warmer summer seasons where drought is an important constraint.

Droght stress (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle. The lack of adequate moisture leading to

drought stress is a common occurrence in rainfed areas, brought about by infrequent rains and poor irrigation. About 13% of the world's 156 million ha of rice is cultivated as rainfed rice under upland conditions where drought stress affects rice growth and reduces grain dry weight [6]. Drought stress affects plant growth and development and ultimately, reduces grain yield of rice. The reduction in yield may depend on developmental stage of the crop. The response of rice yield to soil water status varies with growth stage being most sensitive at flowering, followed by booting and grain filling stage [7]. More reduction in grain dry weight, due to drought stress in flowering stage, is largely resulted from the reduction in fertile panicle and filled grain percentage. Drought stress also promoted the remobilization of stored carbon reserves and water deficit during grain-filling enhanced plant senescence and accelerated grain-filling. Usually, senescence induced by water deficit shortens grain-filling period and can result in reduction in grain weight. According to [8] grain filling pattern had marked influence on final grain weight.

Plant growth is anchored by photosynthesis; however, excess light (EL) can cause severe damage to plants. EL induces photo oxidation, which results in the increased production of highly reactive oxygen intermediates that negatively affect biological molecules and, if severe, a significant decrease in plant productivity [9]. Drought stress directly affects rates of photosynthesis due to the decreased CO<sub>2</sub> availability resulted from stomatal closure [10,11] and/or from changes in photosynthetic metabolism [12].

The occurrence of drought stress affects many of the physiological processes such as photosynthesis and chlorophyll resulting in reduced growth and poor grain filling [13]. In contrast with other crops, rice is particularly more sensitive to water stress especially at reproductive growth stages such as panicle initiation, anthesis and grain filling [14, 15]. Thus, drought stress is affecting about 50% of rice production in the world [16, 2, 17]. Though so many research works in regard to effects of water stress on photosynthesis, grain growth and vield in rice were carried out all over the world including Bangladesh [18-20] but little works on rice in these aspects were done especially in Bangladesh [21,22]. This study, therefore, be carried out to investigate photosynthesis and its related parameters, to study grain growth in six rice genotypes.

#### MATERIALS AND METHODS

A pot experiments were carried out with six rice genotypes viz., Binadhan-13, Kalizira, BRRI dhan 34, Ukunimadhu, RM-100-16 and NERICA mutant at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Control (100% FC) and droght stress (40% FC) was imposed on rice genotypes at grain filling stage and discontinued when the specific stage was over. The soils were collected from BINA farm. The soil was sandy loam in texture having pH 6.67. Each pot contained 13 kg of soils. The fertilizer doses ha<sup>-1</sup> was 160, 65, 120 and 90 kg Urea, TSP, MOP and Gypsum, respectively. The experiment was laid out in a Complete Randomized Design with three replications. Twenty one day old seedlings were transplanted in pots on 25 July in 2013 and 22 July in 2014. Grain dry weights of 10 grains of selected panicles, chlorophyll content (SPAD reading) and photosynthetic rate of flag leaf were measured at 3 days interval from anthesis to maturity. Photosynthesis was recorded using Portable Photosynthesis System LI-6400XT, LI-COR Inc., Lincoln, NE, USA. Chlorophyll content was measured using SPAD meter (Model: SPAD 502). Data on grain dry weight, photosynthesis and chlorophyll content were recorded at anthesis to maturity. The collected data were analyzed statistically

following Completely Randomized Design by MSTAT-C computer package programme developed by [23]. The treatment means were adjudged by Duncan's Multiple Range Test (DMRT).

#### RESULTS AND DISCUSSION

Results showed that drought stress decreased grain dry weight, photosynthetic rate and chlorophyll content of flag leaf significantly in 2013 and 2014 (Table 1). The higher grain dry weight, chlorophyll content and photosynthetic rate was found in genotypes NERICA and Binadhan-13. Grain dry weight increased with age till 24 days after anthesis followed by a Plateau whereas SPAD reading and photosynthesis rate decreased with grain age until physiological maturity. The higher grain dry weight was observed in genotypes NERICA (13.33) and Binadhan-13 (8.64) and lesser was observed in rest of the genotypes RM-100-16 (8.02), Kalizira (7.04), BRRI dhan-34 (6.80) and Ukunimodhu (6.59) (Table 1). Grain dry weight is the ultimate result of grain filling. Grain filling is the final stage of growth in cereals where fertilized ovules develop into caryopses. More reduction in grain yield, due to water stress in flowering stage, is largely resulted from the reduction in fertile panicle and filled grain percentage. In rice water stress at vegetative growth especially booting stage [24] and flowering stage can interrupt floret initiation causing spikelet sterility and poor grain filling resulting in lower grain weight and ultimately reduces rice yield [25,26].

Drought stress effect was statistically significant at  $(P \le 0.05)$  in both the year (Table 2). Under control condition, grain dry weight ranged from 6.54 to 14.20 in 2013 and 6.87 to 15.13 mg grain<sup>-1</sup> in 2014 while under drought it ranged from 6.02 to 13.4 in the former and 6.31 to 14.91 mg grain<sup>-1</sup> in the later year (Table 2). In 2013, grain dry weight reduction was lesser in genotypes Binadhan-13 and NERICA mutant (average of 4.89%) compared to others (average of 7.01%) while in 2014, it was also smaller in Binadhan-13 and NERICA (average of 5.33%) compared to the rest (average of 7.03%) (Table 2). In both the years, at 40% FC the degree of relative reduction in grain dry weight was smaller in Binadhan-13 and NERICA (3.79%, average of 2013 and 2014) than in the four genotypes (Kalizira, BRRI dhan34, RM-100-16 and Ukunimodhu) (7.02%, average of 2013 and 2014) (Table 2) and this result suggests that reserve deposition in the grain was less affected by drought in former than in the latter group of rice genotypes. Thus, the genotypes Binadhan-13, and NERICA appeared to be drought tolerant compared to the rest of the four genotypes.

Table1: Effect of drought on grain growth, chlorophyll content and photosynthetic rate of flag leaf during grain filling period (days after anthesis to maturity) of six rice genotypes in 2013 and 2014

inning period (days after anthesis to maturity) of six rice genotypes in 2013 and 2014									
Treatments	Dry wt ( mg grain <sup>-1</sup> )	Chlorophyll (SPAD value)	Pn rate $(\mu molCO_2 m^{-2} s^{-1})$	Dry wt ( mg grain <sup>-1</sup> )	Chlorophyll (SPADvalue)	Pn rate (μmolCO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )			
	2013			2014					
Drought									
Control	8.61 a	37.76 a	23.16 a	8.791	40.29	25.02 a			
40 % FC	7.76 b	37.36 a	17.37 b	8.415	39.74	18.19 b			
Genotype									
Binadhan-13	8.64 b	39.51 a	19.18 b	8.637 b	41.88 a	20.05 b			
Kalizira	6.70 d	37.59 b	17.04 c	7.042 b	39.84 a	17.80 c			
RM-100-16	7.65 c	36.02 b	16.44 d	8.023 b	38.18 a	17.19 c			
Ukunimodhu	6.28 e	37.44 b	16.90 c	6.592 b	39.68 a	17.66 c			
BRRI dhan-34	6.49 de	37.65 b	16.48 d	6.804 b	39.91 a	17.22 c			
NERICA mutant	13.33 a	37.15 b	35.54 a	14.52 a	40.59 a	39.70 a			
Days after anthesis	2013				2014				
0	1.69 g	39.35 a	24.31 a	1.689 e	42.00 a	26.13 a			
4	2.57 f	39.04 a	22.63 b	2.427 e	41.72 a	24.00 b			
8	5.80 e	38.13 ab	21.86 c	6.678 d	40.84 ab	23.24 с			
12	8.56 d	37.78 abc	20.88 d	8.780 c	40.42 abc	21.89 d			
16	9.98 c	37.28 abc	20.15 e	10.803 b	39.59 bcd	21.10 e			
20	11.20 b	36.67 bc	18.49 f	11.733 b	38.94 bcd	19.50 f			
24	12.81 a	36.38 bc	17.26 g	13.345 a	38.66 cd	18.69 g			
28	12.84 a	35.84 c	16.52 h	13.367 a	37.94 d	18.30 g			
Lsd <sub>0.05</sub>	0.415	1.972	0.405	1.053	1.946	0.666			

Data were separately analyzed for the year 2013 and 2014. In a year in each column, figures having common letter(s) do not differ significantly at  $P \leq 0.05$  as per DMRT.

The reserve mobilization and deposition to the rice endosperm was promoted in tolerant genotypes [27]. Due to water stress at flowering stage largely results in from the reduction in fertile panicle and filled grain percentage [8]. The slow grain filling and low grain weight of inferior spikelet have often been attributed to a limitation in carbohydrate supply [14].

Chlorophyll content in six rice genotypes differed significantly due to drought at  $P \le 0.05$  in the year 2014 (Table 2). Chlorophyll was measured in terms of SPAD value, higher the magnitude greater would be the chlorophyll content. Under control condition, chlorophyll content ranged from 36.07 to 40.01 (SPAD value) in 2013 and 38.23 to 42.41 (SPAD value) in 2014 while under drought it ranged from 35.96 to 39.0(SPAD value) in the former and 37.13 to 42.41 (SPAD value) in the later year (Table 2). In 2013 and 2014, no significant reduction was observed. Genotypes Kalizira and Ukunimodhu showed the lesser reduction (average of 0.63%) than genotypes Kalizira, and Ukunimodhu (average of 2.59%) when compared to control in 2013. In 2014, Genotypes NERICA and Kalizira showed the lesser reduction (average of 0.86%)

than genotypes NERICA and Kalizira (average of 2.63%) compared to control (Table 2).

Results presented in Table 2 showed that combined effect of genotypes and drought on photosynthetic rate (Pn) was significant at  $P \le 0.05$  in both of the years. Under control condition, Pn ranged from 20.13 to 36.25  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in 2013 and 21.05 to 41.28  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in 2014 while under drought it ranged from 12.75 to 34.83 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in the former and 13.32 to 38.11 µmolCO<sub>2</sub>m<sup>-2</sup>s<sup>-1</sup> in the later year (Table 2). In both the years, all the six genotypes showed significant reduction under drought condition over control. Genotypes Binadhan-13 and NERICA showed the lesser reduction (average of 8.77%) than the rest of the genotypes (average of 34.62%) compared to control in 2013. In 2014, genotypes Binadhan-13 and NERICA mutant also showed the lesser reduction (average of 10.54%) than in the rest genotypes (average of 34.68%) compared to control (Table 2). The decrease in chlorophyll content is a commonly observed phenomenon under drought stress [28, 29, 30]. Drought stress reduces the photosynthetic rate by stress-induced stomatal or nonstomatal limitations [31, 32, 33] stated that limited photosynthesis in the flag leaves may affect the reproductive development. Our research findings show that in drought stress condition both photosynthesis rate and chlorophyll content were also decreased at the reproductive stage (Table 2).

Table 2: Combined effect of genotype and drought (40 % of field capacity, FC) on grain dry weight, chlorophyll content and photosynthetic rate of flag leaf during grain filling period of six rice genotypes in 2013 and 2014

		2014					
Genotype	Drought	Grain dry wt	Chlorophyll	Pn rate			
Genotype	level	(mg grain <sup>-1</sup> )	(SPAD value)	$(\mu molCO_2m^{-2}s^{-1})$			
	2013						
Binadhan-13	Control	9.35 c+	40.01 a	23.27 с			
	40% FC	8. 93 c (4.49) ++	39.00 ab (2.52)	20.10 d (13.62)			
Kalizira	Control	6.98 ef	37.77 abc	20.23 d			
	40% FC	6.42 gh (8.02)	37.41 abc (0.95)	13.85 f (31.53)			
RM-100-16	Control	7.89 d	37.98 abc	20.13 d			
	40% FC	7.41 de (6.08)	36.90 bc (2.84)	12.75 g (36.66)			
Ukunimodhu	Control	6.54 fgh	36.07 c	20.32 d			
	40% FC	6.02 h (7.95)	35.96 c (0.30)	13.49 f (33.61)			
BRRIdhan34	Control	6.69 fg	38.09 abc	20.17 d			
	40% FC	6.29 gh (5.98)	37.21 abc (2.31)	12.77 g (36.69)			
NERICA	Control	14.20 a	37.66 abc	36.25 a			
	40% FC	13.4 b (5.28)	36.63 bc (2.73)	34.83 b (3.92)			
	2014						
Binadhan-13	Control	9.35 b	42.41 a	24.33 c			
	40% FC	8.93 b (4.46)	41.34 a (2.52)	21.07 d (13.39)			
Kalizira	Control	7.33 b	40.03 a	21.15 d			
	40% FC	6.76 b (7.74)	39.66 a (0.92)	14.46 e (31.63)			
RM-100-16	Control	8.28 b	40.25 a	21.05 d			
	40% FC	7.77 b (6.18)	39.11 a (2.83)	13.32 e (36.72)			
Ukunimodhu	Control	6.87 b	38.23 a	21.23 d			
	40% FC	6.32 b (8.01)	37.13 a (2.88)	14.09 e (33.63)			
BRRIdhan34	Control	7.02 b	40.38 a	21.10 d			
	40% FC	6.59 b (6.17)	39.45 a (2.30)	13.35 e (36.73)			
NERICA	Control	15.13 a	40.75 a	41.28 a			
	40% FC	14.91 a (6.21)	40.43 a (0.79)	38.11 b (7.68)			

<sup>+:</sup> Data were separately analyzed for the year 2013 and 2014. In a year in each column, figures having common letter(s) do not differ significantly at  $P \le 0.05$  as per DMRT.

#### **CONCLUSION**

In summary, drought stress not only limits grain dry weight, chlorophyll content but also photosynthetic rate. Based on the relative reduction of physiological variables, genotypes Binadhan-13 and NERICA mutant demonstrated better tolerance to drought.

#### **ACKNOWLEDGEMENTS**

We gratefully acknowledge the Division of Crop Physiology, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh for funding and conducting the research..

#### REFERENCES

- FAO. Food and agriculture Statistics Global Outlook. Food and Agricultural Organization of the United Nations 2008. http://faostat.fao.org/Portals/\_Faostat/documents/pd f/world.pdf.
- 2. Dawe D. Agricultural research, poverty alleviation and key trends in Asia's rice economy. In "Charting New Pathways to C4 Rice" (J. E.

- Sheehy, P. L. Mitchell, and Hardy B, Eds.) 2007 pp. 37–53. Intl. Rice Res. Ins., Los Banos, Philippines.
- 3. Easterling WE, Aggarwal PK, Batima P, Brander KM, Erda L, Howden SM, Kirilenko A, Morton J, Soussana JF, Schmidhuber J, Tubiello FN. Food, fibre and forest products. In "Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report ofthe Intergovernmental Panel on Climate Change" (M. L. Parry, O. F. Canziani J. P. Palutikof, P. J. van der Linden, and C.E. Hanson, Eds.) pp. 273–313. Cambridge University Press, Cambridge, United Kingdom.
- 4. Krishnan P, Ramakrishnan B, Reddy KR, Reddy VR: High-Temperature Effects on Rice Growth, Yield, and Grain Quality. Advances in Agronomy. 2011:111 87-206.
- 5. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology. 2005;59:1-6.
- 6. Carlos ACC, Orivaldo A, Rogério PS, Gustavo PM. Grain quality of upland rice cultivars in

<sup>++:</sup> Figures within parenthesis indicate % decrease at 40 % FC compared to control.

- response to cropping systems in the Brazilian tropical savanna. Science of Agriculture. 2008;65:468-473.
- 7. O'Toole JC. Adaptation of rice to drought-prone environments. In: Drought Resistance in Crops with Emphasis on rice. IRRI, Manila, Phillippines.1982;195-216.
- 8. Xie G, Yang J, Wang Z, Zhi Q. Grain filling characteristics of rice and their relationships to physiological activities of grains. Acta Agronomy Sinica. 2001;27:557-565.
- 9. Li Z, Wakao S, Fischer BB, Niyogi KK. Sensing and responding to excess light. Annuals Revew of Plant Biology. 2009;60 239–260.
- 10. Flexas J, Bota J, Galmés J, Medrano H, RibasCarbó M. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiology of Plant. 2006;127:343–352.
- 11. Chaves MM, Flexas J, Pinheiro C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of Botany. 2009;103:551–560.
- 12. Lawlor DW, Cornic G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell and Environment. 2011;25:275–294.
- 13. Samonte S, Wilson LT, McClung AM, Tarpley L. Seasonal dynamics of non-structural carbohydrate in 15 diverse rice genotypes. Crop Science. 2002;41:902-909.
- Tao H, Brueck H, Dittert K, Kreye C, Lin S, Sattelmacher B. Growth and yield formation for rice (Oryza sativa L.) in the watersaving ground cover rice production system (GCRPS). Field Crops Research. 2006;95:1-12.
- 15. Yang J, Peng S, Zhang Z, Wang Z, Visperas RM, Zhu Q. "Grain and dry matter yields and partitioning of assimilates in japonica/indica hybrid rice," Crop Science. 2002;42:766–772.
- Bouman BAM, Peng S, Castaoeda AR, Visperas RM. Yield and water irrigated tropical aerobic rice systems. Agricultural Water management. 2005;74:87-105.
- 17. Mostajean A, Eichi VR. Effects of drought stress on growth and yield of rice (Oryza sativa L.) Cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves American-Evras. Journal of Agriculture and Environmental Sciences. 2009;5:264-272.
- 18. Islam MT. Screening of some transplanted aman rice cultivars under water stress condition. Bangladesh Journal of Training and Development. 2001;14:213-220.
- 19. Zubaer MA, Chowdhury AKMBB, Islam MZ, Ahmed T, Hasan MA. Effects of water stress on growth and yield attributes of Aman rice genotypes. International Journal of Sustainable Crop Production. 2007;2:25-30.

- Ashraf M. Inducing drought tolerance in plants. Recent Biotechnological Advances. 2010;28:169-183
- 21. Sadeghi SM, Danesh RK. Effects of water deficit role at different stages of reproductive growth on yield components of rice. World Applied Sciences Journal. 2011;13:2021-2026.
- 22. Sabetfar S, Ashouri M, Amiri M, Babazadeh S. Effect of Drought Stress at Different Growth Stages on Yield and Yield Component of Rice Plant. Persian Gulf Crop Protection. 2013;2:14-18.
- Rusell DF. MSTAT-C Package Programme. 1986: Crop and Soil Science Department, Michigan State University, USA.
- Pantuwan G, Fukai S, Cooper M, Rajatasereekul, O'Toole JC. Yield responses of rice (Oryza sativa L.) genotypes to drought under rainfed lowlands Selection of drought resistant genotypes. Field Crops Research. 2002;73:169-180.
- 25. Kamoshita A, Rofriguez R, Yamauchi A, Wade L. Genotypic variation in response of rainfed lowland to prolonged drought and rewatering. Plant Production Science. 2004;7:406-420.
- Botwright A, Latte HR, Wade LG. Genotype and environment interactions for grain yield of upland rice backcross lines in diverse hydrological environments. Field Crops Research. 2008;108:117-125.
- Morita S, Yonemaru J, Takanashi J. Grain Growth and Endosperm Cell Size Under High Night Temperatures in Rice (Oryza sativa L.). Annals of Botany. 2005;95:695-701.
- Bijanzadeh E, Emam Y. Effect of defoliation and drought stress on yield components and chlorophyll content of wheat. Pakistan Journal of Biological Science. 2010;13:699-705.
- Mafakheri A, Siosemardeh A, Bahramnejad B. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science. 2010;4:580-585.
- Din J, Khan SU, Ali I, Gurmani AR. Physiological and agronomic response of canola varieties to drought stress. Journal of Animal Plant Science. 2011;21:78-82.
- 31. Kim JY, Mahé A, Brangeon J, Prioul JL. A maize vacuolur invertase, IVR2, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression, Plant Physiology. 2000;124:71–84.
- 32. Saibo NJM, Lourenço Τ, Oliveira Transcription factors and regulation photosynthetic and related metabolism under environmental stresses. Annals of Botany. 2009;103:609-623.
- 33. Rahnama A, Poustini K, Tavakkol-Afshari R, Tavakoli A. Growth and stomatal responses of bread wheat genotypes in tolerance to salt stress. International Journal of Biological Life Science. 2010;6:216-221.