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CARDIORESPIRATORY RESPONSES OF MILITARY LOAD CARRIAGE WITH VARYING WALKING SPEEDS AND GRADIENTS

Madhu Sudan Pal[1], Deepti Majumdar[1], Prakash Chandra Dhara[2] and Dhurjati Majumdar[1]*

[1]Defence Institute of Physiology and Allied Sciences Defence Research & Development Organisation, Min. of Defence, Govt. of India, Lucknow Road, Delhi-110054, India

[2]Department of Human Physiology with Community Health, Vidyasagar University, Midnapore-721102, W. B.

Abstract ■ The present study was undertaken to compare the cardiorespiratory responses during carrying a standard load weight (10.7 kg) in two different modes (compact and distributed) at varying walking speeds and gradients by Indian soldiers and to find out the best mode of load carriage out of these two processes. Ten physically fit infantry soldiers with their mean (SD) age 23.2 (2.62) years, height 172.6 (3.81) cm, weight 65.9 (17.09) kg, maximum aerobic capacity 47.51(4.44) $ml.kg^{-1}.min^{-1}$ walked at 3.5 and 4.5 $km.hr^{-1}$ on a motor driven treadmill for 10 minutes at each of the four gradients (0, 5, 10 and 15%), respectively without and with carrying 10.7 kg load in compact and distributed mode. Heart rate (HR), minute ventilation (VE) and oxygen consumption (VO_2) were determined using $K4b^2$ system. It was observed that distributed mode of carriage demands higher cardiorespiratory responses than compact mode by 2.6, 4.8, 5.2 and 6.7 % for HR; 5.8, 6.3, 7.0 and 7.9 % for VE ; 4.9, 5.6, 6.0 and 6.5 % for VO_2 ; 4.9, 5.7, 6.2 and 6.3 % for $\%VO_2max$ with walking speed of 3.5 $km.hr^{-1}$ at 0, 5, 10 and 15 percent gradient, respectively. However, for 4.5 $km.hr^{-1}$ speed it exceeded by 4.6, 5.4, 6.8 and 8.3 % for HR; 6.0, 6.3, 7.4 and 8.2 % for VE; 5.1, 5.9, 7.1 and 9.0 % for VO_2; 5.0, 5.9, 7.1 and 7.7 % for $\%VO_2max$ at 0, 5, 10 and 15 percent gradient, respectively. It is concluded that cardiorespiratory responses are higher for distributed mode of load carriage than compact mode during varying conditions of walking speed and gradients.

***Keywords** : Load carrying; Gradient walking; Load distribution; Heart rate; Oxygen uptake*

INTRODUCTION

Load carriage is a common activity among humans in all walks of life. However, load carriage in armed forces is considered as more physically and mentally demanding than other occupations. Soldiers are required to carry heavy loads in different terrains and gradients even under adverse climatic conditions and are simultaneously required to maintain effective combat fitness. The physiological cost of load carriage has been investigated in India (Datta et al.1975; Samanta and Chatterjee 1981) and

* *Corresponding author*

elsewhere (Beekley et al. 2007; Lyons et al. 2005; Christie and Scott 2005; Charteris et al. 1989; Hong et al. 2000; Li et al. 2003; Patton et al. 1991). Interestingly, in most of the studies, load was placed as single compact unit and carried on head or as backpack. In some of the studies load was distributed in two halves and placed on the yoke (Datta and Ramanathan, 1971). The position of the yoke varied as per the carrying mode of the local tradition. Considerable research has been devoted to determine the best method of load carriage that minimizes the physical stress on the body (Datta and Ramanathan 1971, Legg 1985, Legg and Mahanty 1985, Kirk and Schneider 1992, Legg et al. 1992, Jacobson and Jones 2000, Lloyd and Cooke 2000). It has been recognized that gradient, together with speed and load are among the main determinants of energy expenditure during walking (Givoni and Goldman 1971, Knapik et al. 1996). In military environment, particular preference of carrying load is not applicable. Soldiers carry load in a set pattern such as some load in backpack, ammunition in military waist belt (web) and arms (rifle) in hand. But limited research (Johnson et al. 1995, Legg et al.1992, Abe et al. 2004) has been carried out on how the distribution of load in different parts of the body will affect the physiological responses of an individual.

Soldiers of the Indian Army carry 10.7 kg of load in various operational conditions either as compact load as Backpack (BP) or as distributed load in combination of Haversack (HS, 4.4 kg), Web (Wb, 2.1 kg) and INSAS rifle (R, 4.2 kg). The purpose of the present study was, therefore, to compare the cardiorespiratory responses of carrying this army standard load in two different modes (compact and distributed) at varying walking speeds and gradients by Indian soldiers and to find out the best mode of load carriage out of these two processes.

METHOD

Subjects :

Ten physically fit male infantry soldiers of Indian Army without any history of musculoskeletal disorders or cardiovascular pathology and with a service experience of at least four years volunteered for this study. Their mean (SD) age, height, weight and maximum aerobic capacity were 23.2 (2.62) years, 172.6 (3.81) cm, 65.9 (7.09) kg and 47.51(4.44) ml.min-1kg-1, respectively. They signed informed consent before participating in the experimental procedure.

Experimental details :

At the beginning soldiers were briefed about the purpose and risk of the study. Initially they were habituated to walking on treadmill (Taeha, Intertrack 6025, Korea) at various speeds, without and with loads at different gradient in the laboratory. Next maximum aerobic capacity (VO_2max) of the subjects was measured in treadmill exercise with regular increase in gradient (Harbor Protocol, Wasserman et al. 1994), keeping the speed constant. During the measurement of maximum aerobic capacity subjects wore vest, underwear and physical training shoes. On the day of load carriage experiment the subjects reported to the laboratory at 8.00 a.m. after light breakfast and load carriage experiments were carried out after allowing one hour rest. They were abstained from smoking or taking any food as long as they were in the laboratory. During the load carriage experiment all subjects wore full Indian Army uniform including combat boot and helmet which weighs about 3.7 kg. Load carriage experiments were carried out on each subject with and without carrying 10.7 kg load (16.2 % body weight) in two different modes at 3.5 and 4.5 $km.hr^{-1}$ walking speeds at 0%, 5%, 10% and 15% gradients on a motor driven treadmill for 10 minutes duration. The two modes of load carriage were: i) Compact mode-

10.7 kg load was carried in a standard military backpack; ii) Distributed mode- 4.4 kg load was carried in the standard military HS on the back, plus 2.1 kg load was carried in Wb in front of waist region and 4.2 kg INSAS rifle was carried in right hand. A balanced order experimental design was employed in which five subjects used the compact load carriage first and other five subjects used the distributed load carriage. (The mode, magnitude and placement of loads are given in Table 1). A total of 240 experiments were carried out on load carriage. The subjects underwent load carriage experiments in different days. About one hour rest was given between two experiments.

Cardiorespiratory measurements:

All load carriage experiments were conducted in controlled laboratory environment of 22°C-25°C, 50%-55% relative humidity; and at the same hour of the day between 9.30 a.m. and 13.30 p.m. for eliminating the specific dynamic action of food for all practical purposes. During the experiment, heart rate (HR), minute ventilation (VE) and oxygen consumption (VO_2) of each of the individuals were determined by the process of breath-by-breath gas analysis using $K4b^2$ system ($K4b^2$, Cosmed S.r.l, Italy). Average of the last 3 minutes HR, VE and VO_2 data of 10 minutes walking trial were considered as individual value.

Statistical analysis :

Paired Student's t-Test was performed to determine the level of significance in different cardiorespiratory responses in comparison to no load condition at different gradients of walking and between two modes of load carriages.

Table 1 : The mode, magnitude and placement of load during load carriage experiment

Condition	Weight (kg)	Placement of load	Mode
A	0.0	No load (NL)	—
B	10.7	10.7 kg backpack (BP)	Compact load
C	10.7	4.4 kg haversack on back + 2.1 kg web in front of waist + 4.2 kg INSAS rifle in right hand (HSWR)	Distributed load

RESULTS

Cardiorespiratory responses, e.g., HR, VE, VO_2 and $\%VO_2max$ during without and with carrying of 10.7 kg load as compact and distributed mode with varying walking speeds (3.5 and 4.5 km.hr-1) and treadmill gradients (0, 5, 10 and 15%) are shown in Tables 2 and 2a. It has been observed that cardio respiratory responses were increased with increase in speed and gradient in each of the no-load and loaded conditions. This increase was significant ($p<0.05$) in each of the gradient and speed conditions between NL and BP, NL and HSWR and BP and HSWR except for HR at 0% gradient at

walking speed of 3.5 km.hr^{-1} Cardiorespiratory parameters showed higher responses during distributed mode of load carriage (HSWR) than compact mode (BP) in both walking speeds and gradients.

The values of HR, VE, VO_2 and $\%VO_2$ max increased from 93.4 to149.1 beats.min^{-1}, 23.9 to 57.3 L.min^{-1}, 9.8 to 28.3 ml.min^{-1}kg^{-1}, and

Table 2 : Mean (SD) of HR (beat.min^{-1}), VE (L.min^{-1}), VO_2 (ml.min^{-1}kg^{-1}) and $\%VO_2$ max while walking at 3.5 km.hr^{-1} speed at different gradients without and with carrying a 10.7 kg load as compact (BP) and distributed (HSWR) mode.

Parameters	Gradient (%)	Load (kg)			Level of significance		
		NL (A)	BP (B)	HSWR (C)	A vs B	A vs C	B vs C
HR	0	89.7 (6.57)	93.4 (6.75)	95.8 (10.64)	NS	NS	NS
	5	96.6 (5.79)	102 8 (6.88)	107.8 (6.64)	***	***	***
	10	106.3 (5.54)	117.2 (2.52)	123.3 (3.06)	***	***	***
	15	132.6 (9.75)	149.1(11.71)	159.1(12.54)	***	***	**
VE	0	22.3 (2.04)	23.9 (2.28)	25.3 (2.04)	***	***	***
	5	28.9 (1.01)	31.4 (1.11)	33.4 (1.14)	***	***	***
	10	37.3 (1.93)	41.8 (3.48)	44.8 (2.36)	***	***	***
	15	50.6 (2.70)	57.3 (5.02)	61.8 (4.71)	***	***	***
VO_2/kg	0	9.4 (0.67)	9.8 (0.65)	10.2 (0.59)	***	***	***
	5	14.3 (0.60)	15.1 (0.85)	16.0 (0.76)	***	***	***
	10	19.6 (0.78)	21.0 (1.26)	22.2 (0.73)	***	***	***
	15	26.0 (2.14)	28.3 (2.13)	30.2 (2.46)	***	***	***
$\%VO_2$max	0	19.9 (1.58)	20.6 (1.79)	21.6 (1.62)	***	***	***
	5	30.4 (2.87)	32.0 (3.21)	33.8 (3.40)	***	***	***
	10	41.6 (4.14)	44.5 (4.83)	47.2 (5.50)	***	***	***
	15	55.3 (7.17)	60.1 (7.61)	63.9 (6.72)	***	***	***

** P<0.01, *** P<0.001, NS Not Significant

Table 2a : Mean (SD) of HR (beat.min^{-1}), VE (L.min^{-1}), VO_2 (ml.min^{-1}kg^{-1}) and %VO_2max while walking at 4.5 km.hr^{-1} speed at different gradients without and with carrying a 10.7 kg load as compact (BP) and distributed (HSWR) mode.

Parameters	Gradient (%)	Load (kg)			Level of significance		
		NL (A)	BP (B)	HSWR (C)	A vs B	A vs C	B vs C
HR	0	93.8 (7.95)	99.1 (7.64)	103.6 (9.26)	*	**	*
	5	110.6(11.14)	118.7(10.96)	125.1(10.65)	***	***	**
	10	130.1(11.07)	144.2 (9.65)	154.0 (8.23)	***	***	***
	15	148.4 (9.70)	169.7 (7.49)	183.8 (4.83)	***	***	***
VE	0	28.3 (2.33)	30.5 (2.21)	32.3 (2.41)	***	***	***
	5	39.1 (3.21)	43.2 (4.41)	45.9 (3.74)	***	***	*
	10	52.2 (5.86)	59.6 (4.12)	64.0 (3.65)	***	***	***
	15	66.2 (4.03)	76.8 (4.27)	83.1 (7.78)	***	***	**
VO_2/kg	0	13.2 (1.02)	14.0 (1.07)	14.7 (1.24)	*	***	**
	5	19.4 (1.59)	20.8 (1.86)	22.0 (1.75)	**	***	***
	10	26.2 (2.09)	28.5 (1.42)	30.5 (2.28)	***	***	***
	15	32.3 (3.81)	35.6 (3.93)	38.8 (2.75)	***	***	*
%VO_2max	0	28.0 (1.99)	29.5 (2.43)	31.0 (2.15)	*	***	**
	5	41.0 (3.84)	43.9 (4.14)	46.5 (3.99)	**	***	***
	10	55.5 (5.47)	60.3 (5.31)	64.6 (6.39)	***	***	***
	15	68.1 (7.91)	76.2 (7.48)	82.1 (6.28)	***	***	*

* P<0.05, ** P<0.01, *** P<0.001, NS Not Significant

20.6 to 60.1 % at 3.5 km.hr^{-1} in 0% to 15% gradients, respectively, for BP. These parameters increased from 95.8 to 159.1 beat.min^{-1}, 25.3 to 61.8 L.min^{-1}, 10.2 to 30.2 ml.min^{-1} kg^{-1} and 21.6 to 63.9 % respectively for HSWR at same speed and gradients. At walking speed of 4.5km.hr^{-1}, HR, VE, VO_2 and %VO_2 max for BP increased from 99.1 to 169.7 beats.min^{-1}, 30.5 to 76.8 L.min^{-1}, 14.0 to 35.6 ml.min^{-1}kg^{-1}, and 29.5 to 76.2% from 0% to 15% gradient. For HSWR these parameters increased from 103.6 to 183.8 beat.min^{-1}, 32.3

to 83.1 $L.min^{-1}$, 14.7 to 38.8 $ml.min^{-1}kg^{-1}$ and 31.0 to 82.1%, respectively with gradients ranging from 0% to 15%.

The percentage increment of HR, VE, VO_2 and $\%VO_2$ max for NL and BP, NL and HSWR and BP and HSWR at different treadmill gradients of 0%, 5%, 10% and 15% at two speeds (3.5 $km.h^{-1}$ and 4.5 $km.h^{-1}$) are given in Table 3. A comparison of increase of these parameters in BP and HSWR shows that the increment was higher in HSWR than BP in each speed and gradient. The degree of increase of all these parameters was higher for HSWR than BP compared to NL.

Table 3 : Percentage increments of HR ($beat.min^{-1}$), VE ($L.min^{-1}$), VO_2 ($ml.min^{-1}kg^{-1}$) and % VO_2max while walking at 3.5 and 4.5 $km.hr^{-1}$ speed respectively at different gradients without and with carrying a 10.7 kg load as compact (BP) and distributed (HSWR) mode.

Parameters	Gradient (%)	Percentage increment					
		NL vs BP		NL vs HSWR		BP vs HSWR	
		3.5 (km/hr)	4.5 (km/hr)	3.5 (km/hr)	4.5 (km/hr)	3.5 (km/hr)	4.5 (km/hr)
HR	0	4.1	5.6	6.8	10.5	2.6	4.6
	5	6.5	7.4	11.6	13.2	4.8	5.4
	10	10.3	10.9	16.0	18.4	5.2	6.8
	15	12.4	14.4	19.9	23.8	6.7	8.3
VE	0	7.3	7.5	13.4	13.9	5.8	6.0
	5	8.6	10.5	15.4	17.4	6.3	6.3
	10	12.3	14.3	20.2	22.8	7.0	7.4
	15	13.1	16.1	22.0	25.5	7.9	8.2
VO_2/kg	0	3.9	5.5	9.0	10.9	4.9	5.1
	5	5.3	7.0	11.2	13.3	5.6	5.9
	10	7.0	8.5	13.4	16.2	6.0	7.1
	15	8.8	10.5	15.9	20.4	6.5	9.0
$\%VO_2$max	0	4.0	5.5	9.0	10.8	4.9	5.0
	5	5.3	6.9	11.3	13.2	5.7	5.9
	10	7.0	8.6	13.6	16.3	6.2	7.1
	15	8.8	11.9	15.6	20.5	6.3	7.7

DISCUSSION

The primary objective of the present study was to find out if any differences exist among the cardiorespiratory responses of Indian soldiers during carrying a same magnitude of load as compact and distributed mode. Malhotra and Sengupta (1965) conducted load carriage experiment (carrying school bags weighing 6.0 lb in four different position, i.e., rucksack, low back, across the shoulders and in the hands) to identify the most economical way of carrying school bags by children. In their experiment the lowest physiological responses (pulse rate, minute ventilation and energy expenditure) were observed in rucksack method and the highest physiological responses were observed in the hand. They concluded that rucksack was the most economical and efficient whereas the hand carriage was the most inefficient method

in terms of energy expenditure. Soule et al. (1978) observed that demands of energy cost during load carriage probably depend on the pattern of load distribution. If the load is well distributed, balanced and close to the centre of the body it demands less energy cost than load is unbalanced positions. Results of their study revealed a lower energy cost when carrying the load in the compact mode and weight was distributed as evenly as possible about the centre of gravity of the body as in the distributed form. Legg et al (1992) compared the heart rate and oxygen uptake of the shoulder and backpack methods of load carrying on a motor driven treadmill with varying gradients. They reported that the metabolic cost of backpacking (26.0 kg load carried on backpack) was significantly lower than for shoulder load carriage (18.4 kg carried on right shoulder and 7.6 kg carried on left shoulder) at walking speed of 4.8 $km.hr^{-1}$ and 0%, 2.5% and 5% treadmill gradients. The results of the present study clearly demonstrates that distributed mode of load carriage demands more cardiac cost and metabolic expenditure than compact mode and supports the findings of previous researchers.

In previous studies of Datta and Ramanathan (1971), Lloyd and Cooke (2000), the principle of keeping the load close to the trunk was followed by placing it in a compact mode (e.g. double pack). In the present study the compact load in BP mode was placed close to the body and was found to be associated with lower cardiorespiratory responses when compared with the distributed mode. The use of compact load (backpack) enabled the load to carry in a more balanced way without involvement of arm and shoulder muscles. Therefore, during its carriage back and trunk muscles were used more efficiently and the physiological responses were lower.

In case of distributed load in this study, haversack is positioned on the back while web similar to front pack remained close to the centre of gravity of the body at waist region. Increase of cardiovascular responses during distributed mode of load carriage than BP might not to be due to haversack and web but from hand carriage of INSAS rifle. The higher cardiorespiratory responses in distributed load carriage were probably due to a combination of factors. The muscular activity of the arm and shoulder would be greater for this method as the hand was being employed to hold and carry the load (R: 4.2 kg). Only one hand was used to hold the rifle while the other was extended and swung for maintaining balance. The raised position of the arms caused extra strain on the cardiovascular system. Exercise with hand held weights might also be associated with isometric hand gripping. Jackson et al. (1973) showed that when an isometric exercise component was added to a dynamic exercise task, cardiovascular responses was elevated above levels noted for the dynamic exercise alone. Graves et al. (1988) observed that the energy cost of walking exercise was increased by 3.8 $ml.min^{-1}kg^{-1}$ for carrying 1.36 kg hand held weights at 6.3 $km^{-1}hr$ speed and 6.3 % gradient Carriage of load in hand increased the energy cost (Graves et al. 1988) and was considered worst in terms of physiological efficiency (Datta and Ramanathan 1971). In our study carrying rifle (4.2 kg) in right hand could have contributed a reasonable increase in cardiorespiratory responses. The degree of relative imbalance and associated changes in gait pattern caused by carrying a rifle in one hand was beyond the scope of this study.

In military environment, the magnitude and mode of load carried by soldiers were operation specific. In non-combat operations, the compact (BP) mode of load carriage was most preferred by soldiers, whereas in combat situation they were required to carry the

essential ensembles (HS, Wb and R) in the distributed mode only. The present study showed that distributed mode of load carriage demanded higher cardiorespiratory responses than the compact mode while carrying the same magnitude of load with increasing walking speeds and gradients. Knowledge of this study would be useful during the design of particular military operation in field and non-field situation, either during real encounter or trials for obtaining the maximum efficiency of the soldiers.

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SALT TOLERANT WILD RICE, *PORTERESIA COARCTATA* TAKEOKA NEEDS MORE ATTENTION

R. N. Mandal[1]* and K. R. Naskar[2]

1. Scientist, CIFA (ICAR), Kausalyaganga, Bhubaneswar-2
2. Pr. Scientist, CIFRI (ICAR), Barrackpore, W.B.

Abstract ■ *Porteresia coarctata* Takeoka, considered as a wild rice relative species, belongs to the family Poaceae (Graminae). It is found to grow in Sundarban mangrove area and spreads over other mangrove ecosystems of India, and South and South-East Asian coastal areas. Its habitats are generally inundated twice a day by tidal water; remain submerged for a long and unsuitable for other vegetation growing in that niche. Morpho-anatomical studies show that this species acclimatizes coastal habitats by modifying its different organs, and thus sustain in the process of succession. Analysis of soil and water, and data of tidal fluctuations reveal that there are correlation among habitats traits and modification of respective organs of this species in halophytic adaptation.

Key words : Porteresia coarctata, morpho-anatomy, habitats and adaptation

INTRODUCTION

Porteresia coarctata Takeoka – a salt tolerant grass species (Roxburgh-1832; Prain-1903a; Mukerjee-1959; Naskar and GuhaBakshi-1987; Banerjee et al.-1989 and Mandal-1996) under the family Poaceae(Gramineae), is morphologically proximate to the taxon *Oryza sativa* L., a cultivated rice species. Earlier, the generic epithet of this taxon had been named as *Oryza* Roxb. (Roxb. Fl. Ind. 2nd ed. 2:206, 1832), but since 1965 that taxon has been segregated from *Oryza* and placed under the new epithet as *Porteresia* Takeoka (Bull Nat. Sci., Mus. Tokyo. 8:406, 1965). Both the genera *Porteresia* and *Oryza* have close morphological affinities, but little dissimilarity distinguishes one another very clearly.

Porteresia vegetation has also another importance in estuarine on shore areas where it occurs its first occupancy as a macrophyte in newly silted up land. Its vegetative growth proliferates rapidly soon after its appearance because of soil particles along with nutrients coming through tidal flow getting deposited in and around it. With its profuse tillers, enormous stolons, gregarious adventitious roots and occasional pseudo tap roots, it forms net like mats that hold up and embed deposited particle, thus playing a vital role in enhance siltation in one way and in checking soil erosion other way.

* *Corresponding author*

In process, this newly formed soil beds provide consolidated ground to other vegetation, which follow it in succession. Considering its importance in coastal areas, the present studies were carried out to ascertain the relationship between its modified organs for halophytic adaptation and its habitats traits.

MATERIAL & METHODS

A survey was carried out to collect the vegetation and observe the morphological traits, including phenology. Collected specimens were made anatomical dissection, then stained following the standard methods, and made permanent slide for microscopic studies. Soil and water samples were collected and analyzed following the standard methods mentioned in APHA and data were recorded in Table-1& 2. Data of tidal levels recorded in Table-3. were collected from the daily News paper, The Statesman.

Morphology: Adventitious roots develop from base of redicle and also from nodes. Stolons, vegetative propagating layer, and pseudo-tap roots, a special anchoring root up to 1.5m long developing in case of loose silted up soil, are the characteristic features of this species. Leaves: linear: Inflorescence: Spikelet; 1st and 2nd glume do not contain flowers, 3rd glume, known as lemma, has flowers, overlapped by 4th glume, known as palea; Flower: hermaphrodite; tepals-2, known as lodicules; stamens-6, free; carpel -I, ovary-1, ovule-I, style-2, stigma-2, feathery. Anatomy: Leaves: isobilateral; epidermis- one layered thick with silica deposition to form uneven surface bearing single celled hairs located at outer groove. Hypodermis and mesophyll – thick layers with having frequent sclerides tissues. Stem: cortex – consisting of parenchymatous tissues with various sized circular cavities bounded by sclerides patch. Root: adventitious roots consist of parenchymatous tissues, while pseudo-tap roots have dense sclerides tissues.

Flowering, fruiting and germination: Flowering and fruiting commence during late June to December. Pollination is performed by wind; Fruit- caryopsis, upto 1.4 cm long; Seed – up to 1.0cm long; Germination – hypogeal.

RESULTS AND DISCUSSION

Analysis of soil and water mentioned in Table-1.&2. shows that habitats are having intermediate properties of saline and sodic soils. High sodium affected soils become almost impervious to water and air, resulting in 'physiologically dry' condition towards vegetation. Its high concentration is also toxic to some plants due to detrimental effect of sodium in soil structure and associated high pH may create deficiencies of the micro nutrients cations; all together cause the loss of soil permeability. Tidal fluctuation recorded in Table-2. shows that vegetation remain submerged most of the times, while water and soil salinity range up to 18.77 ‰ and 26.24 ‰ respectively. In addition, coastal areas very often encounter natural calamities like cyclones, sea surges, strong tidal current, soil erosion etc.

Morpho-anatomical studies show that the following modifications are essential in relation to adaptation in this habitat. *Salt regulation:* leaves having one-celled hairs (Ball and Dutt-1984), which store excess salt, abscise after maturation and thus excrete extra salt. *Toughness and rigidity:* leaves, stems and roots, all have individual sclerides, sclerides patches, silica deposition and stones cells which provide toughness and rigidity to the respective organs in getting protection from wilting and shrinkage and mechanical support to withstand natural calamities like sea surges, cyclones, strong tidal current, soil erosion, etc. *Buoyancy: n*umerous cortical cavities present in leaves and stems facilitate the whole plant to become buoyant during inundation. *Anchor:* pseudo

Table 1 : Chemical parameters of soil

PH	EC mmhos/cm	Water soluble base				Exchangable base				Water soluble				
		Na+ ppm	K+ ppm	Ca+2 ppm	Mg+2 ppm	Na+ me/100g	K+ me/100g	Ca+2 me/100g	Mg+2 me/100g	Cl-ppt	Salinity ppt	SO_4^{-2} ppm	CO_3^{-2} me/l	HCO3- me/100g
7.10-8.00	1.20-4.35	220-5500	100-800	1.05-3.30	0.20-4.60	5.43-27.71	0.32-1.92	1.00-11.0	0.60-13.70	0.79-10.38	1.45-18.77	220-828	Nil	0.50-2.00

Table 2 : Chemical parameters of water

Different seasons	PH	EC mmhos/cm	Na^+ ppm	K^+ppm	Ca+2	Mg+2	Cl-	Salinity	SO_4^{-2}	CO_3^{-2}	HCO_3^-
Pre monsoon	7.3-8.00	12.00-18.00	5500-13000	405-505	14.0-33.20	51.60-100.50	9.07-14.52	16.40-26.24	147-163	Nil-8.80	1.00-2.40 2.40
Post monsoon	6.8-7.87	6.80-7.87	4000-7500	210-325	5.00-12.80	5.40-65.40	2.00-8.49	3.54 15.27	90.00 138.00	Nil-0.72	0.89-4.5

Table 3 : Tidal fluctuation (average)

Mean High Spring (m) (MHWS)	Mean spring (m) (ML) WS	Mean High Neap (m) MHWN	Mean Neap (m)	Mean Sea Water (m) (MSW)
5.94	0.49	4.42	2.14	3.30

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taproots develop and also can penetrate up to 1.5m. deep into soil to provide mechanical support to the upper part of plant while growing in loose soil strata. *Propagation*: apart from sexual propagation, this plant species exhibits clonal propagation by means of stolon, a propagating layer, uniqueness of which enables the vegetation to spread over the vast area even in hostile situation.

CONCLUSION

The uniqueness of adaptation exhibited by *P. Coarctata* Takeoka needs to be incorporated into other rice cultivars by process of biotechnology. The relevant subject specialists may search and segregate those genes responsible for halophytic adaptation and incorporate them into rice cultivars and make them suitable for cultivation in coastal areas. It is indeed a tough job, but only research can bring the prosperity and hope for future. Because huge unutilized coastal areas require to be used for cultivation now and near future to feed the dense population, more than 60% population reside and depends up on coastal area resources.

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CURRENT STATUS OF BIOTECHNOLOGICAL RESEARCH ON RICE (*ORYZA SATIVA* L.)

Sanjib Nandy[1], Nirmal Mandal[2], Sushmita Nandy[3] and Saikat Kumar Basu[4]*

[1]Bioproducts & Bioprocesses, Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB Canada; [2]Department of Biotechnology, Instrumentation & Environmental Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, WB India; [3]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK Canada. [4]Department of Biological Sciences, University of Lethbridge, Lethbridge, AB Canada

Abstract ■ Rice (*Oryza sativa* L.) is one of the most important cereal crops providing food security to a substantial part of the global population. While world rice production has been rising at a similar rate to that of the human population, the hope for improved nourishment of the world's population strongly depends on the development of better rice varieties and improved methods of rice production, processing and utilization. Genetic enhancement through biotechnology is seen as a way to produce more food, reduce pressure on natural resources, and meet population demand. This review highlights some important aspects of biotechnological research on rice over the past 20 years, major technologies already developed and those that are currently used; and the future implications of this rapidly expanding research area.

Key words : rice, biotechnology, genome, genetic transformation

Abbreviations: AFLP: Amplified Fragment Length Polymorphism; BAC: Bacterial Artificial Chromosome; FISH: Fluorescence *In Situ* Hybridization; GISH: Genomic *In Situ* Hybridization; IIRGS: Indian Initiative for the Rice Genome Sequencing; IRG: International Rice Genebank; IRGSP: International Rice Genome Sequencing Project; IRRI: International Rice Research Institute; MAS: Marker Assisted Selection; PCR: Polymerase Chain Reaction; PEG: Polyethylene Glycol; QTL: Quantitative Trait Loci; RAPD: Randomly Amplified Polymorphic DNA; RFLP: Restriction Fragment Length Polymorphism; SSR: Simple Sequence Repeat; STS: Sequence Tagged Site.

INTRODUCTION

Rice (*Oryza sativa* L.) biotechnology and genetic engineering have great potential in accelerating the pace of conventional breeding programmes. Studies of the rice genome have highlighted rice as an ideal model plant for cereal genomics research (Khush 2005). The sequenced rice genome will be able to provide significant information to genetically enhance rice yield and rice quality in the future. Its small genome size and sequence similarities to other grass plants provide it with amenable traits for research (Khush 2005; Vasil 1994). Furthermore, these characteristics provide a

**Corresponding author's email: saikat.basu@uleth.ca*

basis for the study of cereal plants through comparative genomics (Bajaj and Mohanty 2006). Hence, the sequencing and analysis of the rice genome will not only assist in the biotechnology and breeding of rice plants but also be immediately applicable to other economically important crops.

Over the last 20 years, rice biotechnology has made significant strides in several avenues. These include three important approaches, one through tissue cultures: (a) Embryo rescue, (b) Anther culture; second through map construction of rice genome; (a) Molecular marker aided selection; (b) QTL mapping and the third through genetic transformation systems: (a) Protoplast transformation, (b) Biolistic transformation. Cytological analysis, gene tagging; transgenic plant production, and germplasm management are the current major areas of application of biotechnology to improve rice.

1. Tissue culture

One technology that deserves greater attention from both the researchers and policymakers is the use of tissue culture, the most widely used application of which involves creating copies of plants through a process known as micropropagation. Presence of somaclonal variants in rice has reported in early sixties and seventies (Henke *et al.* 1978; Nishi *et al.* 1968). There have been several reports of the regeneration of plants and plants from rice protoplasts as reviewed by Cocking (1989). So far rice is the only one of the four most important crop plants (rice, wheat, corn, sorghum) in which regeneration of plants from protoplast has been achieved (Chaudhury *et al.* 1988; Bajaj and Mohanty 2006; Datta and Datta 2006; Sasaki *et al.* 2005; Shrawat and Lörz 2005).

1a. Embryo rescue

Wide hybridization in cereals is a significant plant breeding tool for the incorporation of desirable characters from wild into the cultivated species. Many wild species of the genus *Oryza* fail to hybridise with cultivated species because of the incompatibility or sometimes fertilization takes place but embryo fails to develop. Genomes of some wild species of rice are strongly non-homologous and gene transfer through conventional breeding method such as backcrossing is highly restricted. Therefore, embryo rescue technique could be employed to explore the possibility of getting a distant hybrid with desirable characteristics. A number of useful genes for disease and insect resistance have been transferred using this technique from wild species to cultivated rice (Brar *et al.* 1991; Jena and Kush 1990, 1984; Rodrangboon *et al.* 2002). Recently embryo rescue technique has been used to transfer several useful genes for resistance to bacterial blight, tungro, yellow stemborer and leaf folder from *Oryza ridleyi* ($2n = 48$), a tetraploid wild species to rice cultivars IR24, IR29, IR56 and IR74 (Ram *et al.* 2003).

1b. Anther culture

Anther culture has been quite useful for the introgression of desirable traits, in overcoming F_1 sterility for the production of haploid lines (Nandy *et al.* 2001; Wan *et al.* 1989) and for the utilization of heterosis in rice (Bishnoi *et al.* 2000). Haploid breeding has been successfully utilized for rice improvement in China (Chen *et al.* 1991; Jain 1997). Hu and Zeng (1984) have developed the technique for the production of gamete derived plants from cultured anthers. The cultivars Xin Xun and Hua Han Zhao were developed through anther culture breeding (Zhang 1989). Two other varieties, namely HuaYa1 and HuaYa 2, took a period of five years to be developed compared to 12 years using conventional pedigree and bulk methods. Morrison and Evans (1988) reported two other high yielding rice varieties developed via anther

culture having a combined cultivation of over 1 million ha in eastern China.

2. Map construction of rice genome

RFLPs (Restriction Fragment Length Polymorphisms) are genetic differences observable at the DNA level characterized by a number of variable length restricted fragments (Chawla 2002). Original documentation of RFLPs resulted from the findings of Grodzicker *et al.* (1974). The first published RFLP map of rice was based on random genomic clones derived from *PstI* library (McCouch *et al.* 1988). To enrich the genetic map for single copy sequences, several cDNA libraries were developed from a variety of plant species. Rice has the smallest genome of any monocot species known till date (ca. 5 X 10^8 bp) (Arumuganathan and Earle 1991; Sasaki *et al.* 2005). Rice is rapidly emerging as the model monocot system for map-based gene cloning due to availability of several well characterized genetic mutants of rice.

A molecular map of rice consisting of over 600 RFLP markers has been developed at Cornell University, USA (Bajaj and Mohanty 2006; Datta and Datta 2006; McCouch 1993; Sasaki *et al.* 2005) and made available to the rice researchers throughout the world. The existing map is based on an interspecific backcross population derived from a cross between *O. sativa* (*indica*) and *O. longistaminata*, developed in the Cote d'Ivoire, Africa. In addition, comparative mapping efforts in rice and maize offer a new opportunity for increasing the reservoir of available markers for rice (McCouch 1993; Qu le and Takaiwa 2004). These maps together provide rice researchers with 1000 RFLP markers mapped on to the 12 chromosomes of rice. Brar and Dhaliwal (1997) have reported that 2300 DNA markers have been mapped in rice genome. Some laboratories in China, Japan and the USA focused their attention to sequence the genomes of both the *japonica* and *indica* subspecies of rice (Bajaj and Mohanty 2006). The shotgun sequencing protocol employs a method which shears the entire genome bases into several thousand long DNA pieces (Chawla 2002).

In two separate papers published on April 5, 2002 edition of Science, a group of 100 scientists in China headed by Yu (2002) at the Beijing Genomics Institute and another group of 55 led by Goff (Bajaj and Mohanty 2006; Datta and Datta 2006; Goff 2002; Qu le and Takaiwa 2004) at the Torrey Mesa Research Institute (a research division of the Swiss agro-chemical company Syngenta International), have published the draft of entire DNA base sequences of *Oryza sativa* L. *sp. indica*. International Rice Genome Sequencing Project (IRGSP) is a consortium of ten countries namely, Brazil, China, France, India, Japan, Korea, Taiwan, Thailand, UK and USA. By becoming member of the IRGSP, India for the first time participated in any genome sequencing project. The NRCPB is one of the two centers involved in the Indian Initiative for the Rice Genome Sequencing (IIRGS), the other one being Delhi University South Campus. Rice genome has a total genetic distance of approximately 1800 cM and 1 cM is equal to approximately 250-300 kb (Saito *et al.* 1991). As a pioneer in large scale genome analysis of rice IRGSP has successfully developed a genetic map, yeast artificial chromosome based physical map, a transcript map and a phage P_1 artificial chromosome/bacterial artificial chromosome sequence ready physical map that serve as common resources for rice genome sequencing (Sasaki *et al.* 2005). After completing the high quality draft sequence by December, 2002 (phase 2), IRGSP also completed the remaining sequencing by December 2004 (phase 3) and is expected to be facilitating advanced research in functional

and applied genomics (Sasaki *et al.* 2005). While the megabase size in *indica* was reported to be 466, and that of *japonica* was 420; the number of genes in *indica* genome has been estimated to be between 46,022 to 55,615 genes, while in *japonica* the suggested range is from 32,000 to 50,000 genes (Bajaj and Mohanty 2006; Sasaki *et al.* 2005). The next trend in rice research will focus on the determination of the function of 40,000-50,000 genes predicted in the rice genome (Khush 2005).

2a. Molecular marker aided selection

Several types of molecular markers are available namely: Restriction Fragment Length Polymorphism (RFLP); Randomly Amplified Polymorphic DNA (RAPD); Polymerase Chain Reaction (PCR) Based Marker viz. Sequence Tagged Site (STS), Simple Sequence repeat (SSR), Amplified Fragment Length Polymorphism (AFLP) etc. Molecular markers are quite powerful to saturate the genetic map as compared with the classical map comprising of morphological markers (Chawla 2002). Gene tagging is the process of locating genes of interest via linkage to molecular markers. In modern plant breeding, Marker Assisted Selection (MAS) individuals carrying target genes are selected in a segregating population based on patterns of tightly linked markers rather than on their phenotypes. RFLP markers have been used to tag a bacterial blight resistance gene derived from the wild species *O. australiensis* in the early generation backcross progeny of *O. sativa* X *O. australiensis*. In order to facilitate further mapping of random markers and gene of interest, an interspecific backcross population derived from *O. sativa* and *O. longistaminata* is maintained vegetatively at IRRI, Philippines and at Cornell, University of Georgia, USA. Integrated map of rice chromosome 10 has been already developed through SSR markers (McCouch 2002), so that it will be more beneficial for germplasm maintenance. RFLP, SSR and AFLP gene-tagging work in rice was directed towards several characters. At present, tight linkage of several resistance genes with RFLP markers has been established due to the untiring efforts of several groups of dedicated workers (Chawla 2002). Gene conferring tolerance or resistance to stresses such as water stress, saline stress and mineral deficiency or toxicity stress is being tagged at IRRI. Single genes governing thermo sensitive male sterility and fertility restoration have been tagged in rice. This achievement has ushered a new chapter in hybrid rice production (Chawla 2002). Beside this, the molecular marker technology has been utilized not only in understanding the phenomena of heterosis and transgressive variation in rice but also in maximizing the efficiency of cross in schemes for developing high performance varieties (Khush 2005; McCouch 1993).

2b. QTL mapping

Most agronomically important characters of crops are inherited quantitatively. The development of RFLP maps (McCouch *et al.* 1988) has made it possible to study the inheritance pattern of different complex traits and to locate individual genetic factors controlling these traits (Tanksley 1993). In rice, Quantitative Trait Loci (QTL) for root morphological characters for drought avoidance (Champoux *et al.* 1995); heading date and plant height (Li *et al.* 1995); different traits of agronomic characters such as plant height, days to heading, days to maturity, panicle length, panicles per plant, spikelets per panicle, grains per panicle, percent seed set, 1000 grain weight, spikelets per plant, grain per plant, grain yield, grain shape and milling quality (McCouch and Doerge 1995; Xiao *et al.* 1996); yield and related characters (Lin *et al.* 1996; Nandy *et al.* 2004) heading date (Yano *et al.* 1997), root

morphology, root distribution and root knot nematode (Yadav et al. 1997) have been identified. Four QTL have been found in F_2 population for seedling vigour (Redona and Mackill 1996); and four other QTL are responsible for the blast resistance in F_4 population (Fukuoka and Okuno 1997a), while another two for hybrid breakdown in F_2 population (Fukuoka and Okuno 1997b). Similarly another four QTL have been identified for the character submergence tolerance and nine for root/shoot ratio of rice (Brar and Dhaliwal 1997). Around 108 recombinants inbreed lines of the Bala X Azucena mapped population revealed the presence of a major gene *AsTol*, which has been mapped on chromosome 6 (Dasgupta *et al.* 2004).

3. Genetic transformation system

During the past 20 years, tremendous progress has been made to develop a high-frequency, routine and reproducible genetic transformation protocol of rice either through by *Agrobacterium*-mediated transformation technologies or biolistic DNA transfer. Using these modern technologies, a large number of agronomically important traits, including quality improvement and increased nutritional value, have been introduced in rice plant. Rice with its relatively small genome size, ease of transformation, well known genetics and cytological studies, availability of a dense physical map and molecular markers, together with its complete genome sequence is considered now a model monocot system. It is being used to understand several fundamental problems of plant physiology, growth and developmental processes ranging from elucidation of a single gene function to whole metabolic pathway engineering (Chawla 2002).

3a. Protoplast transformation

Transformation of monocotyledons by *Agrobacterium* is not a general process (Chawla 2002). In the past monocots, particularly rice plants were considered to be recalcitrant to this technology and they were outside the *Agrobacterium* host range (Sharawat and Lörz 2005). However, transformation methods based on the use of *Agrobacterium* are still preferred for following properties: easy to handle, higher efficiency, more predictable pattern of foreign DNA integration and low copy number of integration (Sharawat and Lörz 2005). Chen *et al.* (1988) developed a protocol for consistent and large-scale production of fertile transgenic rice plants, which was very useful for transformation by *Agrobacterium* in rice. The production of transformed *japonica* cultivar by co-cultivation of mature embryos with *Agrobacterium* was described by Raineri *et al.* (1990). Successful application of such a method has been reported to basmati cultivars of *indica* rice after only minor modifications (Rashid *et al.* 1996). Immature embryos were also good starting materials for *Agrobacterium* mediated transformation of *indica* (Khanna and Raina 1999) and *japonica* varieties (Aldemita and Hodges 1996). Yokoi *et al.* (1998) produced chilling tolerance of photosynthesis and unsaturation of fatty acids in rice by introducing the *GPAT* gene. Rice seeds were obtained with iron fortification by using soybean ferritin gene (Goto *et al.* 1999; Wakasa *et al.* 2006) and succeeded in engineering the provitamin A (2-carotene) biosynthetic pathway into carotenoid free rice endosperm (Datta and Datta 2006;Wakasa *et al.* 2006;Ye *et al.* 2000). Zhai *et al.* (2004) on the basis of their extensive research on 24 T-DNA-*Xa21* flanking sequence reported three distinct classes of T-DNA loci in rice. The three classes are: typical T-DNA integration with distinct left and right borders, T-DNA integration associated with adjacent vector backbone sequences and T-

DNA integration involved with complicated recombination in the flanking sequences (Zhai *et al.* 2004).

First transgenic calli was obtained after polyethylene glycol (PEG)-induced DNA uptake of the *nptII* gene into root-derived protoplasts, followed by selection on kanamycin (Zhang and Wu 1988). Datta *et al.* (1992, 2006) published the first report of the recovery of transgenic *indica* rice plants from cultivar Chinsurah Boro II. Detailed protocols have been described by Hodges *et al.* (1991) and Li *et al.* (1990). Electroporation has also been widely used to introduce naked DNA into protoplasts (Chawla 2002). Toriyama *et al.* (1988) first used this method for the production of Yamahoushi cultivar transgenic rice via anther culture-derived protoplasts with the aminoglycoside phosphotransferase II (*aph(3')II*) gene, conferring resistance to the antibiotic kanamycin. In another earlier study, electroporated protoplasts from the cell suspensions of Taipei-309 leaf base calli with the 35S promoter were fused to the *nptII* gene (Zhang *et al.* 1991). Out of six regenerated plants, two were positive for *nptII* activity. Xu and Li (1994) obtained fertile transgenic *indica* rice plants using seed embryo cells. Chaudhury *et al.* (1988) observed transient expression of *gus* gene in intact seed embryos of *indica* rice.

3b. Biolistic method of gene transfer

Microprojectile bombardment, also called the biolistic method or the particle gun method has been used in many laboratories for plant improvement (Chawla 2002). Sanford (1990) described the concept of Microprojectile bombardment in details. Bombarded tissues of rice plant were plated on regeneration media supplemented with appropriate selective agents to obtain transformed embryos or other transgenic organized tissues, such as shoots and roots (Christensen *et al.* 1992). A gene transfer procedure were reported for the model *indica* rice variety TN1, using bombardment of embryogenic callus (Sivamani *et al.* 1996). Jain (1997) optimized the biolistic method for transient gene expression and production of agronomically useful transgenic basmati rice plants. Many biolistic devices (e.g. particle gun) have been developed, including both commercial and lab-built models. Moreover, there is a possibility to get a relatively inexpensive simple particle gun especially for rice-producing developing countries (Bajaj and Mohanty 2006; Sudhakar *et al.* 1998).

The success of plant transformation depends very much on promoter sequences (Vasil 1994). To express the transgenes in plant cells, appropriate promoter sequences have to be introduced alongside the gene to ensure efficient transcription of mRNA (Bajaj and Mohanty 2006; Finch *et al.* 1991). A large number of promoters have been used in rice transformation and several promoter sequences have been isolated from monocots for specific use in cereal species for the efficient expression of the transgenes. Unfortunately, the levels of gene expression produced by this promoter in cereals were less than in dicots (Hauptmann *et al.* 1988). CaMv 35S (Nillson *et al.* 1996), EMU, Actin1, ubiquitin-1 and Adh1 has been shown to produce highest levels of expression in rice (Ignacimithu *et al.* 2000). Other promoters such as His, LHcP, reCS, Pin, Rolc, RTBV, Osg6B, OsgrP, RSs1, PEPc, BP10, Glu, Ltp have also been used successfully (Ignacimithu *et al.* 2000). Between 1988 and 2003, *nptII*, hygromycin resistance (*hyg*), and phosphinothricin phosphotransferase (*bar*) selectable marker genes were found to be useful for rice transgenic (Wu 2000). The expression of the transgene (*OASA1D*) encoding a feedback-sensitive Ksubunit of rice anthranilate synthase in rice by Wakasa *et al* (2006) resulted in higher accumulation of amino acid tryptophan in calli and leaves. Such

metabolic manipulation towards production of better quality and quantity of proteins in rice could improve the protein intake of a large section of our global population (Khush 2005).

OTHER MAJOR AREA OF RICE BIOTECHNOLOGY

1. Cytological analysis

Kuwada (1910) first reported the chromosome number of cultivated rice, $2n = 24$, using both mitotic and meiotic cells. Many cytological studies followed to elucidate the characteristics of the rice chromosomes. Laser microdissection, Genomic *In Situ* Hybridization (GISH) and Fluorescence *In Situ* Hybridization (FISH) seems to be most useful new technologies in rice chromosome research. The mapping of Bacterial Artificial Chromosome (BAC) clones on rice chromosomes using FISH has been recently reported (Bajaj and Mohanty 2006). Several rice chromosomal analyses have been done by different researchers across the globe (Brar *et al.* 1991; Fukui 2006; Ram *et al.* 2003).

2. Gene Tagging

A molecular marker very closely linked to a gene can act as a "tag" which can be used for indirect selection of the gene in breeding programme. Earliest gene tagging work in rice focused on finding markers closely linked to single genes for disease and insect resistance, such as resistance to blast, bacterial blight rice tungro, gall midge, brown plant hopper, green leaf hopper and white backed plant hopper (Sasaki *et al.* 2005). Rice genome map can be used to locate and tag genes of economic importance such as disease & pest resistance, salinity & drought tolerance, other qualitative characters and wide compatibility. More than 40 rice genes have already been tagged with DNA markers. If different major genes specifying resistance to several insect pests of rice are tagged with molecular markers, it would be possible to follow the inheritance of all theses genes simultaneously without any cumbersome entomological test under field condition for scoring resistant plants. This would be particularly valuable in a breeding programme of developing rice varieties having resistance to several insects (Kush 2005).

3. Transgenic plant production

The first *Bt* rice plants were produced more than 14 years ago (Fujimoto *et al.* 1993) and is now already under field trials (High *et al.* 2004). The expression of mannose-specific lectin gene (*GNA*) has been used extensively in transgenic rice for protection against a number of economically important homopteran, coleopteran and lepidopteran insects (Nagadhara 2004).

In rice, partial resistance to fungal diseases through conventional breeding has been achieved and could be increased further by using genetic engineering approaches. In this context, the recently cloned *Pi-Ta* gene, a member of putative cytoplasmic NBS-receptor class *R* gene holds much promise for resistance against rice blast (Bryan *et al.* 2000). Field trials of *Xa21* transgenic rice plants suggested a significant increase in yield because of less damage caused by *Xanthomonas oryzae* (Tu *et al.* 2000). Field trials of transgenic rice expressing genes for bacterial resistance, herbicide tolerance, as well as to assess the gene flow of transgenic rice using the herbicide tolerance gene as a marker, have also been initiated. Transgenic rice plants resistant to rice dwarf virus, rice hoja blanca virus, rice tungro virus were also developed by several researcher (Bajaj and Mohanty 2006).

Transgenic rice with Tolerance to water deficiency (Roy and Wu 2001), salt tolerance (Ohta *et al.* 2002), cold tolerance (Matsumura *et al.* 2002), heat stress (Yamanouchi *et al.*

2002) and submerged tolerance (Rahman *et al.* 2001) also been developed. The need of the hour is to develop multigene transgenics aiming towards resistance against multiple pests and diseases. The availability of complete rice genome sequence has opened up the way. Commenting on the immense benefits that rice breeders may derive from the unraveling of base sequences, it was pointed out that the genes for synthesis of vitamin A are already present in the *japonica* rice genome in an inactivated state. In the light of present findings, all is needed is to activate those genes by means of suitable promoters instead of inserting foreign genes from an altogether unrelated species, such as daffodil as was done by Ingo Potrykus (Ye *et al.* 2000; Yoshihara *et al.* 2005) to evolve "Golden rice". The rice genome project will usher in a new era in the improvement of cereals in general and rice in particular. The transgenic rice plants termed 'Golden Rice 2' showed an increase in total carotenoids of up to 23-fold compared to the original "Golden Rice", and displayed a preferential accumulation of β-carotene (Paine *et al.* 2005). Rice has also been transgenically improved to contain greater quantities of various amino acids, such as glycine, lysine, tryptophan, cysteine, and methionine. Similarly, improvements in starch biosynthesis and oil quality have also been addressed (Bajaj and Mohanty 2006; Wakasa *et al.* 2006; Zhai *et al.* 2004) In another recent study, Kim *et al.* (2006) cloned a glucosyltransferase cDNA (RF5) from rice and expressed that in the bacteria *E. coli* using real time PCR strategy.

4. Germplasm Management

Wild germplasms abound in genetic variation. The tremendous range of naturally occurring genetic variation makes rice one of the most widely adapted crop species known. The genetic resources of rice will continue to be used by plant breeders and researchers worldwide to develop new rice varieties that will contribute to the required increase in rice productivity by the year 2025. International Rice Research Institute (IRRI) at Manila, Philippines has been at the forefront of international efforts to collect and conserve the genetic resources of rice, now held in trust in the International Rice Genebank (IRG). Without advanced knowledge of the functional roles of cultivated and wild rices, the improvement programme will neither effectively utilize the rice gene pool to produce varieties that will contribute to increase the productivity nor even efficiently conserve these important resources in gene banks. It is important to strengthen linkages between *ex situ* conservation and farmer's use of germplasm conserved in genebanks, and the *in situ* conservation of wild species. Particularly with the new interest in biotechnology and genetic engineering and hunt for QTL, germplasm conservation through cryo-preservation technology, is sometimes thought to be reducible to storage of technology. These are the sources of past, present and future and are equally essential for the development of their counterpart. Cryptic genetic variation often goes undiscovered by the breeders because target characters are not necessarily identified in the germplasms (Bajaj and Mohanty 2006; Khush 2005). Molecular markers have been utilized to tap hidden source of genetic variation in rice. Efforts have been made to tag alleles in the wild germplasm that contribute to trangressive variation in wild crosses. Once identified, these novel alleles could be transferred to the improved and high yielding backgrounds (Bajaj and Mohanty 2006; Datta and Datta 2006; Sasaki *et al.* 2005; Shrawat and Lörz 2005).

FUTURE PROSPECTS

The above discussion in rice plant improvement

through biotechnology could witness dramatic changes in the coming years, both in terms of the commercial release of transgenic rice containing the existing gene resource and the discovery of new genes by utilizing the advances in rice genomics. Based on current status, future goals can be achieved by: combining several agronomically beneficial genes and introducing them into the same plants to maximize the desired effects and multigene transgenics aiming towards resistance against multiple pests and diseases.

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UTILITY AND EFFICACY OF UTILIZING BODY MASS INDEX AND PERCENT BODY FAT IN THE EVALUATION OF NUTRITIONAL STATUS AMONG UNIVERSITY STUDENTS.

Samiran Bisai[1], Kaushik Bose[1]* and Debidas Ghosh[2]

1. Department of Anthropology, Vidyasagar University, Midnapore 721102, West Bengal, India.
2. Department of Bio-Medical Laboratory Science & Management, Vidyasagar University Midnapore-721102, West Bengal, India.

Abstract ■ A cross-sectional study of 101 (25 male and 76 female) university students was undertaken to determine their anthropometric characteristics and nutritional status. Standard procedures and techniques were followed. Internationally accepted cut-off points were utilized to evaluate nutritional status based on the body mass index (BMI) and percent body fat (PBF). This paper presents important findings regarding the utility and efficacy of BMI and PBF in the evaluation of nutritional status.

INTRODUCTION

Overweight and obesity is an emerging important public health problem throughout the world (WHO, 1995) and its prevalence rate has largely increased over the last two decade in both developed and developing countries (Doll et al. 2002). Obesity is associated with several chronic diseases and mortality. Socioeconomic differences act as a risk factor through which differences contribute to morbidity and mortality (Martikainen and Marmot 1999). The existence of health differences among different socioeconomic classes in India has already been reported (NFHS II, 2000; NFHS-III, 2007). The prevalence of overweight and obesity (BMI = 25.0 kg/m^2) in India has rapidly increased from 12.8 % in 1998-99 to 14.8% in 2005-06 (NFHS-II, 2000; NFHS-III, 2007).

Anthropometry is single most portable, universally acceptable, inexpensive and non-invasive method available to assess the size, proportion and composition of the human body (WHO 1995). Currently, the body mass index (BMI) is used widely as an indicator of the risk of overweight and of presence of overweight, because of the relative ease and accuracy of the basic measurement (Himes and Dietz 1994). However, the BMI has limitations; it tends to have high specificity, but low and variable sensitivity in different ethnic groups. Further, the validity of BMI across diverse populations has not been evaluated (Himes and Bouchard, 1989). It is calculated with the formula: weight in kg divided by height in meter squared. This index was developed by Lambert Adolphe Jacques Quetelet, a Belgian Mathematician, in

* *Corresponding author : Department of Anthropology, Vidyasagar University, Midnapore – 721 102, West Bengal, India, e-mail: banda@vsnl.net*

the 19th century (Quetelet 1869). The BMI has been widely accepted and used as an assessment adult nutritional status, with the highest and lowest limits of normality being based on statistical criteria relating to the higher rate of morbidity and mortality of people having BMI higher or lower then normal values (WHO 1995, Bellizzi and Deitz 1999, Willet et al 1999, Stevens 2000).

This global epidemic is well described in the children and adolescent populations. However, scanty data is available in respect to the prevalence of overweight and obesity among university students in West Bengal. In view of this, the present investigation was undertaken to assess the nutritional status among university students of self-finance nutrition and dietetic course under the distance mode affiliated to Vidyasagar University, Midnapore. In particular, this study attempted to test for the utility and efficacy of utilizing BMI and percent body fat (PBF) in the evaluation of nutritional status.

METHODOLOGY

This cross-sectional study was conducted three days held during 14th May – 16th May, 2007. A total of 101 individuals were included in the present study: 25 men and 76 women. The data on university students were collected from personal contact programme (PCP) under distance mode self finance nutrition and dietetic course through practical classes over a period of 3 hours. These practical classes, held over three days, were undertaken for the assessment of adult nutritional status. All subjects belonged to the higher socio-economic class.

All anthropometric measurements were recorded following the standard techniques of Lohman et al (1988). Height and weight were recorded to the nearest 0.1 cm and 0.5 kg, respectively. Technical errors of measurements (TEM) were computed and they were found to be within acceptable limits (Ulijaszek and Kerr 1999). BMI was computed using the following standard equation:

BMI = Weight in kg) / height in meter square.

Nutritional status was evaluated using internationally accepted BMI guidelines (WHO, 1995). The following cut-off points were used:

Chronic energy deficiency (CED)	:	BMI < 18.5
Normal	:	BMI = 18.5 – 24.9
Overweight	:	BMI = 25.0 – 29.9
Obesity	:	BMI ≥ 30.0

We followed the World Health Organization's classification (1995) of the public health problem of low BMI, based on adult populations worldwide. This classification categorises prevalence according to percentage of a population with BMI < 18.5.

1) Low	(5–9%	: warning sign, monitoring required.
2) Medium	(10–19%):	poor situation.
3) High	(20–39%):	serious situation.
4) Very high	(≥ 40%) :	critical situation.

Percentage of body fat (PBF) was computed using the following equation (Deurenberg et al 1991):

PBF = (1.20 * BMI) + (0.23 * Age) - (10.8 * Sex) – 5.4

Where, Sex: Male = 1, Female = 0.

Furthermore, the WHO recommended sex specific PBF cutoff values were used for obesity. The following cut-off points were:

Sex	Obesity
Male	BF ≥ 25%
Female	BF ≥ 35%

The distributions of the height, weight and body mass index were not significantly skewed. Student's t-tests were performed to test for differences in mean anthropometric characteristics by sex of the subjects. Correlation and regression analyses were done to test for the association between age, BMI

and PBF. Odds ratio (OR) was calculated to measure the risk for being CED and overweight-obesity. Sensitivity, specificity, positive predictive value, negative predictive value and efficiency tests were done for screening obesity using two criteria. All statistical analyses were undertaken using the SPSS Statistical Package. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The characteristics of the study sample are presented in ***Table 1.*** The mean age of men and women was 28.28 years (SD = 8.16 years) and 23.22 years (SD = 1.42 years), respectively. Means for height, weight, BMI and PBF of the men were 164·00 cm (SD = 6.60 cm), 58.62 kg (SD=11.86 kg), 21.74 kg/m^2 (SD=4.08 kg/m^2) and 16.39 % (SD = 5.21 %), respectively. The corresponding values for women were 154·19 cm (SD = 5.81 cm), 55.90 kg (SD = 9.60 kg), 23.48 kg/m^2 (SD = 3.40 kg/m^2) and 28.12 % (SD=4.38), respectively. On average, men were heavier than women, but this difference was statistically not significant ($t = 1.157$, $p > 0.05$). Mean height was significantly higher among men ($t = 7.077$, $p < 0.05$). In contrast, mean BMI and PBF were significantly higher in women ($p < 0.05$).

Table 2 presents the nutritional status of the subjects based on BMI. The prevalence of CED, normal, over weight and obesity were 8·91%, 62.38%, 21.78 % and 6.93 %, respectively. The prevalence of CED was higher in men (20.0 %) compared to women (5.26%). Based on WHO classification, the prevalence of CED among men was high. From the public health point of view the situation was serious. In women, the CED situation was better than men required only monitoring. Women had 3.38 fold lower risk of being CED compared with men (OR = 3.38, 95 % CI: 0.68 – 17.52). The

Table 1: Anthropometric characteristics of the study subjects.

Variables	Male (n = 25) Mean ± SD	Female (n = 76) Mean ± SD	Mean Difference	t-value
Height (cm)	164.00 ± 6.60	154.19 ± 5.81	9.81	7.077*
Weight (kg)	58.62 ± 11.86	55.90 ± 9.60	2.72	1.157
BMI (kg/m^2)	21.74 ± 4.08	23.48 ± 3.40	–1.74	2.034*
PBF (%)	16.39 ± 5.21	28.12 ± 4.38	–11.73	11.079*

SD = Standard deviation. * Significant sex difference; $p < 0.05$.

Table 2: Nutritional status based on BMI of the study subjects.

BMI category	Status	Male (%)	Female (%)	Total (%)
< 18.5	CED	20.00	5.26	8.91
18.5 – 24.99	Normal	68.00	60.53	62.38
25.0 – 29.99	Overweight	8.00	26.32	21.78
≥ 30.0	Obese	4.00	7.89	6.93

CED = Chronic energy deficiency.
Chi-square $_{(df = 3)}$ = 8.06, $p<0.05$ (Sex difference of nutritional status).
OR (CED Vs Normal) = 3.38 (95 % CI: 0.68 – 17.52).
OR (Overweight & obesity Vs Normal) = 3.20 (95 % CI: 0.78 – 15.24).

prevalence of overweight and obesity were higher in women (26.32% and 7.89%, respectively) compared to men (8.00 % and 4.00 %). They had 3.20 times higher risk of being overweight and obese (OR = 3.20, 95 % CI: 0.78 – 15.24) compared to being normal.

The prevalence of obesity by sex of the subjects is shown in *Table 3*. The prevalence of obesity in women was similar (7.89%) using both criteria (BMI and PBF).

Table 3: Prevalence of obesity based on PBF.

Sex	Obesity (%)
Male	8.00
Female	7.89

Screening test indicated that the efficiency rate by these two methods were 100%. There was no misclassification. In men, the prevalence of obesity was higher when PBF was used for classification of obesity. Only 4 % misclassification was observed. The efficiency rate indicated 96 % individuals were correctly screened using both these criteria. However, sensitivity (50%) results indicated that there was poor association to detect obese individuals. But, the negative predictive power and efficiency was more than 95 % to screen actual cases (**Table 4 & Table 5**).

In both men and women, BMI was not significantly correlated with age; hence, linear regression analyses between BMI (independent variable) and PBF (dependent variable) were done. *Table 6* presents the results of the linear regression between BMI and PBF in both sexes. In both men ($B = 1.193$, $t = 12.43$, $p < 0.001$) and women ($B = 1.213$, $t = 116.26$, $p < 0.001$), BMI had significant impact on PBF. BMI accounted for 86.5 % and 99.4% variation in PBF among men and women, respectively.

Table 4: Prevalence of obesity using BMI and PBF among men.

	PBF < 25.0	PBF = 25.0	Total
BMI < 30	23	1	24
BMI = 30	0	1	1
Total	**25**	2	**25**

Correlation coefficient (r) = 0.93, p<0.001.

Sensitivity = 50 %, Specificity = 92 %, Efficiency = 96 %.

Table 5: Prevalence of obesity using BMI and PBF among women.

	PBF < 35.0	PBF = 35.0	Total
BMI < 30	70	0	70
BMI = 30	0	6	6
Total	**70**	**6**	**76**

Correlation coefficient (r) = 0.99, p< 0.001.

Sensitivity = 100 %, Specificity = 100 %, Efficiency = 100 %.

Table 6: Linear regression analyses of BMI with PBF by sex of the subjects.

Sex	Constant	B	SeB	Beta	T	Adj R^2
Male	-9.543	1.193	0.096	93.3	12.43*	86.5
Female	-0.355	1.213	0.010	99.7	116.26*	99.4

BMI = Independent variable, PBF= Dependent variable, *p < 0.001.

The relationship between BMI and PBF is shown in ***Figures* 1** (men) **& Figure** *2* (women). There was strong positive correlation (r, Pearson correlation coefficient) between BMI and PBF in both men ($r = 0.93$, $p < 0.001$) and women ($r = 0.99$, $p < 0.001$).

Figure 1: Relation between BMI and PBF in men.

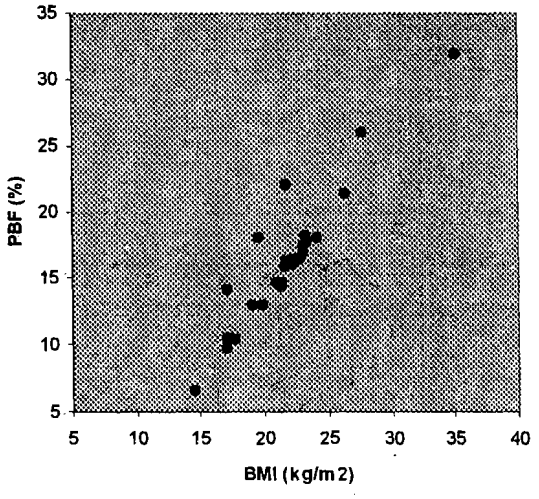
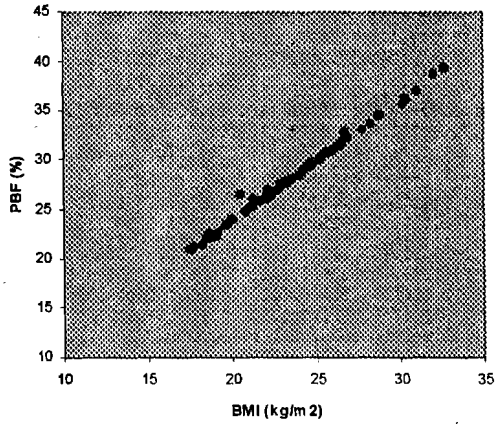


Figure 2: Relation between BMI and PBF in women.



In conclusion, this study provided strong evidence that both BMI as well as PBF can be used to evaluate nutritional status among these students. Similar studies should be conducted among larger samples to fully validate this finding. More importantly, similar studies should be conducted among various ethnic groups of India to determine whether this finding is ethnic-specific to Bengalees or is also applicable among them.

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BLOOD UREA, A CRYOPROTECTENT AGENT DURING HIBERTATION AND ITS SEASONAL VARIATION IN INDIAN COMMON TOAD – *DUTTAPHRYNUS MELANOSTICTUS* (SCHNEIDER, 1799)

Suman Pratihar*, Jayanta kr. Kundu

Department of Zoology .Genetics and Molecular biology. Vidyasagar University, Midnapore. West Bengal-721102

Abstract ■ In this investigation, effect of cold stress on blood urea concentration was studied in the Indian common toad *Duttaphrynus melanostictus* .Seasonal variation of blood urea level was also analyzed. Our observation clearly indicated that the urea level increased significantly in the hibernating toads. Seasonal study of blood urea concentration also reflected variations in the blood urea level through out the year .During the hibernating phase,increased urea concentration in blood acts as a cryoprotectent agent ,which help the hibernating animals to sustain themselves through the prolonged winter.

INTRODUCTION

Hibernation is a unique physiological condition, known best for suppression of metabolism and body temperature, which is thought to promote survival during periods of food shortage .Effect of cold stress can also produce remarkable changes in blood urea concentration (Pasanen 1977, Jorgenensen 1997, Costanzo and Lee 2005,) which probably help the animal to cope with low temperature and act as a cryoprotectent agent.

In the present investigation, effect of cold stress on blood urea concentration in natural population of the Indian common toad, *Duttaphrynus melanostictus* has been envisaged. This toad is very common in West Bengal and is available in plenty in natural habitat during summer, but is found to be hibernating in mud holes & corner of the rooms during winter.

MATERIALS AND METHODS

Animal

5 Adult common Indian toads, each weighing 80-100 gm, were collected from a selected site in Midnapur (22°15′ N 87°39′ E) throughout the year as hibernating (Jan-Feb), in stages of arousal from hibernation (April-May), reproductive (June-July) and pre-hibernating phase (Nov). Blood samples were drawn via cardiac puncture at the time of euthanasia, using a microcollection system, and collected in tubes.

METHODS

From hibernating and non-hibernating individuals blood samples were drawn via cardiac punc-

* *Corresponding author, E-mails: pratihar_vu@yahoo.co.in*

ture immediately after euthanasia, according to the ethical guideline laid of the Committee for the Purpose of Control & Supervision of Experimental Animals (CPCSEA) under the Animal Welfare Division of Government of India on the use of animals in scientific research. Blood urea was estimated photometrically by DAM method (Urea reacts with diacetylmonoxime in the presence of an activator to take a pink color .This is measured at spectrophotometer at 540nm against blank) using the standard kit (Merck- Diagonistica-PDLFT0082).

STATISTICAL ANALYSIS

Statistical analysis was done using Microcal Software, Inc. Version: 6.0. Biochemical experiments were performed at least three times with 5 toads in each experimental group. Students t-test was performed to compare the means at $P<0.05$ significance level.

RESULTS

Variation of urea concentration was studied through out the year(Fig.1) .During the hibernating phase(Jan-Feb),urea conc. was 149.8mg/

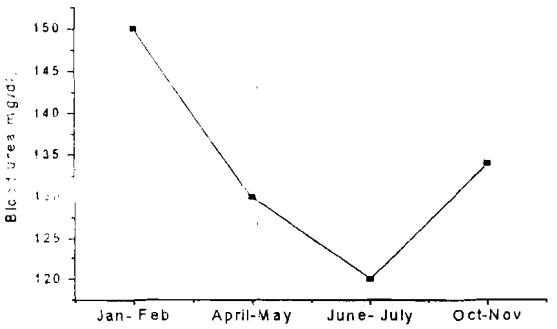

Figure 1: Seasonal variation of blood urea concentration. Blood urea expressed in mg/dl.

dl ,when air temperature was 10°c-12°c.It was 130.2mg/dl in the month April-May(air temparature38°c-40°c) when the animal got out from their hibernation. In rainy season (June-July), it was further reduced to 120.6mg/dl and at the beginning of winter (Oct-Nov), the urea concentration increased to 134mg/dl (Table:1).

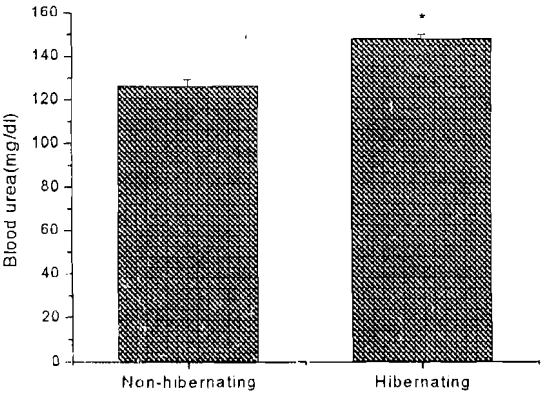

Figure 2: Comparison of blood urea (expressed in mg/dl) in non-hibernating and hibernating frogs. At the 0.05 level the two means are significantly different (* $P<0.05$).

DISCUSSION

Blood urea level significantly increased in the hibernating animals as compared to the non hibernating toads (Fig.2). Available reports indicate that urea ,the end product of protein metabolism is the predominant organic osmolyte, which accumulates during dehydration in hibernation (Jorgensen 1997).Elevated urea level in serum as an effective cryoprotective agent (Costanzo and Lee 2005). Here, decreased hydration is accompanied by a marked reduction in the resting state of oxygen consumption,which is inversely correlated with urea concentration. So, present findings therefore strongly indicate a strong link between elevated urea level and cryoprotection, during hibernation. Perhaps, cold stress triggers changes in gene expression of urea sensitive enzymes .During the rainy season, urea concentration probably decreased due to their much effect for reproductive success and protein anabolism of the animals .At the beginning of the winter during the months of

Table. 1 Seasonal variation of blood urea (mg/dl) concentration in Indian Common toad (n=5).

Sample no.	Jan-Feb	April-May	June-July	Oct-Nov
1.	148mg/dl	130mg/dl	116mg/dl	134mg/dl
2.	152mg/dl	132mg/dl	120mg/dl	136mg/dl
3.	150mg/dl	128mg/dl	118mg/dl	130mg/dl
4.	146mg/dl	128mg/dl	124mg/dl	138mg/dl
5.	153mg/dl	133mg/dl	125mg/dl	132mg/dl
Mean.	149.8mg/dl	130.2mg/dl	120.6mg/dl	134mg/dl

Oct-Nov (pre-hibernating period) urea concentration further increased .In those months they would probably were making them metabolically competent for the coming harsh winter.

In summary it may be concluded that in the Common Indian toad the protein metabolism and urea level elevation plays a significant role in maintaining their physiological status steady particularly during hibernating period .And that during hibernation,urea plays as a cryoprotectent agent to help them to cope up with the low temperature and sustained life. Seasonal variation in urea level is related to the availability of their food and is a part of metabolic strategy.

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A REVISION OF *EQUISETITES RAJMAHALENSIS* OLDHAM & MORRIS

Pankaj K. Pal* and Moumita Basu

Palaeobotany and Palynology Section, Botany Department
Burdwan University, Golapbag, Burdwan - 713 104

Abstract ■ Specimens of *Equisetites rajmahalensis*, the only known representative of extinct Equisetaceae in India, have been worked out in detail with an emendation of the circumscription of the taxon. As it is difficult to assign a fossil to any of the two extant genera of Equisetaceae, *Equisetum* Linnaeus and *Hippochaetae* Milde, the comprehensive name *Equisetites* Sternberg has been used to describe the Indian material. The paper also records for the first time the occurrence of the species in the Dubrajpur Formation.

Keywords : Equisetites rajmahalensis, Rajmahal Formation, Dubrajpur Formation, Upper Jurassic-Lower Cretaceous

INTRODUCTION

Specimens resembling modern *Equisetum*, collected from the Rajmahal Formation, were originally described as *Equisetites rajmahalensis* by Oldham & Morris (1863). Subsequently, the species was also reported from the Bhuj Formation of Kachchh Basin (Roy, 1968). The generic name *Equisetites* was instituted by Sternberg (1833) for fossils comparable with modern *Equisetum* Linnaeus. Later workers (Harris, 1961; Gould, 1968) opined that as no morphological difference is found between *Equisetum* and *Equisetites*, the fossils may be described under the generic name *Equisetum*. This view was followed by later workers and accordingly the Indian material was described as *Equisetum rajmahalense* (Bose and Sah, 1968; Sengupta, 1988). However, Sen and Sen (1973) proposed that *Equisetum sensu* Linnaeus comprises two distinct genera *Equisetum sensu stricto* and *Hippochaetae* Milde 1865. The idea was first conceived by Milde (1865) and later substantiated by Campbell (1928), Rothmaler (1944), Manton (1950) and finally by Sen and Sen (1973). Rothmaler (1944) pointed out that though many interspecific hybrids occur within each of the two genera of the modern Equisetaceae (*Equisetum sensu stricto* and the *Hippochaetae* Milde), yet intergroup hybrids are totally absent. Sen and Sen's (1973) conclusion is based on morphological, anatomical, embryological, cytological and physiological differences. The generic status of

* *Corresponding author, E-mail : pankaj_bot@yahoo.com*

Hippochaetae has also been corroborated by DNA homological data of *Equisetum sensu stricto* and *Hippochaetae* Milde (Sadhukhan, 1975).

Because it is difficult to assign a fossil specimen (particularly that preserved as an impression or a cast or a mould) to any of the two extant genera of Equisetaceae, therefore the comprehensive generic name *Equisetites* Sternberg has been preferred to describe the Indian material.

MATERIAL AND METHODS

The material of the present work is constituted of a good number of specimens collected from Balbhadri Hill (*loc. typ.*), Bindaban and Chunakhali localities of the Rajmahal Formation and the Khatangi Hill locality of the Dubrajpur Formation. Some specimens described by previous workers have also been studied. The specimens are preserved as impressions or as moulds. Buried parts of some specimens were degaged when required. The specimens were studied under Leica Wild M3B Stereobinocular microscope using strong incident light.

SYSTEMATIC DESCRIPTION

PTERIDOPHYTA
SPHENOPSIDA
EQUISETALES
EQUISETACEAE
GENUS EQUISETITES STERNBERG 1833
Equisetites rajmahalensis Oldham & Morris
Pl. 1, Figs 1-5

1863 *Equisetites rajmahalensis* Oldham & Morris, Pl.2, figs. 2-5, pl. 35, figs 3,4.
1869 *Equisetum rajmahalense* Schimper p. 276.
1877 *Equisetum rajmahalense* Schimper : Feistmantel, p.11.
1933 *Equisetites rajmahalensis* Oldham & Morris : Sahni & Rao, p. 188.
1938 *Equisetites* sp. : Jacob, p. 152.
1947 *Equisetites rajmahalensis* Oldham & Morris : Ganju, p. 56,pl.1, fig. 1.
1947 *Equisetites* sp. Oldham & Morris : Ganju, P.57, pl.1, fig. 2.
1966 *Equisetites rajmahalensis* Oldham & Morris : Surange, p. 56, fig. 30A-B.
1968 *Equisetum rajmahalense* (Oldham & Morris) Feistmantel : Bose & Sah, p. 18, pl.1 figs 1-6.
1968 *Equisetum rajmahalense* (Oldham & Morris) Feistmantel : Roy, p. 108, pl. 1, figs 1-2; Text-fig.1.
1975 *Equisetites rajmahalensis* O. and M. : Sharma, p. 84, pl. 1, fig. 3.
1984 *Equisetites rajmahalensis* Oldham & Morris, Bose & Banerji, p.8, pl. 2, fig.1; Text-fig. 3E-G.
1988 *Equisetum rajmahalense* (Oldham and Morris) Schimper : Sengupta, p.50, pl. 3, figs 9,10.

Emeded diagnosis - Rhizome horizontal, about 2.5 cm wide, obscurely marked with irregular longitudinal ridges and grooves; nodes not well marked but distinguished by small diaphragms (considered to be rotated); length of internodes about 7 cm; diaphragm 5 mm wide consisting of a rim about 1 mm wide with about 28 obscure tubercles, centre appearing sunken.

Aerial stem typically 4 cm wide; internodes up to 1.8 cm long but sometimes shorter; leaf sheath 1-1.5 cm long, composed of up to 30 segments (on whole sheath), segments consisting of leaf portions about 2 mm wide at base and tapering to 1.5 mm above where they are normally broken of, connected by sunken flanges which die away to a point below; leaf sheath segments marked by numerous stomatal pits; surface of internode smooth, not pitted (free leaves not observed, cones unknown).

Localities & Horizons -Balbhadri Hill (*loc. typ.*), Bindaban, Chunakhali, Bartala, Borio, Sakrigalighat, Onthea, Chilgojhuri and Nipania

localities of the Rajmahal Formation; Kakadbhit, Chawad River and Dharesi localities of the Bhuj Formation and Khatangi Hill locality of the Dubrajpur Formation.

Age - Upper Jurassic - Lower Cretaceous.

Lectotype - No. 4487 of the Geological Survey of India, Calcutta.

DISCUSSION AND COMPARISON

Present observations are based on a fresh collection of about a dozen specimens collected from Bindadan and Balbhadri Hill localities of the Rajmahal Formation and the Khatangi Hill locality of the Dubrajpur Formation. Also, specimens described by some previous authors have been studied. *Equisetites rajmahalensis*, the only representative of Sphenopsida in the Indian Upper Jurassic-Lower Cretaceous, is rather uncommon in occurrence and not known in position of growth. The stems are preserved in the form of moulds of external surface. The commissural flanges are conspicuous, appearing as straight narrow ridges and the intervening leaf-segments appear as sunken strips. The segments of the leaf-sheath are covered with numerous irregularly arranged tubercles representing the casts of stomatal pits. Thus, *Equisetites rajmahalensis* possessed sunken stomata and in this feature it resembles modern *Hippochaetae*. Bose and Sah (1968) mentioned a stem, 8 mm wide; no such stem is known to us. Trivedi and Sukh-Dev (1982) pointed out that pl.1, figs 4, 6 of Bose and Sah (1968) should be viewed upside down and therefore they furnished a fresh description of the leaf-sheath. While doing so, the authors (Trivedi and Sukh-Dev, 1982) mentioned that the leaf teeth are short and pointed. However, the free leaf teeth of *Equisetites rajmahalensis* are not known at all. In all the so far known specimens of *E. rajmahalensis*, including its selected lectotype, the leaves are always broken off which led us to believe that they probably diverge from the stem. The orientation of figure of Surange (1966, Fig. 30B) is correct though Trivedi and Sukh-Dev (1982) erroneously mentioned that it should be viewed supside down.

Equisetites rajmahalensis had been known from the Rajmahal and Bhuj formations of India. Occurrence of the species in the rocks of Dubrajpur Formation is hereby recorded for the first time.

Equisetites rajmahalensis is most similar to *E. columnare* Brongniart from English Middle Jurassic (Harris, 1961). But *E. columnare* has 50-60 leaf segments (though fewer on slender stem); its commissural furrows extend downward further. Its free leaves are also commonly broken off (because they diverge from the stem), but have been traced to an acute apex. Several features of *E. columnare* cannot be compared because those are unknown in *E. rajmahalensis*. Feistmantel (1877) made detailed comparison with *E. muensteri* Sternberg, a species with much narrower stems, from European Rhaetic and Liassic.

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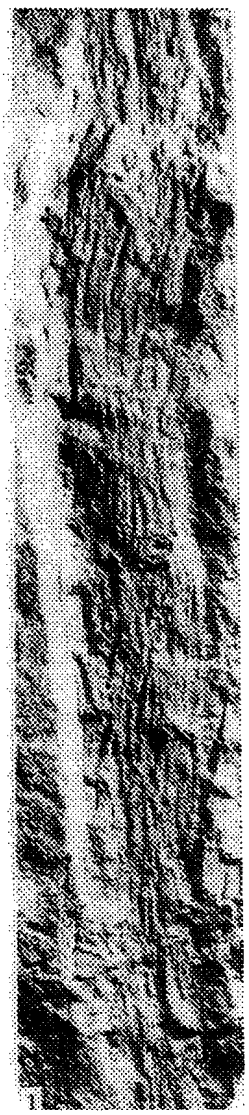

Fig. 1

Fig. 2

Fig. 3

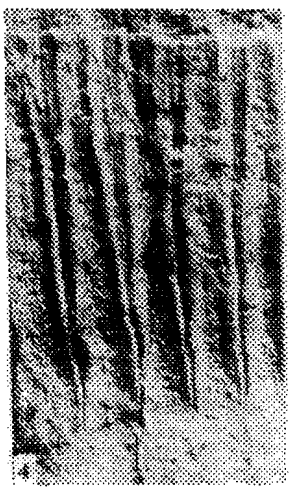

Fig. 4

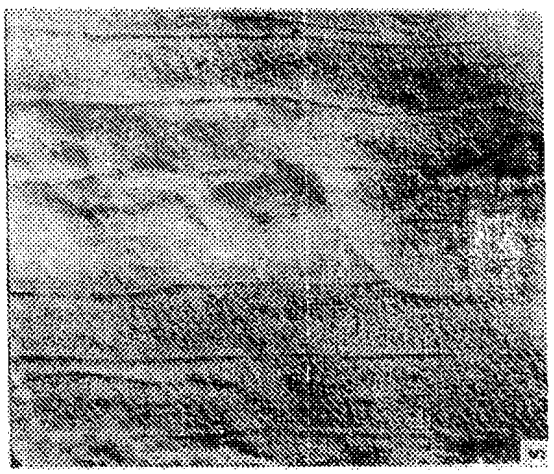

Fig. 5

Plate 1, Figs 1-5 *Equisetites rajmahalensis* Oldham & Morris. **Fig. 1** - Rhizome showing internodes with irregular ridges and grooves, nodal regions with small diaphragms, specimen no. 30174/257 of the Birbal Sahni Institute of Palaeobotany, x 1. **Fig. 2** -Aerial stem showing internode and nodes bearing leag-sheaths, specimen no. 43/5 (from Khatangi Hill locality of the Dubrajpur Fm), X 1. **Fig. 3** - Aerial stem, part of Specimen no. 16712 of the Birbal Sahni Institute of Palaeobotany, XI. **Fig. 4** - Part of leaf-sheath in Fig. 3 magnified, x 6. **Fig. 5** - Magnified view of leaf-sheath showing stomatal pits, specimen no. 9/1 (from Balbhadri Hill locality of the Rajmahal Fm.), X 16.

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