International Journal of Farm Sciences **6(4):** 290-298, 2016

Biochemical traits of some rice varieties grown under waterlogged and submerged conditions

AK PADHIARY, B KAR*, S ROUT, PK BEHERA*** and S SAHOO****

Krishi Vigyan Kendra, RRTTS Campus (OUAT)
Chiplima, Sambalpur 768026 Odisha, India
***Department of Seed Science and Technology, ***Department of Agronomy**
Orissa University of Agriculture and Technology, Bhubaneswar 751003 Odisha, India
****School of Forestry and Environment**
Sam Higginbottom Institute of Agriculture Technology and Sciences
Allahabad 211007 Uttar Pradesh, India
Email for correspondence: sandeeprout1988@gmail.com

ABSTRACT

A field experiment was conducted during Kharif 2012 at Adaptive Research Station, Sakhigopal, Puri, Odisha to study the biochemical traits of different rice varieties grown under waterlogged and submerged conditions. Total chlorophyll content of the leaf was found to be highest (2.52 mg/g FW) in Varshadhan; the similar trend was also recorded in choirophyll-a and Chlorophyll-b content among the cultivars and starch content was highest in Varshadhan (145.46 mg/g). Considering all the parameters the Varshadhan among all the varieties may be recommended to farmers for cultivation under waterlogged and submerged conditions.

Keywords: Biochemical; rice; submerged; waterlogged; varieties

INTRODUCTION

Rice is one of the most important cereal crops widely cultivated in the varied diverse ecosystems. India is one of the world's largest producers and consumers of rice next only to China (Sharma 2010). In Odisha around 93 per cent area is covered with rice crop during Kharif season which is generally sown in June-July and harvested in November-December. It is considered as a staple food of 65 per cent of Indian population. Rice production in India is an important part of the national economy. In India lowland rice area is about 14.4 Mha which accounts for 32.4 per cent of the total area under the rice crop in the country. The submersed rice ecosystem in India represents 26 per cent of the total cultivated area. Among the abiotic stresses mainly waterlogging, light, temperature, soil salinity and drought may adversely affect plant growth and performance (Dalmia and Sawhney 2004). Deep water rice is defined as rice that is flooded deeper than 15 cm for one month or longer during the growing season (Catling 1985) with turn flooding that grows in the very deeply flooded areas. In Odisha flooding usually occurs in 3 stages of plant growth and can last for 7 days to one month. The stipulation that flooding must sustain for at least one month is to be distinguished deep water rice area from other flood-prone areas. In the coastal belt the water may rise up to more than 50 cm by tide action and the flash flood areas where rice may be temporarily submerged for only a few days. Most deep water rice survives by elongation of the stem whereas other rice types lack these characteristics and are destroyed by deep water.

Rice grown in more than 50 cm water for one month or longer period during the cropping season is called deep water rice. They are of two types: traditional tall and floating. Traditional tall varieties are tall with long leaves and grown in water depth between 50-100 cm whereas floating rice is grown in 100 cm or deeper situation. In the coastal belt of Odisha rice grown in low lying areas during monsoon is called deep water rice. The deep water rice grown in Asian and African countries is of different type. The growth of deep water rice varieties largely depends upon factors such as flooding region, micro-relief and soil type etc. In view of above facts the present experiment was planned and conducted at the Adaptive Research Station, Sakhigopal, Puri, Odisha during the wet season of 2012 with the objective to evaluate the biochemical traits of different rice varieties grown under waterlogged and submerged conditions.

MATERIAL and METHODS

The field experiment was conducted during Kharif 2012 at Adaptive Research Station, Sakhigopal, Puri, Odisha to study the biochemical traits of different rice varieties grown under waterlogged and submerged conditions. The soil of experimental field of Sakhigopal farm is clay loam and texture slightly acidic in nature. The location is situated in 19°48' North latitude and 85°52' East longitude 20 km away from the Bay of Bengal with an altitude of 6 m amsl. The climate is relatively warm and humid in nature with short mild winter. The average annual rainfall of the region is 1408.8 mm which is received from southwest monsoon. Rice varieties taken as treatments in the experiment were Sarala, Hanseswari, Varshadhan, Bankoi, Champa and CR-925-2. The seeds were procured from the Adaptive Research Station, Sakhigopal, Puri for the experiment. The sowing in the nursery bed was done on 7 June 2012. The required amount of FYM and phosphatic fertilizer were well mixed with the soil of the nursery before sowing. The sowing was done in lines. The irrigation channels were prepared surrounding the seed beds. Frequent sprinkler irrigation was provided to seedbed initially and after germination irrigation management was done in such a manner that the raised seed beds remained moistened without any standing water over their surface for one week. Thereafter standing water was maintained up to 3 cm.

For the better growth of seedlings minimum N fertilizer was applied in the seed beds. Before seven days of rooting of seedlings granular pesticide as per as recommendation was applied in seed beds in order to avoid diseases and pests after the transplanting. After 21 days of sowing the seedlings were uprooted for transplanting on 5 July 2012. The main land (50 x 40 m) was ploughed with the tractor after harvest of the previous crop and FYM @ 5 tons/ha was spread over the field. The field was again cross-ploughed and levelled properly. Two days prior to transplanting for each sowing the irrigation was given to plot size of 400 sq m (50 x 8 m) for puddling by the power tiller and a little standing water was maintained in the field. Further main plot (50 x 8 m) was divided into three stripes representing three replications. Before transplanting of seedlings and basal dose of the 15 kg N, 30 kg P_2O_5 and 30 kg K_2O per hectare were applied and mixed thoroughly in soil during puddling. Rest nitrogen was top-dressed twice. The first top dressing of nitrogen @ 30 kg/ha in the form of urea was applied after 15 days of transplanting and second top dressing at 112 days after transplanting. Twenty one day old seedlings of rice varieties were transplanted with a spacing of 20 x 10 cm having two seedlings per hill. Hand weeding was done at 15 and 35 days after transplanting. Due to waterlogged condition the weed population was minimum and suppressed. To control the insect and pests thaiman granules @ 10 kg/ha were applied along with the first top dressing of nitrogen. After receding of submergence stem borer attack was observed and monocrotopus @ 1 l/ha was applied. The crop was harvested on 5 December 2012 after it attained physiological maturity. The plant material for the biochemical evaluation was collected from five randomly selected competitive plants in each replication for all the parameters at growing stage and tagged for recording a representative sample of the entire population. After harvesting grains were collected to determine starch and protein.

Total chlorophyll content in the leaves was determined by using the method given by Arnon (1949), peroxidase content in the plant as per Sadasivan and Manickam (1996), superoxide dismutase (SOD) as per Alscher et al (1997), proline as per Sadasivam and Manickam (1996) and Gilmour et al (2000), carbohydrate (NSC) as per Yoshida (1981) and starch using the Anthrone reagent (Anon 1999). The observations were statically analyzed following the RBD as outlined by Panse and Sukhatme (1961).

RESULTS and DISCUSSION

Chlorophyll content: The chlorophyll-a content of leaves measured at flowering stage (before submergence) showed maximum (1.98 mg/g FW) in Hanseswari followed by Sarala (1.97 mg/g FW) whereas the minimum value (1.43 mg/g of FW) was recorded in Champa (Table 1). After submergence highest Chl-a content was found in Hanseswari (1.89 mg/g FW) followed by Varshadhan (1.81 mg/g FW) with reduction of 5.23 and 4.5 per cent respectively as compared to flowering stage (before submergence). Chlorophyll-b content at flowering stage (before submergence) was highest (0.66 mg/g FW) in Hanseswari followed by Sarala (0.62 mg/g FW) and Varshadhan (0.55 mg/g FW) and lowest (0.45 mg/g FW) in Champa. Similar trend was observed after the submergence as highest value was exhibited by Hanseswari (0.63 mg/g FW) and lowest (0.39 mg/g FW) in Champa. Total chlorophyll content at flowering was highest in Hanseswari (2.76 mg/g fresh wt) followed by Sarala (2.68 mg/g FW) and Varsadhan (2.58 mg/g FW) and minimum (1.98 mg/g FW of leaf) in Champa.

It was revealed that the concentration of chlorophyll content decreased in almost all the varieties after the submergence. The chlorophyll reduction is accompanied by carbohydrate reserve before the submergence in the leaves which helps in minimum shoot elongation and regulates the plant hormone GA and ethylene production. The ethylene triggers gene expression and enzyme activity of chlorophyllase which degrades the chlorophyll. Due to reduction in ethylene production in tolerant varieties chlorophyll reduction was less in submerged tolerant genotypes (Das et al 2005, Sarkar et al 2006).

Carbohydrate content: Data presented in Table 2 indicates that the carbohydrate content of the shoot before the submergence was recorded maximum (15.63%) in case of Varsadhan followed by Hanseswari (15.26%) and Sarala (14.76%) and minimum (11.26%) in CR 925-2. After submergence Varsadhan had maximum carbohydrate content (14.30%) followed by Hanseswari (13.81%) and Sarala (13.06) and minimum in CR 295-2 (9.71 %). Submergence tolerance in rice is not only governed by a single factor but by a number of factors. It was found that high carbohydrate content (15.63%) was noted in Varsadhan followed by Hanseswari (15.26%) the lowest in Bankoi (11.26%).

Higher levels of initial carbohydrate act as buffer stock and its continued availability during submergence is critical for the survival and growth of rice under submerged condition. The metabolic energy needed by the plant during submergence is primarily supplied from stored carbohydrate present in the tissue. Irrespective of varieties it was found that there was a reduction of carbohydrate content of shoot. It was perhaps due to the depletion of the

Table 1. Variation in chlorophyll content before and after submergence of rice varieties

Variety	Chlorophyll					
	a		b		Total	
	BS	AS	BS	AS	BS	AS
Sarala	1.97	1.73 (-12.18)	0.62	0.55 (-11.29)	2.68	2.31 (-13.8)
Hanseswari	1.98	1.89 (-5.23)	0.66	0.63 (-5.45)	2.76	2.42 (-12.31)
Varshadhan	1.91	1.81 (-4.54)	0.55	0.52 (-4.54)	2.58	2.52 (-2.32)
Bankoi	1.66	1.26 (-24.09)	0.52	0.45 (-13.46)	2.29	1.78 (-22.27)
Champa	1.43	1.32 (-7.69)	0.45	0.39 (-13.33)	1.98	1.72 (-13.13)
CR-925-2	1.54	1.42 (-7.8)	0.50	0.44 (12.0)	2.16	1.90 (-12.03)
Mean	1.75	1.57	0.55	0.49	2.41	2.10
SEm±	0.02	0.04	0.01	0.05	0.08	0.02
$CD_{0.05}$	0.07	0.13	0.04	0.02	0.05	0.04
CV	2.33	4.76	4.53	1.66	2.74	0.97

Figures in the parentheses indicate percentage of increase or decrease over previous observation,
BS= Before submergence, AS= After submergence

Table 2. Variation in carbohydrate, starch, sucrose and proline content in response to submerged condition of rice varieties

Variety	Carbohydrate (%)		Starch (mg/g)		Sucrose (µg/g)		Proline
	BS	AS	BS	AS	BS	AS	
Sarala	14.76	13.06 (-11.5)	137.96	34.70 (-74.8)	146.02	48.13 (-67.0)	9.5
Hanseswari	15.26	13.81 (-9.5)	142.96	46.06 (-67.7)	152.18	52.76 (-65.3)	9.33
Varshadhan	15.63	14.30 (-8.5)	145.46	58.36 (-59.8)	162.62	58.26 (-64.1)	8.43
Bankoi	13.16	10.81 (-17.8)	134.30	30.55 (-77.2)	130.26	32.56 (-75.1)	15.23
Champa	12.76	10.85 (-14.9)	134.83	32.00 (-76.2)	132.77	37.17 (-72.8)	10.90
CR-925-2	11.26	9.71 (-13.7)	135.66	34.58 (-74.5)	145.43	40.72 (-72.0)	10.96
Mean	13.80	12.09	138.53	39.37	144.88	44.93	10.72
SEm±	0.23	0.41	0.54	7.13	0.50	0.33	0.34
$CD_{0.05}$	0.72	1.31	1.72	22.47	1.60	1.05	1.07
CV	2.90	6.02	0.68	31.37	0.61	1.30	5.49

Figures in the parentheses indicate percentage of increase or decrease over previous observation,
BS= Before submergence, AS= After submergence

photosynthetic rate under submerged condition and it was based on loss of chlorophyll fluorescence. It may be also lowering stomata conductance, inter-cellular CO_2 concentration as well as denaturing of the photosynthetic machinery. Moreover inundation owing to the submergence also limits the carboxylation by low/intermediate intercellular CO_2 concentration that may also subside the RUBP-carboxylase activity rather more favouring the oxygenation (Buchanan et al 2004). This deviation ratio of carboxylation to oxygenation under submergence is more serious for switching over the tissues to make it more prone to photo-respiration.

In submergence rice genotypes when the leaves, stem and roots are completely submerged the rate of depletion of carbohydrates is very slow than the susceptible varieties. Drastic reduction of carbohydrates leads to high rate of anaerobic fermentation and production of ethanol at toxic level (Setter and Laureles 1996). The results are in agreement with the findings of Chaturvedi et al (1996).

Starch content: From the data (Table 2) it is revealed that maximum starch content in shoot was recorded in Varshadhan (145.46 mg/g) followed by Hanseswari (142.96 mg/g) and minimum in Bankoi (134.30 mg/g). After submergence maximum value (58.36 mg/g) was exhibited by Varshadhan followed by Hanseswari (46.06 mg/g) and minimum in Bankoi (30.55 mg/g). Chaturvedi et al (1996) also found that tolerant genotypes of rice had higher total initial starch content and lower rate of depletion during submergence than the susceptible genotypes.

Sucrose content of shoot: Varshadhan had highest sucrose content of the shoot (162.62 µg/g) followed by Hanseswari (152.18 µg/g) and minimum in Bankoi (130.26 µg/g) before submergence. It was maximum (58.26 µg/g) in Varshadhan followed by Hanseswari (52.76 µg/g) after the submergence and minimum in Bankoi (32.56 µg/g) (Table 2). Sucrose is the predominant translocating product of current photosynthesis and is stringently under the control of CO_2 flux into the leaves. Perhaps due to up-regulated sucrose synthetase, hydrolysis of sucrose enhanced ethanol production and improved energy charge in association with haemoglobin induction in this glycolytic pathway which increased survival under anoxia due to submergence. The present findings are in line with the work of Sairam et al (2008).

Proline accumulation: The accumulation of proline was lowest (8.43 mg/g FW) in case of Varshadhan followed by Hanseswari (9.33 mg/g FW) and Sarala (9.50 mg/g FW) and maximum in CR 925-2 (10.96 mg/g FW) under submerged condition. On the whole, among the cultivars significant difference was recorded (Table 2). Chaubey et al (1996) inferred that accumulation of proline is maximum in susceptible varieties under submerged condition. Proline accumulation is considered as a sign of submergence injury the concentration of which builds up under stress due to hydrolysis of proteins. Alia and Sarathi (1993) and Shibasaka and Tsuji (1988) endorsed the views of Chaubey et al (1996) that suppression of mitochondrial electron transport and normal growth of mitochondria are two reasons for accumulating proline under submerged condition. The tolerant cultivars under submerged condition perhaps have better energy utilization due to mitochondrial activity for which proline accumulation is less.

Catalase enzyme activity: Data presented in Table 3 reveal that Catalase enzyme activity before submergence was maximum in Hanseswari (22.48 mmol H_2O_2 decomposed/min g FW) followed by Varshadhan (22.40 mmol H_2O_2 decomposed/min g FW). The catalase activity of the genotypes was more or less same before submergence but there was general reduction in catalase activity after the submergence in all the varieties. The percentage of reduction was minimum in Varshadhan (87.27%) and maximum in Champa (89.71%).

Peroxidase enzyme activity: The activity of the peroxidase enzyme was maximum (47.27 OD/g FW) in Sarala followed by Champa (47.03 OD/g FW) (Table 3) and lowest in Hanseswari (46.64 OD/g FW) before submergence. Peroxides activity was maximum (12.66 OD OD/g FW) in Varshadhan and minimum in Bankoi (10.71 OD/g FW) after submergence.

Super oxide dismutase (SOD) enzyme: Data in Table 3 indicate that the activity of the SOD enzyme before submergence was maximum in Varsadhan (168.42 units/g) followed by Sarala (168.08 units/g) and minimum in CR 925-2 (160.20 units/g). There was greater variation with regard to enzyme activity among the cultivars. It was also revealed that under waterlogged condition the activity of SOD was more or less similar.

Although the plant is well adapted to the aquatic environment it has low survival if complete submergence persists for several days. When the plants are de-submerged after a period of submergence a sudden explosive to air possibly produces free radicals of oxygen which may induce membrane lipid peroxidation, protein denaturation and change in DNA leading to cell death.

From the present investigation it is clear that the activities of catalase (CAT), peroxides (PER) and superoxide dismutase (SOD) were very similar in all the genotypes including tolerant and intolerant cultivars before submergence. However during submergence and after exposure to air the activities of these enzymes were higher only in the tolerant cultivars in comparison to susceptible varieties (Chaubey et al 1996).

Ushimaru et al (1992) reported that antioxidative enzymes were co-regulated in such that they showed higher activities at higher O_2 tension. It appeared that the activities of oxygen detoxification enzymes in previously submerged plants were restored to some extent in tolerant cultivars and peroxidase. The greater activities of CAT and PER under submerged condition in tolerant cultivars was possible due to higher absorption of O_2 from the

Table 3. Variation in synthesis of enzyme catalase, peroxidise and super oxide dismutase in response to submerged condition of rice varieties

Variety	Catalase (mmol H_2O_2 decomposed/min g FW)		Peroxidase (OD/g FW)		SOD (units/g FW)	
	BS	AS	BS	AS	BS	AS
Sarala	22.23	2.63 (-88.1)	47.27	11.95 (-74.72)	168.08	65.18 (-61.22)
Hanseswari	22.48	2.82 (-87.50)	46.64	12.54 (-73.11)	167.28	71.40 (-57.31)
Varshadhan	22.40	2.85 (-87.27)	46.88	12.66 (-72.99)	168.42	70.46 (-58.16)
Bankoi	22.35	2.40 (-89.26)	46.85	10.71 (-77.13)	166.88	61.33 (-63.24)
Champa	22.37	2.41 (-89.71)	47.03	11.30 (-75.97)	167.03	60.93 (-63.52)
CR-925-2	22.37	2.44 (-89.1)	47.00	11.57 (-75.38)	160.20	61.95 (-61.33)
Mean	22.37	2.59	46.94	11.95	166.32	65.21
SEm±	0.05	0.03	0.07	0.08	0.08	0.99
$CD_{0.05}$	0.18	0.12	0.24	0.27	0.26	3.14
CV	0.44	2.59	0.29	1.28	0.09	2.65

Figures in the parentheses indicate percentage of increase or decrease over previous observation,
BS= Before submergence, AS= After submergence, SOD= Super oxide dismutase

surrounding water. The tolerant cultivars had higher root activity hence could transport more O_2 through the shoot to the roots under complete submergence (Sarkar and Bera 1997). The present study is supported by the findings of Monk et al (1989).

CONCLUSION

In the present study it was found that among the varieties Varshadhan had maximum carbohydrate and starch content and activity of antioxidant enzymes such as catalase, peroxidase, SOD (super oxide dismutase) which were utilized by the plant under submerged condition for its metabolic activity. Therefore Varsadhan can be recommended to the farmers for cultivation under waterlogged and submerged conditions.

REFERENCES

Alia and Sarathi PP 1993. Suppression in mitochondrial electron transport is the prime cause behind stress induced proline accumulation. Biochemical and Biophysical Research Communications **193:** 54-58.

Alscher RC, Donahue JL and Cramer CL 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiologia Plantarum **100(2):** 224-233.

Anonymous 1999. Official methods of analysis. 16th edn, Association of Analytical Chemists, pp 440-446.

Arnon DI 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris.* Plant Physiology **24(1):** 1-15.

Buchanan BB, Gruissem W and Jones RL 2004. Biochemistry and molecular biology of plants. 2nd edn, American Society of Plant Physiologists, Rock Ville, Maryland, USA.

Catling HD 1985. An agro-ecological characterization of the deep water areas in the Chao Phya basin of Thailand with a subdivision into five regions. International Rice Research Institute, Los Banos, The Philippines.

Chaturvedi GS, Ram PC, Singh AK, Ram P, Ingram KT, Singh BB, Singh RK and Singh VP 1996. Carbohydrate status of the rainfed lowland rice in relation to submergence, drought and shade tolerance. In: Physiology of stress tolerance in rice (VP Singh, RK Singh, BB Singh and RS Zeigler eds), Proceedings, International Conference on Stress Physiology of Rice, 28 Feb - 5 March 1994, Lucknow, Uttar Pradesh, India, pp 103-135.

Chaubey CN, Dwivedi JL, Gregorio GB, Mendoza R, Mishra SB, Palaklang W, Patena CC, Puckridge DW, Quillon CC, Senadhira D and Thakur R 1996. Breeding flood-prone rice. Thailand-IRRI Collaborative Programme for Improving Flood-prone Rice, Prachinburi Rice Research Institute, Prachinburi, Thailand,116p

Dalmia A and Sawhney V 2004. Antioxidant defence mechanism under drought stress in wheat seedlings. Physiology and Molecular Biology of Plants **10(1):** 109-114.

Das KK, Sarkar RK and Ismail AM 2005. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. Plant Science **168:** 131-136.

Gilmour SJ, Sebolt AM, Salazar MP, Everard JD and Thomashow MF 2000. Overexpression of the arabidopsis CBF_3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiology **124(4):** 1854-1865.

Monk LS, Fagerstedt KV and Crawford RMM 1989. Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. Physiologia Plantarum **76(3):** 456-459.

Panse VG and Sukhatme PV 1961 Statistical methods for agricultural workers. 2nd edn, ICAR, New Delhi, India, 361p.

Sadasivan S and Manickam A 1996. Pigments. In: Biochemical methods, 2nd edn, New Age International (P) Ltd Publishers, New Delhi, India, pp 190-191.

Sairam RK Kumutha D, Ezhilmathi K, Deshmukh PS and Srivastava GC 2008. Physiology and biochemistry of waterlogging tolerance in plant. Biologia Plantarum **52(3):** 401-412.

Sarkar RK and Bera SK 1997. A comparison of the submergence response of elongating and non-elongating flood tolerant deep water rice. Indian Agriculture **41:** 299-303.

Sarkar RK, Ready JN, Sharma SG and Ismail AM 2006. Physiological basis of submergence tolerance in rice and implications for crop improvement. Current Science **91(7):** 899-906.

Setter TL and Laureles EV 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. Journal of Experimental Botany **47(303):** 1551-1559.

Sharma SD 2010. Rice: origin, antiquity and history. CRC Press, Taylor and Francis Group, Boca Raton, FL and Science Publishers, Enfield, New Hampshire.

Shibasaka M and Tsuji H 1988. Respiratory properties of mitochondria from rice seedlings germinated under water and their changes during air adaptations. Plant Physiology **86:** 1008-1012.

Ushimaru T, Shibasaka M and Tsuji H 1992. Development of O_2-detoxification system during air adaptation to submerged rice seedlings. Plant and Cell Physiology **33(8):** 1065-1071.

Yoshida S 1981. Fundamentals of rice crop science. International Rice Research Institute, Los Banos, Leguna, Philipines, 269p.

Received: 19.12.2016 ***Accepted: 31.12.2016***