International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(5): 731-738, October 2016
DOI: 10.5958/2230-732X.2016.00096.6

BIOTECHNOLOGY

Variation in total polyamine content in some native rice cultivars of North Kerala, India in response to salinity stress

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Paper No. 498 Received: 16-3-2016 Accepted: 6-9-2016

Abstract

Phytohormone like low molecular weightaliphatic polycationic compounds known as polyamines have an essential role inplant growth and development and they respond to various environmental stress factors. Maintaining crop yields under adverse environmental stresses is a major challenge facing modern agriculture where polyamines play an important role. The total polyamine content in some native rice cultivars of North Kerala under salinity stress has been analysed presently and the results show that the total polyamine content in all the cultivars got increased in relation to the increase in the salt concentration applied. The highest total polyamine content was noted in the cultivar *Orkazhama* followed by *Kuttusan* and *Kuthiru* which were collected from a traditional saline rice tract of North Kerala. The highest percentage of increase in total polyamines was noted in the cultivar *Kunhutty* followed by *Kuttusan* and *Veliyan*. *Kunhutty* and *Veliyan* are cultivars collected from a non saline rice tract. In all the cultivars the total polyamine content got increased starting from the 10mM NaCl treatment itself. Among the cultivars studied, *Orthadian* showed the lowest amount of total polyamine content and *Kuthiru* showed the lowest percentage of increase in relation to increase in salinity. This result shows that salinity induces the accumulation of polyamines in rice plants and this may be a mechanism to contribute tolerance and resistance to salt stress. The rate of variation is cultivar specific. Identification of suitable genotypes and their improvement can be used as a potential method to evolve salinity resistant rice genotypesfor different geographical zones.

Highlights

- Total polyamine content in all the rice cultivars studied got increased in relation to the increase in the salt concentration applied.
- The highest total polyamine content was noted in the cultivar *Orkazhama* followed by *Kuttusan* and *Kuthiru* which were collected from a traditional saline rice tract of North Kerala.
- The highest percentage of increase in total polyamines was noted in the cultivar *Kunhutty* followed by *Kuttusan* and *Veliyan*.
- In all the cultivars the total polyamine content got increased starting from the 10mM NaCl treatment itself.

Keywords: Abiotic stress, native rice cultivars, *Oryza sativa*, polyamines, salinity

Plant productivity is strongly influenced by both biotic and abiotic stresses. Plant development and productivity are negatively affected by these environmental stresses which are the primary cause of crop loss worldwide, reducing the average yields of major crop plants by more than 50% (Alcázar *et al*., 2006). Plants encounter a variety of environmental constraints throughout the entire developmental stages and among these salinity is one of the most important. Most cultivated plant species are highly sensitive to salt stress and they either die or display reduced productivity when they are exposed to long periods of salt stress (McKersie and Leshem, 1994). Plants respond to environmental fluctuations through physiological, developmental and biochemical changes to cope with biotic as well as abiotic stress conditions (Mahajan and Tuteja 2005; Fujita *et al.*, 2006). It is further predicted that these stresses will become more intense and frequent with climate change.

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and is rated as a salt sensitive crop (Maas and Hoffman 1976; Shannon *et al*. 1998; Grover *et al*. 2001). Salinity affects all stages of growth and development of the rice plant and the crop's responses to salinity varies with growth stages and intensity and duration of exposure to salinity (Widawsky and O'Toole 1990; Shimamoto 1999; Minhas and Grover 1999). Relatively, rice is considered as more sensitive to salts during early seedling stage than at reproductive stage (Flowers and Yeo 1981; Lutts *et al*., 1995). Excess salt content in the growth medium adversely affects all major metabolic activities in rice including cell wall damage, accumulation of electron dense protein particles, plasmolysis, cytoplasmic lysis, damage to sub cellular organelles like endoplasmic reticulum, accumulation of citrate, malate and inositol in leaf blades, increase in proline levels by 4 to 20 fold, decrease in Fv/Fm ratio, reduction in photosynthesis and overall decline in seed germination and seedling growth (Yeo *et al*., 1985; Lutts *et al*., 1995; Garcia *et al*., 1997; Khan *et al*., 1997; Pareek *et al*., 1997; Sivakumar *et al*., 1998), leading ultimately to reduced growth and diminished grain yield.

Salt stress is a complex environmental constraint consisting of osmotic as well as ionic components. The osmotic component is caused due to the decrease in the external osmotic potential of the soil solution and the ionic component due to the accumulation of ions which become toxic at high concentrations. Salt induced increase in endogenous polyamine content has been reported in various species (Erdei *et al*. 1996; Aziz *et al*. 1999). Polyamines are small ubiquitous and positively charged aliphatic compounds that have an impact on most if not all the processes linked to plant growth and development. They play a key role in the control of cell proliferation and cell differentiation and have also a strong influence on seed germination, flowering, fruit ripening and leaf senescence in plants (Chen and Kao 1991; Koetje *et al*., 1993; Galston *et al*., 1997; Martin-Tanguy 1997; Ali 2000). The most commonly found polyamines in plants include putrescine, spermidineand spermineand may be present either in a free soluble form or conjugated to phenolic compounds (Martin-Tanguy 1997) or linked to macromolecules such as proteins (Besford *et al*., 1993) or DNA (Van den Broeck *et al*. 1994). It has been reported that polyamines may assume complex functions in relation to resistance to various abiotic and biotic stresses (Martin-Tanguy 1997; Bouchereau *et al*., 1999). Differences in the response of different polyamines under salt stress have been reported among and within species. Krishnamurthy and Bhagwat (1989) reported that salttolerant rice cultivars drastically accumulated high levels of spermidine and spermine resulting in enhanced level of total polyamine content with a relative decrease in putrescine content. Abiotic stresses induce variation in the content of less common polyamines like caldopentamine, homocaldohexamine, norspermine, norspermidine (Roy and Ghosh 1996), cadaverine (Slocum 1991) and tyramine (Aziz *et al*., 1999) also.

Variation in total polyamine content in seven native cultars of rice collected from the traditional rice tracts of North Kerala, India in response to progressive salt stress has been analysed presently using a spectrophotometric method. Progressive stress has been applied mimicking the variation in the natural saline rice tracts of the study area.

Materials and Methods

Germination of seeds and growing of plant materials

The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India located at 11°35′N latitude and 75°48′E longitude in the first crop season of 2013. Seven native cultivars of rice including five cultivars namely *Orthadian, Orkazhama, Kuthiru, Kuttusan* and *Chovvarian* collected from one of the saline rice habitats of North Kerala and two native rice cultivars namely *Kunhutty* and *Veliyan* collected from one of the nonsaline rice habitats of North Kerala were used for the study. Enough number of healthy and mature caryopses taken from a single plant per cultivar were washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water and allowed to germinate in 10cm diameter Petri dishes covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day onwards. On the 10th day, required number of the germinated seedlings were transferred to coloured plastic pots of 25cm diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after the establishment of the seedlings the smallest among the two were removed. The plants were maintained in the experimental poly house of the Department of Botany under wetland conditions, always maintaining 3 cm of water above the soil level. The soil was fertilized with 1g N: P: K =18: 18: 18 per pot at fortnightly intervals starting from the 30th day. Weeding was done manually whenever required. Plants were grown in Randomized Block Design with three replications.

Experimental treatments

The experimental treatment was started from the 45th day onwards using aqueous solutions of sodium chloride as detailed in Table 1.

Table 1: Details of salinity treatment applied in the case of the different rice cultivars

Sl. No.	Treatment
T1	Control
T2	10mM (0.91dSm^{-1}) on 45th day
T3	10mM (0.91dSm^{-1}) on 45th day & 30mM (2.74dS m^{-1}) on 53rd day
T4	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day & 50mM (4.57dSm^{-1}) on 61st day
T5	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dS m^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day & 70mM (6.39dSm^{-1}) on 69th day
T6	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day, 70mM (6.39dSm^{-1}) on 69th day & 100mM (9.13 dSm^{-1}) on 77th day
T7	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74 dSm^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day, 70mM (6.39dSm^{-1}) on 69th day, 100mM (9.13dSm^{-1}) on 77th day & 200mM (18.26dSm^{-1}) on 85th day

Extraction and determination of polyamines

Rice leaf tissues of 100mg of both control and treated plants were weighed separately (Sartorius, Germany), crushed with liquid nitrogen (LN_2) andhomogenized in 1.5ml of (5%,v/v) icecoldperchloric acid ($HClO_4$, Merck) using a clean mortar and pestle. After extraction the extracts were incubated at 4°C for 24 hours, samples were centrifuged at 15000×g for 40 minutes at 4°C using a refrigerated centrifuge (Sigma, Germany). The clear supernatant fractions containing acid soluble polyamines were neutralized with 5M KOH, centrifuged again at 1000×g for 5 minutes and the supernatant was used for total polyamine content determination.

Spectrophotometric method of polyamine estimation

Estimation of total polyamines was carried out as proposed by Naik *et al*. (1981) and Federico *et al*. (1991). The standard reaction mixture contained 0.4M sodiumphosphate buffer, pH 6.8, 0.1 unit of polyamine oxidase and 0.2ml of the sample to be assayed. After incubation at 37°C for 10 min the reaction was terminated by the addition of 0.2ml of 10% trichloro acetic acid (TCA). The suspension formed was then centrifuged at 10000×g for 10 minutes. The Δ^1-pyrroline formed during the reaction was estimated according to the method of Naik *et al*. (1981). To 1ml of the supernatant 1ml of ninhydrin reagent (250mg ninhydrin, 37.6mg hydrindantin, 6ml acetic acid and 4ml of 6M ortho-phosphoric acid) and 1.5ml acetic acid were added. The assay tubes were incubated at 100°C in a boiling water bath for 30 minutes to develop the colour. The tubes were then cooled and 2.5 ml acetic acid was added to make up the volume to 6.0 ml. The colour was read in a spectrophotometer and then the optical density of the samples was determined at 510nm. Spermidine was used as the standard. Calculation was done using the formula:

$$\text{Total polyamine content} = \frac{\text{Standard OD}}{\text{Standard con}} \times \text{Sample OD} \times \frac{1}{w} \times \frac{V}{v}$$

where, W = weight of the tissue used; V = total extraction volume; v = aliquote volume used for assay. Total polyamine content is expressed in mg/g FW.

Results and Discussion

Total polyamine content estimated in the case of the different samples and the trend of variation have been recorded in Table 2 and Figures 1&2. From the above it is clear that the total polyamine content in all the cultivars got increased in relation to the increase in salt concentration applied. Among the cultivars the highest total polyamine content was noted in *Orkazhama* followed by *Kuttusan* and *Kuthiru*. These genotypes were collected from a saline rice tract of North Kerala. The highest percentage of increase in total polyamines was noted in the cultivar *Kunhutty* (162.50%) followed by *Kuttusan* (143.72%) and *Veliyan* (124.19%). *Kunhutty* and *Veliyan* are cultivars collected from a non saline rice tract. In all the cultivars the total polyamine content got increased starting from the 10mM NaCl treatment itself. Among the cultivars studied, *Orthadian* showed the lowest amount of total polyamine content and *Kuthiru* showed the lowest percentage of increase (84.12%) in relation to increase in salinity. This result shows that salinity induces the accumulation of polyamines in rice plants and this may be a mechanism to contribute tolerance and resistance to salt.

Table 2: Total polyamine content in different rice cultivars under salinity stress (mg/g FW)

Treatments	Mean ± SE (mg/g FW)	CD @ 5%	Percentage of increase over control
Orthadian			
0mM	0.875 ± 0.02	0.13	0.00
10mM	0.885 ± 0.01		1.14
30mM	1.080 ± 0.02*		23.42
50mM	1.135 ± 0.01*		29.71
70mM	1.330 ± 0.01*		52.00
100mM	1.570 ± 0.02*		79.43
200mM	1.860 ± 0.02*		112.57
Chovvarian			
0mM	0.920 ± 0.01	0.14	0.00
10mM	1.080 ± 0.02*		17.39
30mM	1.235 ± 0.01*		34.24
50mM	1.520 ± 0.02*		65.22
70mM	1.645 ± 0.01*		78.80
100mM	1.835 ± 0.02*		99.46
200mM	1.995 ± 0.02*		116.85
Kuttusan			
0mM	0.915 ± 0.01	0.13	0.00
10mM	1.100 ± 0.02*		20.22
30mM	1.265 ± 0.01*		38.25
50mM	1.480 ± 0.01*		61.75
70mM	1.835 ± 0.01*		100.55
100mM	2.060 ± 0.02*		125.14
200mM	2.230 ± 0.02*		143.72
Kuthiru			
0mM	1.165 ± 0.02	0.14	0.00
10mM	1.290 ± 0.02*		10.73
30mM	1.400 ± 0.01*		20.17
50mM	1.575 ± 0.01*		35.19
70mM	1.800 ± 0.02*		54.51
100mM	1.930 ± 0.02*		65.67
200mM	2.145 ± 0.02*		84.12
Orkazhama			
0mM	1.120 ± 0.02	0.04	0.00
10mM	1.185 ± 0.01*		5.80
30mM	1.375 ± 0.01*		22.77
50mM	1.760 ± 0.02*		57.14
70mM	1.970 ± 0.01*		75.89
100mM	2.180 ± 0.01*		94.64
200mM	2.345 ± 0.02*		109.36
Kunhutty			
0mM	0.720 ± 0.01	0.04	0.00
10mM	0.775 ± 0.01*		7.64
30mM	0.920 ± 0.01*		27.78
50mM	1.085 ± 0.01*		50.69
70mM	1.270 ± 0.01*		76.39
100mM	1.540 ± 0.02*		113.89
200mM	1.890 ± 0.02*		162.50
Veliyan			
0mM	0.930 ± 0.01	0.09	0.00
10mM	1.095 ± 0.01*		17.74
30mM	1.270 ± 0.01*		36.56
50mM	1.485 ± 0.01*		59.68
70mM	1.730 ± 0.01*		86.02
100mM	1.865 ± 0.01*		100.54
200mM	2.085 ± 0.01*		124.19

*shows significant variation at *P≤0.05*

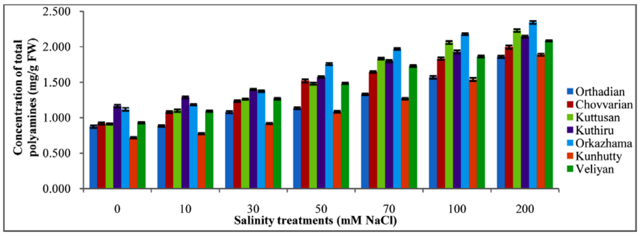

Fig. 1: Variation in the total polyamine content in different rice cultivars under salinity stress

Polyamines are low molecular weight natural compounds with aliphatic nitrogen structure and exist in almost all organisms from bacteria to plants and animals (Hussain *et al.*, 2011). For plant growth and development, polyamines are widely implicated in cell division and differentiation, root elongation, floral development, fruit ripening, leaf senescence, programmed cell death, DNA synthesis, gene transcription,protein translation and chromatin organization. Polyamines also play significant roles in almost all diverse environmental stresses including salt, drought, low and high temperature, wounding, ozone, flooding, heavy metal, acid and oxidative stresses (Galston *et al*., 1997; Bais and Ravishankar 2002).

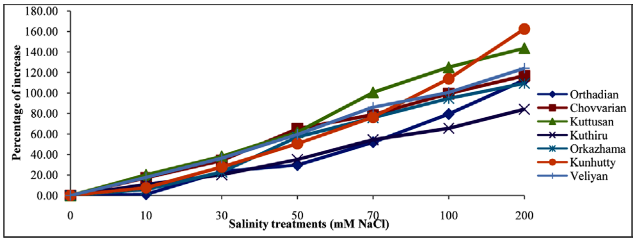

Fig. 2: Percentage of increase in the total polyamine content under different salinity levels

On one hand, polyamine biosynthetic and metabolic pathways, as well as polyamine levels, are modulated by multiple abiotic stresses (Zhang *et al*., 2011; Alet *et al*., 2012; Tavladoraki *et al*., 2012). Studies have shown that polyamine accumulation occurs under abiotic stresses including drought, salinity, extreme temperatures,oxidative stress,water logging, flooding, hypoxia, UV-B, heavy metals, ozone,osmotic stress, mechanical wounding and herbicide treatment (Pang *et al*., 2007; Groppa and Benavides 2008; Gill and Tuteja 2010; Alcázar *et al*., 2010). Hence, high cellular levels of polyamines correlate with plant tolerance in a wide array of environmental stresses. Our results showed accumulation of polyamines in the rice genotypes under salinity stress. Modulated levels of polyamines may act either asasignalora messenger to transmit the perceived signals from thesensors to articulate the plant's behavioral response in order to avoid or overcome stress (Igarashi and Kashiwagi 2000). Polyamines, besides respondingto external stimuli by their modulated titers, also alterion channels (Takahashi and Kakehi 2010), stimulate special kind of protein synthesis (Igarashi and Kashiwagi 2000), regulate abscisicacid and indole-3-acetic acid pathways which inturn enhances tolerance to metal toxicity (Choudhary *et al*., 2012a) and modulate levels of antioxidants like glutathione, ascorbic acid, proline, and glycine-betaine and antioxidant enzymeslike glutathione reductase, superoxide dismutase, catalase and peroxidase (Choudhary *et al*. 2012b) to impart stress tolerance. At cellular pH, polyamines behaveas cations interacting with anionic macromolecules such as DNA, RNA, phospholipids and proteins (Kumar *et al*. 1997)and are thoughtto stabilize membranes and scavenge free radicals (Velikova *et al*. 1998; Chang and Kao 1997). The exact component of saltstress that is responsible for polyamine accumulation is not very clear despite numerous reports elucidating this essential clue (Flores 1991; Tiburcio *et al*., 1997; Bouchereau *et al*., 1999; Capell *et al*., 2004; Zapata *et al*., 2004).

However, studies have shown that salt tolerant rice cultivars maintain a higher level of polyamines (Basu and Ghosh 1991; Krishnamurthy and Bhagwat 1989). In our results also the total poly amine level in the case of the cultivars from the saline rice tract was comparatively higher. It has been reported that salt tolerant rice cultivars were capable of maintaining high concentrations of polyamines like spermidine and spermine whereas sensitive cultivars were rich in putrescine (Krishnamurthy and Bhagwat 1989; Roy *et al*., 2005). Exogenous application of spermine and spermidine significantly prevented the leakage of electrolytes and amino acids from roots and shoots of rice subjected to salinity and a positive correlation between salt tolerance and accumulation of higher levels of polyamines was observed by Chattopadhyay *et al*. (2002). Mutlu and Bozcuk (2005) have also reported the potential role of polyamines in overcoming the adverse effects of salinity in plants. Mansour and Al-Mutawa (1999) have reported that the cellular alterations induced by NaCl in wheat roots were alleviated by the polyamines spermidine and spermine where as putrescine was ineffective. The role of polyamines in plant defence to water stress varies with polyamine forms and stress stages. According to Yang *et al*. (2007) in adapting to drought it would be good for rice to have higher levels of polyamines as well as early accumulation of free polyamines under water stress.

Conclusion

In conclusion it can be stated that accumulation of polyamines in response to saltstress as observed presently is an adaptation to cope up with the stress and accumulation of higher amount of polyamines along with other osmoprotectants makes the cultivars capable of performing well under moderate levels of salt stress. The rate of variation is cultivar specific. Identification of suitable genotypes and their improvement can be used as a potential method to evolve salinity resistant rice genotypes for different geographical zones.

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