RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2010.36.286

ScienceAsia 36 (2010): **286–291**

Changes of anthocyanin cyanidin-3-glucoside content and antioxidant activity in Thai rice varieties under salinity stress

Wipavadee Daiponmak[a,*], **Piyada Theerakulpisut**[b], **Pornthap Thanonkao**[c], **Apichart Vanavichit**[d], **Preecha Prathepha**[a]

[a] Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham 44000, Thailand
[b] Department of Biology, Faculty of Science, Khonkaen University, Khonkaen 40002, Thailand
[c] Department of Biotechnology, Faculty of Technology, Khonkaen University, Khonkaen 40002, Thailand
[d] Rice Science Centre, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

*Corresponding author, e-mail: wipavadee.d@msu.ac.th

Received 18 Dec 2009
Accepted 23 Oct 2010

ABSTRACT: This study was designed to determine the anthocyanin cyanidin-3-glucoside content and antioxidant activity of rice varieties under salinity stress. Two classes of Thai rice lines: cyanic (Riceberry, Kham, and Khamdoisaket) and acyanic (KDML 105, Sinlek, and BC2F7#62-56) were hydroponically grown. Seedlings were grown for 16 days and were supplied with salinity nutrient solution with 0 (control) and 60 mM NaCl for 11 days. The total phenolic content and the antioxidant activity, including 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging, ferric reducing antioxidant power, and lipid peroxidation were also determined by colorimetric assay. Anthocyanin pigment cyanidin-3-glucoside was quantified by high performance liquid chromatography. The results show that salinity stress in Khamdoisaket and KDML 105 cultivars significantly ($p < 0.05$) increased the total phenolic content, antioxidant activity, and cyanidin-3-glucoside content. Riceberry, Kham, and Sinlek cultivars, showed a moderate increase. The salt tolerant variety, BC2F7#62-56, showed only a slight but not significant increase in antioxidant activity and virtually unchanged lipid peroxidation, total phenolic content, and anthocyanin cyanidin-3-glucoside content under salinity stress. These results suggest that the increased total phenolic content, antioxidant activity, and anthocyanin cyanidin-3-glucoside content in rice seedling might be a protection mechanism against salinity stress. BC2F7#62-56 seems to have potential tolerance to salinity stress. This capability could be related to unchanged antioxidant activities, lipid peroxidation, total phenolic content, and anthocyanin cyanidin-3-glucoside content.

KEYWORDS: DPPH, FRAP, lipid peroxidation, Sinlek, Khamdoisaket

INTRODUCTION

High salt or soil salinity is a major environmental stress in the world's cultivated land that limits plant growth, metabolism, and productivity. About 20% of irrigated agricultural land is adversely affected by salinity[1,2]. Plant responses to salinity stress depend on the osmotic and toxic effects of salt and on the degree and duration of the stress[3]. The primary consequence of salinity stress is the ion imbalance and hyperosmotic stress in plants, which often lead to secondary stresses such as oxidative damage. Salinity stress in plants also leads to increased production of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide, and hydroxyl radicals[4]. These oxygen species cause oxidative damage to membrane lipids, proteins, and nucleic acids[5,6]. Plants use both enzymatic and non-enzymatic mechanisms to scavenge ROS and therefore help protect them from destructive oxidative reactions. Antioxidant systems of plants include enzymes such as superoxide dismutase, catalases, and ascorbate peroxidase and non-enzymatic components such as ascorbic acid, glutathione, phenolic compounds, and anthocyanins[7–9]. Anthocyanins are a group of natural colourants that belong to the family of flavonoids. Anthocyanin accumulation that occurs as a response to biotic and abiotic stress situations can be up to 40% in maize. In strawberry cultivars, the amount of anthocyanin and total phenolic compounds increases substantially under long-term salt stress[10]. Furthermore, soluble phenols, anthocyanins, and flavones accumulate in salt tolerant sugarcane[11]. Enhanced synthesis of secondary metabolites under stressful

conditions is believed to protect the cellular structures from oxidative damage[12]. In black rice, cyanidin-3-glucoside (Cy-3-Glc) has been reported to be one of the major antioxidant compounds[13]. Anthocyanin cyanidin-3-glucoside shows strong superoxide radical scavenging activity[14].

Although there have been several studies reporting salinity stress in various plants, little is known about the content of anthocyanin and antioxidant activity in rice with salt stress. We hypothesize that salinity stress could affect the amount of anthocyanin cyanidin-3-glucoside and antioxidant activity. Therefore, this study was designed to determine the anthocyanin cyanidin-3-glucoside content and antioxidant activity of six rice cultivars in response to salinity stress. Results from this study are fundamental for understanding the responsive adaptation to planting areas of soil salinity. This might be used in plant breeding to improve rice nutritional value with high anthocyanin.

MATERIAL AND METHODS

Chemicals

Gallic acid monohydrate (3,4,5-trihydroxybenzoic acid), Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 1,1,3,3-tetraethoxypropane (MDA), Kuromanin chloride (cyanidin 3-O-glucoside), trichloroacetic acid, and 2-thiobarbituric acid were purchased from Sigma (St. Louis, Mo., USA). Ferric chloride hexahydrate ($FeCl_3 \cdot 6\,H_2O$) and ferrous sulphate heptahydrate ($FeSO_4 \cdot 7\,H_2O$) were purchased from BDH Chemical (Dagenham, UK). 2,4,6-Tri(2-pyridyl)-s-triazine was purchased from Fluka (Deisenhofen, Germany).

Conditions of the plants after treatment of salinity stress

Two classes of Thai rice lines including cyanic (Riceberry, Kham, and Khamdoisaket) and acyanic (KDML 105, Sinlek, and BC2F7#62-56) were hydroponically grown. Methods of growing plants, salinization of nutrient solutions, and evaluation of salt stress symptoms were modified from those of Gregorio et al[15]. The experiment was conducted in a greenhouse. Rice seeds were heat-treated for 5 days in a convection oven set to 50 °C to break seed dormancy. Thereafter, the surface of the seeds was sterilized with 1.5% (w/v) calcium hypochlorite for 30 min and rinsed well with distilled water, imbibed for 24 h, and allowed to germinate over water-soaked sterile gauze in Petri dishes for 48 h. Two pregerminated seeds per hole were placed on a Styrofoam seedling float located above a 20 l rectangular plastic tray containing demineralized water for 3 days. When seedlings were well established, demineralized water was replaced by non-saline nutrient solutions[16]. The plants were hydroponically grown in a glasshouse for 11 days, during which the nutrient solution was renewed every 3 days. After seedlings had been growing for at total of 16 days, nutrient solution was replaced with nutrient solution containing 0 (control) and 60 mM of NaCl. They were then grown in the glasshouse under natural day/night light at a temperature of 30 ± 2 °C for a further 11 days. At the end of this time the leaves were collected, frozen in liquid nitrogen and stored at -70 °C.

Extraction of crude antioxidant

Two grams of leaves were extracted into 20 ml of methanol in a shaker at room temperature for 1 h. Subsequently, the extracts were filtered through a Whatman No. 1 filter paper. Organic solvent was removed by rotary evaporation at 40 °C in vacuo (Buchi Rotavapor R-200, USA) until dry. The dried extract was redissolved in 1 ml methanol and stored at -20 °C until analysis.

Total phenol content assay

Phenol content was determined using a modification of the Folin-Ciocalteu procedure[17]. Briefly, 0.5 ml of the crude extract was placed into test tubes, and then 2.5 ml of Folin-Ciocalteu's reagent was added. After 3 min, 2.0 ml of sodium carbonate (7% w/v) were added and the volume was made up to 10.0 ml by adding distilled water. The contents of the tubes were mixed and allowed to stand for 30 min. Absorbance at 765 nm was measured spectrophotometrically (GENESYS 20, USA). The total phenolic content was expressed as gallic acid equivalent in milligrams per gram of fresh weight.

Determination of antioxidant activities of DPPH free radical-scavenging assay

The leaves extraction and the DPPH radical scavenging activity were estimated according to the method of Cheung et al[18] and Choi et al[19]. Aliquots of 0.5 ml of 0.1 mM DPPH methanol were mixed with 0.5 ml of the crude extracts. The mixtures were vigorously shaken and left to stand for 30 min under subdued light. The absorbance at 517 nm was measured against water as a blank. The radical scavenging activity is measured as a decrease in the absorbance of DPPH and antioxidant capacity was expressed as Trolox equivalents (μmol/g fresh weight).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay[20] was carried out with some modifications. We mixed 300 mM acetate buffer, pH 3.6 with 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine and 20 mM $FeCl_3 \cdot 6\,H_2O$ in the ratio of 10:1:1 to give the working FRAP reagent. The FRAP reagent was freshly prepared as required. We mixed 3 ml of working FRAP reagent with 100 µl of Fe(II) standard (25–1000 µM of $FeSO_4 \cdot 7\,H_2O$) and 5 µl of crude extract in the test tube. This was then vortexed, left for 10 min at room temperature, and the absorbance at 593 nm was read. The antioxidant capacity was expressed as a FRAP value (µmol Fe^{2+}/g fresh weight).

Determination of lipid peroxidation

The lipid peroxidation in leaves was determined by measuring the amount of malondialdehyde (MDA) formation according to the thiobarbituric acid method[21]. The crude extract preparation was mixed with the same volume of 0.5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95 °C for 25 min and the reaction was stopped by quickly placing on ice. The cooled mixture was centrifuged at 10 000g for 10 min and the absorbance of the supernatant was read at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA concentration was determined from its extinction coefficient of 155 $mM^{-1}cm^{-1}$, and the concentration was expressed as mM/g fresh weight.

Quantification of anthocyanin cyanidin-3-glucoside content by HPLC

Anthocyanin in leaves of rice was extracted according to a method described in Ref. 22 using acidified methanol that provided the highest extraction efficiency[23,24]. A fresh leaves sample (0.1 g) was extracted in 2 ml of acidified (1% v/v HCl) methanol for 24 h at 4 °C with occasional shaking. The crude extracts were filtered through a 0.45 µm syringe filter prior to HPLC analysis. Anthocyanin was separated and quantified with a chromatograph (Shimadzu) equipped with a diode array detector. An Apollo-C18 column (5 µm; 4.6 × 250 mm, Agilent) was used at 40 °C. The mobile phase consisted of aqueous 5% formic acid (A) and 100% methanol (B). The flow rate was 0.6 ml/min, and detection for anthocyanin was set at 520 nm. The gradient applied for analysis was 90% A and 10% B at the separation time of injection (20 µl) of 20 min which was shifted to 35% B for 4 min and returned to the remainder of the run time. Total run time was 28 min. Cyanidin-3-glucoside injection at a concentration of 2–25 µg/ml was used as the standard.

Statistical analysis

Data were expressed as mean ± SD, based on three replications. The significance of differences between the mean values of control and salt-grown samples were statistically evaluated by a paired sample test using SPSS for Windows, version 12.0 at $p < 0.05$ level.

RESULTS AND DISCUSSION

Effect of salinity on the total phenolic content

The total phenolic content in the leaves of six *Oryza sativa* varieties subjected to 11-day salinity treatments are shown in Table 1. Khamdoisaket and KDML 105 showed a significant increase in accumulation of total phenolic content under salinity treatment, whereas the coloured line, Riceberry, and the colourless Sinlek showed only a moderate increase. A slight increase in total phenolic content was observed in Kham and the salt-tolerant BC2F7#62-56 with increasing salinization. Phenolic compounds are some of the most effective antioxidative constituents in edible and non-edible plant materials including fruits, vegetables, herbs, cereals, tree materials, plant sprouts, and seeds[25]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential[26]. The exposure of rice seedlings to salinity stress leads to an increase in total phenolic content in leaves of almost all genotypes. The results suggest that the increasing of total phenolic content in rice genotypes as a result of salt stress is believed to protect the plant from oxidative damage. Salinity stress-induced total phenolic content accumulation has also been previously observed in strawberry[10] and sugarcane[11].

Effect of salinity on antioxidant activities (DPPH and FRAP assays)

The antioxidant activities of crude methanolic extracts of the six varieties after a 11-day exposure to salinity were determined by DPPH and FRAP assays (Table 1). DPPH radical scavenging activity was significantly increased in KDML 105, Khamdoisaket, Sinlek, and Kham after salinity stress. Riceberry and BC2F7#62-56 did not significantly increase in DPPH radical scavenging activity. The FRAP value showed similar trends in DPPH radical scavenging activity in all the genotypes tested (Table 1). Riceberry, Khamdoisaket, Sinlek, KDML 105, and Kham showed significantly increased FRAP values with salinity stress

Table 1 Effect of salinity on six rice varieties.

Rice	Total phenolic content (mg Gallic/g fresh weight)		DPPH activity (μmol Trolox/g fresh weight)		FRAP activity (μmol Fe^{2+}/g fresh weight)		MDA content (mM/g fresh weight)		Cyanidin-3-glucoside content (mg/g fresh weight)	
	control	60 mM	control	60 mM	control	60 mM	control	60 mM	control	60 mM
RB	0.44 ± 0.05^{a}	0.72 ± 0.01^{a}	0.47 ± 0.04^{a}	0.58 ± 0.08^{a}	2.10 ± 0.27^{a}	3.72 ± 0.66^{b}	10.3 ± 0.4^{a}	16.8 ± 1.7^{b}	0.71 ± 0.01^{a}	1.03 ± 0.16^{a}
K	0.50 ± 0.02^{a}	0.56 ± 0.01^{a}	0.45 ± 0.02^{a}	0.68 ± 0.02^{b}	2.11 ± 0.43^{a}	3.40 ± 0.12^{b}	14.2 ± 2.5^{a}	34.8 ± 5.2^{b}	0.79 ± 0.43^{a}	1.35 ± 0.03^{a}
KD	0.83 ± 0.05^{a}	1.49 ± 0.02^{b}	0.62 ± 0.01^{a}	1.29 ± 0.25^{b}	5.80 ± 0.71^{a}	10.04 ± 2.12^{b}	11.7 ± 1.3^{a}	26.3 ± 1.1^{b}	1.67 ± 0.35^{a}	9.95 ± 0.41^{b}
KM	0.41 ± 0.04^{a}	0.73 ± 0.03^{b}	0.50 ± 0.07^{a}	0.80 ± 0.07^{b}	2.15 ± 0.32^{a}	3.87 ± 0.69^{b}	11.2 ± 1.4^{a}	22.5 ± 2.9^{b}	0.61 ± 0.16^{a}	1.31 ± 0.18^{b}
SL	0.50 ± 0.08^{a}	0.73 ± 0.09^{a}	0.48 ± 0.05^{a}	0.71 ± 0.01^{b}	2.00 ± 0.29^{a}	3.66 ± 0.02^{b}	9.9 ± 2.1^{a}	17.5 ± 3.1^{b}	0.69 ± 0.05^{a}	1.02 ± 0.07^{a}
BC	0.55 ± 0.04^{a}	0.57 ± 0.01^{a}	0.50 ± 0.06^{a}	0.63 ± 0.01^{a}	2.08 ± 1.01^{a}	2.87 ± 0.22^{a}	19.1 ± 1.5^{a}	17.2 ± 4.1^{a}	0.77 ± 0.18^{a}	0.77 ± 0.08^{a}

Values are expressed as means $\pm$ SD of triplicate measurements. Statistical differences at $p < 0.05$ have been calculated for each t test. Means with different letters are significantly different. (RB = Riceberry, K = Kham, KD = Khamdoisaket, KM = KDML 105, SL = Sinlek, BC = BC2F7#62-56).

but BC2F7#62-56 did not. The stable DPPH radical is widely used to evaluate the free radical scavenging activity of hydrogen-donating antioxidants in several plant extracts[27]. The FRAP assay is a direct test of total antioxidant power. Electron-donating antioxidants can be described as reductants, and inactivation of oxidants by reductants can be described as a redox reaction[28]. Our present results showed that the DPPH and FRAP radical scavenging activities of salt stress significantly increased in leaves of almost all varieties except the salt-tolerant BC2F7#62-56. The observation of hydrogen donors capable of scavenging DPPH or electron donors of FRAP radicals accumulation of rice varieties after salinity stress suggested that this may be a possible mechanism for their antioxidant activities.

Effect of salinity on lipid peroxidation

The effect of increasing salinity stress on MDA formation in the leaves of the six rice varieties after 11 days of salinity treatment is shown in Table 1. With an increasing level of salinity stress, the MDA content increased in Riceberry, Kham, Khamdoisaket, KDML 105, and Sinlek, indicating a significant increase in lipid peroxidation. The salt-tolerant BC2F7#62-56 did not exhibit this increase in lipid peroxidation. MDA is regarded as a maker for the evaluation of lipid peroxidation or the damaging of polyunsaturated fatty acid in the membrane with increased salinity stress. Salt-stress induced both free radical formation and lipid-membrane peroxidation in leaves of almost all varieties except the salt-tolerant BC2F7#62-56.

Effect of salinity on anthocyanin cyanidin-3-glucoside content

A typical of chromatogram of cyanidin-3-glucoside is shown in Fig. 1. The UV spectrum of the extract compounds derived from HPLC and pure standard showed a peak at the retention time of 4.31 min. The

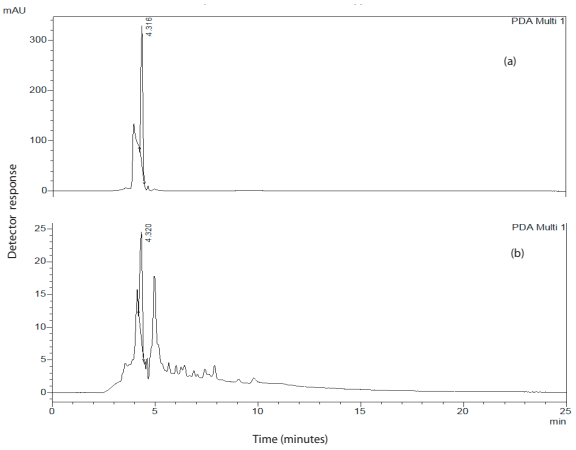

Fig. 1 HPLC chromatogram (520 nm) for (a) cyanidin-3-glucoside standard (b) the leaf extract compounds.

anthocyanin cyanidin-3-glucoside content of Khamdoisaket and KDML105 increased significantly after salt treatment (Table 1). A slight increase in anthocyanin cyanidin-3-glucoside content was observed in Riceberry, Kham and Sinlek, whereas anthocyanin cyanidin-3-glucoside content was unchanged in BC2F7#62-56. For anthocyanin extraction, the acidified methanol had the highest extraction efficiency[23,24]. Cyanidin-3-glucoside is the most common anthocyanidin (aglycone). These results suggest that anthocyanin is an important flavonoid as a modulator of salinity stress and plays an important role in the prevention of stress-induced oxidative damage in leaves of almost all rice varieties except for the salt-tolerant BC2F7#62-56. These results were supported by Eryılmaz[29], reporting that there was a correlation in the increases of anthocyanins with salt stress in tomato and red cabbage seedlings.

Salt-tolerant varieties have been found to be less affected by free radical formation and lipid peroxida-

tion that disrupt the membrane integrity[7,30]. Rice-tolerant varieties accumulated less anthocyanin than susceptible varieties under salt stress[31]. Based on antioxidant activities, lipid peroxidation, and anthocyanin content measured in our studies, BC2F7#62-56 was able to cope with salinity stress. This capability could be related to the unchanged antioxidant activities, lipid peroxidation, total phenolic content, and anthocyanin cyanidin-3-glucoside content.

In conclusion, this investigation suggests that the increased synthesis of total phenolic content, the antioxidant activity, and anthocyanin cyanidin-3-glucoside content in rice seedlings exhibited a protective mechanism against the cellular structures from oxidative damage. BC2F7#62-56 seems to have a potential tolerance to salinity stress. Further research on the expression of genes involved in anthocyanin biosynthesis is necessary to investigate the molecular mechanism of the response to salinity stress response in rice.

Acknowledgements: We wish to thank Pathumthani Rice Research Institute and Rice Science Centre, Kasetsart University, Kamphaeng Saen Campus and the Department of Agronomy, Faculty of Agriculture, Chiang Mai University for the supply of rice seeds. This work was supported by the Ph.D. programme in Biodiversity at Walai Rukhavej Botanical Research Institute, Mahasarakham University and the TRF/BIOTEC Special Programme for Biodiversity Research and Training Grant.

REFERENCES

1. Barrett-Lennard EG (2000) Salt of the Earth: time to take it seriously. In: Cadman H (ed) *The Food and Environment Tightrope*, Australian Centre for International Agricultural Research, Canberra pp 135–45.
2. Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants — where next? *Aust J Plant Physiol* **22**, 875–84.
3. Hasegawa P, Bresson A, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annu Rev Plant Physiol Plant Mol Biol* **51**, 463–99.
4. Halliwell B, Gutteridge JMC (1986) *Free Radicals in Biology and Medicine*, Oxford Univ Press, London.
5. Davies KJA (1987) Protein damage and degradation by oxygen radicals. *J Biol Chem* **262**, 9895–901.
6. Fridovich I (1986) Biological effects of the superoxide radical. *Arch Biochem Biophys* **247**, 1–11.
7. Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Sci* **135**, 1–9.
8. Lee DH, Kim YS, Lee CB (2001) The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Plant Physiol* **158**, 737–45.
9. Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol* **70**, 1–9.
10. Keutgen AJ, Pawelizik E (2008) Quality and nutritional value of strawberry fruit under long term salt stress. *Food Chem* **107**, 1413–20.
11. Wahid A, Ghazanfar A (2006) Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *Plant Physiol* **163**, 723–30.
12. Buchanan BB, Gruissem W, Jones R (2000) *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists, Maryland.
13. Ryu SN, Park SZ, Ho CT (1998) High performance liquid chromatographic determination of anthocyanin pigments in some variety of black rice. *J Food Drug Anal* **6**, 729–36.
14. Ichikawa H, Ichiyanagi T, Xu B, Yoshii Y, Nakajima M, Konishi T (2001) Antioxidant Activity of Anthocyanin Extract from Purple Black Rice. *J Med Food* **4**, 211–8.
15. Gregorio GB, Dharmawansa S, Mendoza RD (1997) Screening rice for salinity tolerance. IRRI Discussion Paper Series No. 22, International Rice Research Institute, Manila, pp 1–30.
16. Yoshida S (1981) *Fundamentals of Rice Crop Science*, International Rice Research Institute, Los Baños.
17. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphotungstic acid reagents. *Am J Enol Viticult* **16**, 2349–51.
18. Cheung LM, Cheung PCK, Ooi VEC (2003) Antioxidant activity and total polyphenolics of edible mushroom extracts. *Food Chem* **81**, 249–55.
19. Choi Y, Jeong HS, Lee J (2007) Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem* **81**, 249–55.
20. Benzie IF, Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth Enzymol* **299**, 15–27.
21. Stewart RC, Bewley JD (1980) Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol* **65**, 245–8.
22. Ithai N, Reddy AR (2004) Rice flavonoid pathway genes, *OsDfr* and *OsAns*, are induced by dehydration, high salt and ABA, and contain stress responsive promoter elements that interact with the transcription activator, OsC1-MYB. *Plant Sci* **166**, 1505–13.
23. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–47.
24. Naczk M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharmaceut Biomed Anal* **41**, 1523–42.
25. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999) Antioxidant Activity of Plant Extracts Containing Phenolic Compounds.

J Agr Food Chem **47**, 3954–62.
26. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995) The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radic Res* **22**, 375–83.
27. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Tech* **28**, 25–30.
28. Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agr Food Chem* **53**, 4290–302.
29. Eryılmaz F (2006) The relationships between salt stress and anthocyanin content in higher plants. *Biotechnol Biotechnol Equip* **20**, 47–52.
30. Sairam RK, Srivastava GC (2002) Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Sci* **162**, 897–904.
31. Djanaguiraman M, Ramadass R, Devi DD (2003) Effect of salt stress on germination and seedling growth in rice genotypes. *Madras Agr J* **90**, 50–3.