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Oxidative stress and changes in antioxidant and biochemical constituents in papaya (*Carica papaya* L.) under salt stress

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ABSTRACT

Six papaya cultivars viz., Pusa Dwarf, Surya, Solo, CO5, Tainan and Red Lady were subjected to saline water salt stress continuously for a period of six months with saline water irrigation having an EC value of 0.6, 2.0 and 4 dsm^{-1}. Among these, Red Lady was more sensitive while Tainan resisted salt stress. Under salt stress of 4 dsm^{-1}, yield reduced by 10% in Tainan and by 24% in Red Lady compared to unstressed controls. T.S.S. measurement showed that quality of fruits was not affected by saline irrigation in both cvs. Malondialdehyde levels estimated after six months period of stress, as thiobarbituric acid reacting substances, did not increase in Tainan in contrast to substantial increase in Red Lady under stress conditions. There was substantial increase in levels of antioxidant compounds namely, carotenoids, phenols and flavonoids in Tainan compared to Red Lady. In Tainan there were significant increases in reducing and total sugars and sucrose under conditions of stress in contrast to sharp decreases in Red Lady. Under conditions of stress, there was considerable accumulation of total and reducing sugars and sucrose, across the varieties, possibly contributing to osmotic adjustment. Association of salt stress tolerance in Tainan with soluble sugar accumulation could be used as a breeding tool for selecting salt tolerant papaya genotypes.

Key words : Oxidative stress, antioxidants, salt stress, *Carica papaya*

INTRODUCTION

Salinity is a major abiotic stress adversely affecting productivity and quality. Papaya (*Carica papaya* L.) is next only to mango as a rich source of pro-vitamin A (Subhas Chander and Rao, 2004). Growth of certain papaya cultivars under salt stress and some biochemical parameters associated with salt stress tolerance were studied and the results are reported.

MATERIAL AND METHODS

Six papaya cultivars, viz., Pusa Dwarf, Surya, Solo, CO5, Tainan and Red Lady were subjected to salt stress continuously for six months with saline water irrigation having EC value of 0.6, 2.0 and 4.0 dsm^{-1} during the year 2004-05. Among the six varieties, cv. Red Lady was more sensitive to salt while cv. Tainan was resistant to salt stress by excluding the sodium cation from the plant system. On this basis, these two cultivars were selected for further biochemical analysis.

Sixth leaf from the top of the tree in these two cultivars (Red lady and Tainan) was taken for biochemical analysis after 20 saline irrigations imposed at intervals of 10 days. The cleaned samples were cut into 0.5 cm squares, mixed thoroughly and dried at 60°C in an oven. Dried samples were powdered in a mixer and stored for biochemical analysis. Estimation was done on a duplicate set of samples. Oxidative stress in the samples due to salt stress was measured as a change in malondialdehyde content, estimated at six months of stress, as thiobarbituric acid reacting substances (TBARS) as described by Egert and Tevini (2002). Five hundred mg of control and stressed samples was extracted with 10 ml of a mixture of 10 ml 5% aqueous TCA and 1 ml of 0.05% methanolic BHT. The homogenate was centrifuged and 2 ml of supernatant was mixed with 4 ml of saturated solution of TBA. The mixture was heated in a boiling water for bath 30 min, cooled and centrifuged and TBARS measured at 532 nm in a spectrophotometer. 0.5 g control and stressed samples of both cultivars were repeatedly extracted with AR grade acetone, filtered and combined and made upto 100ml. The acetone extracts were directly used for estimation of total

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carotenoids as described by Egert and Tevini (2002). Similarly 80% ethanol extracts of the samples were prepared and 50 ml portions of those extracts were defatted by extraction with hexane thrice. Total phenols were estimated in defatted extracts as per the method described by Sadasivam and Manickam (1996). Flavonoids were estimated in the same extracts by the method of Kim *et al* (2003). The undefatted 80% alcohol extracts were used to estimate soluble carbohydrates. Reducing sugars and total sugars after inversion and, sucrose specifically, were estimated as described by Ashwell (1957).

RESULTS AND DISCUSSION

The response of cvs. Tainan and Red Lady to saline water irrigation is shown in Table 1. Average fruit weight was 1.3 kg in 'Red Lady' and 1.4 kg in 'Tainan'. The yield reduced by 10% in cv. Tainan and 24% in cv. Red Lady, when salt stress was 4.0 dsm^{-1}, compared to the unstressed control. However, quality of fruit was not affected by saline irrigation in both the cvs. as evidenced from TSS data. The yield parameters and quality in both cvs. did not differ significantly from control when salt stress was 2 dsm^{-1}. CV values reveal considerable variation in yield and number of fruits under salinity, particularly in Red Lady.

Exposure of plants to excessive levels of salts results in increased production of reactive oxygen species (ROS) in plants. ROS include the superoxide radical (O^-_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen, which come from endogenous sources as byproducts of normal and essential reactions such as energy generation in mitochondria and detoxification reactions. (Harinasut *et al*, 2003).

Excess levels of ROS are the initiators of a chain reaction that leads to degradation of cellular components. Damage is brought about by the oxidation of photosynthetic pigments, membrane lipids, proteins and nucleic acids by ROS. This state of damage caused by ROS is denoted by the term oxidative stress. One major characteristic of oxidative stress is increased lipid peroxidation wherein the polyunsaturated fatty acids (PUFA) in the plant cells are oxidized. The end product of PUFA oxidation is malondialdehyde (MDA). MDA estimation serves as a measure of the degree of oxidative stress experienced by the tissue (Hodges and Forney 2000).

MDA estimation in cv. Red lady at two levels of stress (Table 2) revealed an increase of 3.5% and 44.8% MDA over the control. This was in accordance with the salt sensitive trait of the cv. Red Lady observed in the field. In contrast, there was no change in MDA content in cv. Tainan subjected to the same degree of stress. Thus, there was practically no oxidative stress in the plants indicative of the salt tolerant trait of the variety. MDA levels of tissues

Table 1. Response of papaya to saline water irrigation

Sl. No.	Parameter	cv. Tainan Control (0.6 dsm^{-1})	cv. Tainan Saline treatment (2.0 dsm^{-1})	cv. Tainan Saline treatment (4.0 dsm^{-1})	cv. Red Lady Control (0.6 dsm^{-1})	cv. Red Lady Saline treatment (2.0 dsm^{-1})	cv. Red Lady Saline treatment (4.0dsm^{-1})	C.D. (*P=0.05%*)	CV %
1	Yield (tonnes/ha)	60	58	54	55	49	42	2.95	27.0
2	Average fruit weight (kg/fruit)	1.40	1.40	1.30	1.30	1.20	1.15	0.15	14.5
3	Number of fruits	45	44	40	39	37	30	3.90	26.3
4	T.S.S.	12.3	11.3	11.9	13.8	14.0	14.0	0.70	13.5

Table 2. Oxidative stress and antioxidant compounds in two papaya cvs. Red Lady and Tainan, susceptible and tolerant respectively to salinity stress

Sl. No.	Parameter	cv. Tainan Control	cv. Tainan T1 (Saliniy 2 dsm^{-1})	cv. Tainan T2 salinity 4 dsm^{-1})	cv. Red Lady Control	cv. Red Lady T1 (Salinity 2 dsm^{-1})	cv. Red Lady T2 (salinity 4 dsm^{-1})
1	Oxidative stress/ Malondialdehyde (MDA, in terms of A_{532}/40 mg dry leaf powder)	0.143	0.140	0.145	0.116	0.120 (+3.5%)	0.168 (+44.8%)
2	Total carotenoids (mg/g dry leaf)	65.510	85.96 (+31.2%)	75.59 (+15.4%)	68.920	69.900 (+ 1.3%)	56.280 (-18.3%)
3	Total phenols (mg gallic acid/g dry leaf)	28.930	39.73 (+ 37.3%)	32.07 (+ 10.9%)	28.200	31.270 (+ 10.9%)	21.670 (-23.2%)
4	Total flavonoids (mg catechin/g dry leaf)	6.730	10.14 (+50.7%)	7.59 (+12.8%)	6.910	7.670 (+ 11%)	4.730 (-31.6%)

also served inversely as a measure of cellular membrane integrity (Basra *et al*, 1997).

Plants contain antioxidant compounds which play an important role in detoxifying and regulating levels of ROS. These include carotenoids, ascorbic acid, glutathione, α-tocopherol, phenols and flavonoids (Harinasut *et al*, 2003). Under conditions of various types of stress, plants protect themselves by synthesis of increased levels of various antioxidant compounds (Mandhania *et al*, 2006). Carotenoids are important as antioxidant compounds. They protect chloroplasts against photosensitized oxidation by quenching singlet oxygen, i.e., they function as radical scavengers, effectively binding the ROS and preventing cellular damage (Bosland and Votava, 2000). Results in Table 2 show increased formation of carotenoids (+ 31.2% to 15.4% over control) under conditions of salinity stress in papaya cv. Tainan as compared to cv. Red Lady. The increased carotenoid concentration under of salt stress in Tainan could be a contributory factor for the tolerant trait of the variety. A drought tolerant wheat genotype under water stress, similarly, had the highest carotenoid content (Sairam and Saxena, 2000). Decrease in carotenoid concentration in cv. Red Lady under stress is indicative of increased oxidative stress, possibly contributing to the salt sensitive nature of the cultivar.

Phenolic and flavonoid antioxidants act by free radical scavenging (Subhas Chander and Rao, 2004). Tomato lines having a high level of polyphenols had the most powerful antioxidant potential (Minaggio *et al*, 2003). Results presented in Table 2 show that in papaya cv. Tainan, there was an increase of 37.3% and 10.9% in total phenol content of samples from salt stress of 2 dsm^{-1} and 4 dsm^{-1}, respectively, over the content in unstressed control. In cv. Red Lady, the increase was only 10.9% over control under low salt stress of 2 dsm^{-1}, and, it decreased by 23.2% under high stress of 4 dsm^{-1}. In view of the stress tolerance shown by cv. Tainan under field conditions, increased total phenolic content under stress in this case could be one of the detoxification systems that the plant has developed to limit oxidative damage due to excess formation of ROS, by radical scavenging, which is considered crucial for tolerance (Sarad *et al*, 2004).

Flavonoids are low molecular weight, polyphenolic compounds found in plants. Recent studies provide evidence that accumulation of antioxidant compounds such as flavonoids is one component of a whole set of antioxidant defenses, which help plants to withstand environmental stress (Munne-Bosch, 2005). The cv. Tainan contained 50.7% and 12.8% more flavonoids over control under salt stress of 2.0 dsm^{-1} and 4 dsm^{-1} respectively (Table 2). The corresponding increase in the cv. Red Lady was only +11% over control under 2 dsm^{-1} salt stress and under higher salt stress of 4 dsm^{-1}, the flavonoid content decreased by 31.6%. Thus flavonoids accumulation in the cv. Tainan could be contributing to the salt tolerance by free radical scavenging.

There was ‘considerable’ to ‘substantial’ accumulation of sugars in both the cultivars under stress conditions. There were also some sharp differences. Across the varieties there was an accumulation of 26.1% more reducing sugars in 2 dsm^{-1} stressed samples, compared to the control. Also, across varieties, total sugars increased by 12.1% and 10.4% and sucrose increased by 10.2% and 19.5% over the control under 2 dsm^{-1} and 4 dsm^{-1} stress conditions, respectively (Table 3). Thus, salt stress is associated in general with higher sugar levels, more specifically sucrose content.

In cv. Tainan, there was a significant increase of (i) 44.8% and 78% reducing sugars (ii) 15.6% and 45.2% total sugars and (iii) 18.9% and 21.5% sucrose under 2 dsm-

Table 3. Sugar accumulation in salt stressed papaya across varieties

Sl. No.	Treatment	Sugar level (mg/g dry leaf powder) Reducing sugars	Total sugars	Sucrose
1	Control	19.06	37.68	51.76
2	T1 (2 dsm^{-1})	24.04	42.24	57.02
3	T2 (4 dsm^{-1})	14.90	41.58	61.84
4	C.D. (*P=0.05*)	1.93	1.19	4.37
5	CV%	5.76	1.70	4.45

Table 4. Sugar accumulation in salt stressed papaya cvs. Red Lady and Tainan

Sl. No.	Parameter	cv. Tainan Control	T1 (Salinity 2 dsm^{-1})	T2 (salinity 4 dsm^{-1})	cv. Red Lady Control	T1(Salinity 2 dsm^{-1})	T2 (salinity 4 dsm^{-1})	C.D. (*P=0.05*)	CV%
1	Reducing sugars (mg/g dry leaf)	10.24	14.83	18.23	27.88	33.24	11.57	2.73	5.76
2	Total sugars (mg/g dry leaf)	33.93	39.24	49.25	41.43	45.23	33.91	1.69	1.70
3	Sucrose (mg/g dry leaf)	52.01	61.85	63.18	51.51	52.18	60.51	6.19	4.45

[1] and 4 dsm^{-1} stress conditions, respectively, over control (Table 4). In contrast, in cv. Red Lady, lesser increase of 19.2% more reducing sugars, 9.2% more total sugars and 1.3% more sucrose over control was observed under 2 dsm^{-1} stress, and, under higher salinity of 4 dsm^{-1}, there was 58.5% decrease in reducing sugars and 18.2% decrease in total sugars, compared to the control. There was a significant increase of 7.8% in sucrose content in cv. Tainan, compared to cv. Red Lady across treatments. CV% values reveal variation in reducing sugar and sucrose content under salinity, both across varieties and between cvs. Tainan and Red Lady.

Thus, there was significant and substantial accumulation of reducing and total sugars and sucrose levels in salt stressed cv. Tainan in contrast to lesser increase or sharp decrease in similarly stressed cv. Red Lady. Thus, cv. Tainan, which resisted salt stress in the field, is associated with increased soluble sugar accumulation. Salt stress resulted in increase in sucrose content in tomato significantly (Ko *et al*, 1999). This has been shown to be due to increased sucrose phosphate synthase (SPS) gene expression under conditions of salt stress. Soluble carbohydrates have a potential role in adaptation to drought and salt stress and, sucrose is believed to be instrumental in maintaining membrane phospholipids in the liquid-crystalline phase and in preventing structural changes in soluble proteins (Kerepesi and Galiba, 2000).

The accumulation of sugars observed is in accordance with the role ascribed to such accumulated solutes in contributing to osmotic adjustment under conditions of stress, leading to maintenance of water uptake and cell turgor and removal of free radicals and stablization of macromolecules, organelles and membranes (Neto *et al*, 2004). Drought and salt tolerant genotypes of wheat accumulated more soluble carbohyderate than did sensitive ones (Kerepesi and Galiba, 2000). Both ionic and nonionic stresses increased the concentration of reducing sugars, sucrose and fructans. Under salt stress conditions, salt tolerant cultivars accumulated soluble carbohydrate fructan, which decreased in salt sensitive cultivars. Kerepesi and Galiba (2000) conclude that water soluble carbohydrates content could be a useful marker for selecting genotypes that are more drought or salt-tolerant. The reducing and total sugar and sucrose content in papaya cultivars Red Lady and Tainan show the same trend (Table 4). As in the case of wheat, soluble sugar content increased in the tolerant cv. Tainan, as against much lesser increase and very considerable decrease in the sensitive cv. Red Lady. Thus, this information on soluble sugar accumulation could be useful as a breeding tool for selecting salt-tolerant papaya genotypes.

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