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Antioxidant Potential of Some Mango (*Mangifera indica* L.) Cultivars Growing under Salinity Stress

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THE CURRENT study was conducted during season 2013 on one-year-old mango seedlings of Golek, Misk, Hindi Besinnara, Sukkary and Zebda cvs. to investigate the activities of both enzymatic and non-enzymatic components of the antioxidant system in mango under salinity stress. Plants were subjected to three levels of salinity (0, 1000 and 2000 mg L^{-1}). The activities of peroxidase, (POD), catalase, (CAT), polyphenyl oxidase (PPO), membrane stability index were determined and leaf content of reduced glutathione, chlorophyll, ascorbate and total sugars were determined. Activities of antioxidant enzymes, membrane stability index, ascorbate and reduced glutathione contents were significantly altered by salinity treatments. Leaves of salt-stressed plant exhibited a greater activity of peroxidase and polyphenyl oxidase while reduced activity of catalase. Reduced glutathione and membrane stability index increased by increasing salinity level. Salinity effect was less evident on chlorophyll and total sugar content. The differences between the studied cultivars were evident in most of the studied parameters. Zebda cultivar showed a higher antioxidant potential which provide a resistance mechanism agent salinity stress.

Keywords: Mango, Salinity, Antioxidant enzymes, Membrane stability index, Ascorbate, Reduced glutathione

Salinity is one of the major environmental stresses limiting plant growth and productivity. Saline soils occupy 7% of the land surface and salinization of the cultivated lands will results in 50% land loss by the middle of the 21th century (Wang *et al.,* 2003). Mango considered as very sensitive to soil salinity (Abo-Rekab Zeinab, 2014). In addition, the scarcity of water and the increased world population necessitates the use of high salinity water in agriculture (Chartzoulakis, 2005). The adverse effect of salinity on plant growth results from the increased osmatic potential of soil solution and the toxic effects of some elements (Yamaguchi and Blumwald, 2005). In addition, salinity stress induces oxidative stress through generation of reactive oxygen species (Khanna-Chopra and Selote, 2007), ROS such as supperoxidase radicals, hydroxyl radicals and hydrogen peroxide (Mittler, 2002). The ROS cause severe damage to the plant cell through peroxidation of membrane lipids, denaturation of proteins and cellular oxidative damage (De Azevedo Neto *et al.,* 2006). Recent studies showed that salinity tolerance in plant may be due to their resistance to oxidative stress (Gueta-Dahan *et al.,* 1997). The exposure of plants to environmental

stress, genotypes with high level of antioxidants is more resistance against the oxidative damage (Parida *et al.*, 2004). A correlation between salinity stress and efficiency of antioxidant defense system was reported in various crop species such as rice (Demirel, T. and Turkan, I. 2005) beet (Bor *et al.*, 2003), barley (Perez-Lopez *et al.*, 2010) and tomatoes (Dogan *et al.*, 2011). The plant defense mechanism including antioxidant enzymes such as catalase, peroxidase, glutathione reductase, and polyphenol oxidase also, a non-enzymatic antioxidant and a cellular osmoregulation are involved (Niknam *et al.*, 2003, Agarwal & Pandey, 2004, Demir & Kocaliskan, 2001 and Asada, 2006). The non-enzymatic phyto-chemical involved in salinity resistance including sugars which act as osmolytes (Moghaieb *et al.*, 2004) and the phenolic and flavonoid compound which correlated with the antioxidant activity. Because of genotype differ in their response to salinity, selection of high salinity tolerance genotype is a potential solution for the salinity problem (Ashraf and Harris, 2004).The main objective of the current study is to select tolerant mango genotypes to salinity conditions.

Materials and Methods

The present study was carried out during 2013 season at the nursery and laboratory of pomology department, faculty of agriculture, Cairo University.

Plant material and treatments

Seeds of the poly-embryonic mango cultivars namely Golek, Misk, Sukkary, Zebda and Hindi Besinnara cvs. were sown in July 2012 after extraction from the endocarp and washing twice in tap water. Germinated seedlings were allowed to grow under greenhouse conditions, two months later the sexual seedling were removed and nucellar seedlings were transplanted into polyethylene black bags filled with sand and beat moss (3:1). The seedlings were left under greenhouse conditions until March 2013.Two hundred seventy plant of each tested cultivar was selected and divided into three groups for three salt treatments, each treatment included 90 plants divided into three replicates (30 plants per each replicate). The seedling was treated for 90 days with three levels of salinity (0, 1000 and 2000 mg L^{-1}) the salt treatments were added with nutrient solutions consisting of 20% of Hoagland solution strength.

Plant sample preparation

The plant samples (weighting 0.1-0.4 g) were stored at -20 ºC, and then processed as described by Ni *et al.* (2001). Briefly, the enzymes from the frozen plant samples were extracted using cold potassium phosphate buffer (0.1M, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone and 1% (v/v) Triton X-100. The samples were macerated with 1 ml of the extracting buffer. Samples were further ground with another 1 ml of extracting buffer. In total, 2ml of the extracting buffer was used for each sample. An aliquot (1.5ml) of the extract was centrifuged at 10000g for 10 min at 4 ºC. the supernatant was frozen for future enzyme activity assays.

Peroxidase activity

Peroxidase was determined according to Vetter *et al*. (1958). A sample of (200 µl), in which the color is to be formed, the following reagents are added. 1ml of 1% o-phenylenediamine (in 95% ethyl alcohol, fresh every 4 hours) and 1 ml of 0.3% hydrogen peroxide (in distilled water). The reaction is allowed to proceed for 5 min at which time it is stopped by adding 2 ml of saturated sodium bisulfate. The reagent blank for each sample is prepared by adding the dye, followed by the sulphite, and then the hydrogen peroxide. The enzyme is inhibited by the sulfite so that it is inactive when the hydrogen peroxide is added. The starch in the sample and the blank is flocculated by adding 25ml of 95% ethyl alcohol. The starch suspension must be swirled continuously during addition of alcohol, so that good flocculation occurs. The samples are then centrifuged at approximately 3000 r.p.m. for 5 minutes. The clear supernatant is decanted into a colorimeter tube and its absorbance recorded at 430 mµ. The colorimeter is set at 100% transmittance with the corresponding blank for each sample. The enzyme activity was expressed as the change in absorbency at 430 m (ΔOD_{430})/ minute/g fresh weight.

Catalase activity

Catalase activity was measured using biodiagnostic Kit No. CA 2517 which is based on the spectrophotometric methods described by Aebi (1984). Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min by catalase inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-Dichhloro-2 hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

Polyphenyl oxidase activity

Polyphenyl oxidase activity was determined according to a modification of Ishaaya (1971), in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, pH 7), 200 µl enzyme solution and 200 µl catechol solution (2%). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25ºC). Enzyme reaction was initiated by adding catechol solution. Then after exactly1 min, the optical density was determined. Zero adjustment was against sample blank. The polyphenol oxidase activity was determined as O.D. units $x10^3$ at absorption of 405 nm.

Glutathione reduced (GSH)

The assay of glutathione reduced level was performed using biodiagnostic Kit No. GR 25 11 which is based on the method of Duron and Kelly (1963). It depends on the reduction of 5,5-diothiobis 2-nitrobenzoic acid with glutathione to produce a yellow color whose absorbance is measured at 405 nm.

Determination of ascorbate content

For the extraction and estimation of ascorbate, the method of Oser (1979) was used. Tissue was ground in 5% (w/v) metaphosphoric acid, centrifuged at 10,000g for 10 min and the supernatant was used for estimation of ascorbate. The reaction mixture consisted of 2% Na-molybdate, 0.15 N H_2SO4, 1.5 mM Na_2HPO_4 and tissue extract. It was mixed and incubated at 60ºC in water bath for 40 min, cooled, centrifuged at 3,000g for 10 min and absorbance was measured at 660 nm.

Membrane stability index (MSI)

Root tissue (100 mg) was cut into small pieces and immersed in 10 mL of distilled water. Conductivity of the solvent was measured after incubating the tubes at 40 °C for 30 min (C1) and at 100 °C for 10 min (C2). MSI was calculated by the equation:

$MSI = [1 – (C1/C2)] \times 100$ (Shairam and Srivastava, 2002).

Total sugars and Chlorophyll content

Total sugars were colorimetrically estimated using phenol-sulphoric acid method (Dubios *et al.*, 1956) and total Chlorophyll was measured by SPAD meter (Minolta- SPAD- 502 made in Japan) the results were expressed as APAD units.

Experimental design and data analysis

The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using MSTAT-C statistical package (Freed *et al*., 1990) software, and means of the treatments were compared by Least Significant Difference (L.S.D.) according to Duncan (1955) at significance level of 0.05.

Results and Discussions

Catalyase Activity

The effect of salinity treatments on catalase activity in leaves of the studied mango cultivars as shown in Table 1 indicated that salinity treatments reduce the activity of catalase compared with the control treatment. The reduction of catalase activity was obvious in all the studied mango cultivars except Zebda cv. which maintains high activity of the enzyme regardless the salinity level. This may be attributed to the salinity resistance of Zebda cv.

TABLE 1. Effect of different salinity treatments on catalase activity (U/mg fresh weight) of the studied mango cvs.

Cultivars	Salinity			Mean
	0	1000	2000	
Golek	74.00 ab	61.39 de	53.87 fg	63.08 C
Zebda	78.31 a	78.20 a	79.57 a	78.69 A
Sukkary	77.61 a	65.17 cd	49.75 g	64.18 BC
Misk	76.52 a	75.84 a	57.27 ef	69.88 B
Hindi Besinnara	75.43 a	67.75 bc	56.43 ef	66.53 BC
Mean	76.37 A	69.67 B	59.37 C	

The effect of salinity on catalase activity was reported previously (Luo & Liu, 2011, Parida & Jha, 2010 and De Azevedo Neto *et al*., 2006). Catalase eliminate the H_2O_2 by breaking it into H_2O and O_2 (Sharma and Dubey 2005).

Peroxdase activity

Data showed in Table 2 showed the effect of salinity treatments on peroxidase activity (POD), the enzyme activity was rapidly increased with increasing salinity concentration, the peroxidase activity was higher in Zebda cv. compared with the other cultivars regardless the salinity level. Hindi Besinnara cv. recorded the lowest level of peroxidase activity.

TABLE 2. Effect of different salinity treatments on peroxidase activity (U/mg fresh weight) of the studied mango cvs.

Cultivars	Salinity			Mean
	0	1000	2000	
Golek	449.3 fg	1202 c	2862 a	1505 A
Zebda	969.3 c-e	1330 c	2590 a	1630 A
Sukkary	709 ef	787.7 d-f	2622 a	1373 A
Misk	228 g	1130 cd	2827 a	1395 A
Hindi Besinnara	757 d-f	1269 c	1784 b	1270 A
Mean	622.5 C	1144 B	2537 A	

The peroxidase has an important role in scavenging of H_2O_2.itis also involved in the growth and development process (Dionisio-Sese and Tobita, 1998).

Polyphenyl oxidase activity

Salinity treatments led to a significant increase in Polyphenyl oxidase (PPO) activity (Table 3) Polyphenyl oxidase activity increased with the increase in salinity level in all the studied cultivars. Zebda and Golk cvs. recorded the highest activity of Polyphenyl oxidase while Hindi recorded the lowest one. It has been reported that salinity increase the activity of Polyphenyl oxidase **(**Agarwal and Pandey, 2004). The PPO enzyme is responsible for the oxidation of the phenolic compound (Sheen and Calvert, 1969) it is also has an important role in the defense mechanism agents of salt stress (Tuna *et al*., 2008).

TABLE 3. Effect of different salinity treatments on Polyphenyl oxidase activity (O.D. units x10^3 min / g fresh weight) of the studied mango cvs.

Cultivars	Salinity			Mean
	0	1000	2000	
Golek	517.3 efg	756.7 de	1283 ab	852.3 B
Zebda	959.7 cd	1146 bc	1470 a	1192 A
Sukkary	437 gh	565.7 efg	747.7 de	583.4 C
Misk	258.3 h	325 gh	683.3 ef	422.2 C
Hindi Besinnara	239.7 h	390.3 gh	443 fgh	357.8 C
Mean	482.4 B	636.8 B	925.5 A	

Ascorbate content

As shown in Table 4 salinity treatments has a slight effect on ascorbate content in mango cultivars under salinity treatments. However the response of the studied cultivars differed under the different salinity treatments. The effect of salinity on ascorbate was obvious in Zebda and Sukkary cvs. while Hindi Besinnara cv. had the highest ascorbate concentration regardless the salinity treatments.

TABLE 4. Effect of different salinity treatments on ascorbate content (µmol g^{-1} fresh weight) of the studied mango cvs.

Cultivars	Salinity			Mean
	0	1000	2000	
Golek	0.0566 a-d	0.0633 ab	0.0466 b-f	0.0555 A
Zebda	0.0433 c-f	0.0366 ef	0.0333 f	0.0377 B
Sukkary	0.0533 a-e	0.0433 c-f	0.0333 f	0.0433 AB
Misk	0.0566 a-d	0.0400 d-f	0.0600 a-c	0.0522 AB
Hindi Besinnara	0.0666 a	0.0566 a-d	0.0466 b-f	0.0566 A
Mean	0.0553 A	0.048 A	0.0440 A	

Reduced Glutathione

The change in the reduced glutathione level of the studied mango cultivars under the effect of salinity stress is shown in Table 5 there was a gradual increase in the reduced glutathione level with increasing salinity stress compared with the control treatment. However there was a remarkable difference in the cultivar responses i.e. Zebda cv. had the highest reduced glutathione level compared with the other cultivars, while Hindi Besinnara cv. had the lowest level. The reduced glutathione has a role in scavenging the ROS (Mittler, 2002). According to Asada (2006) reduced glutathione (GSH) react with the hydroxyl free radical to protect cell proteins from damage. So it plays a crucial role in the defense system against environmental stresses by preventing oxidative damage in plant cell (Asada, 1999)

TABLE 5. Effect of different salinity treatments on reduced glutathione (µmol g^{-1} fresh weight) of the studied mango cvs.

Cultivars	Salinity			Mean
	0	1000	2000	
Golek	150 h-k	173 ghi	222.7 de	181.9 C
Zebda	28.3 def	256 cd	327 ab	267.1 A
Sukkary	154 hij	207.3 efg	294 bc	218.4 BC
Misk	161.3 hij	181 fgh	341.3 a	227.9 AB
Hindi Besinnara	111.7 k	137 ijk	129.3 gk	126 D
Mean	159.1 B	190.9 B	262.9 A	

Membrane stability index.

The membrane stability index of the studied mango cultivars was assessed to study the degree of membrane damage due to salinity treatments (Table 6). Membrane stability index increased with increasing concentration of NaCl. The percentage of ion leakage was much higher in Misk and Sukkary cultivars as compared to the other cultivars. However, the differences between the studied cultivars were non significant**.** In crop plants, cell membrane stability has been widely used as criteria to differentiate stress tolerant and susceptible cultivars and in some cases higher membrane stability could be correlated with abiotic stress tolerance (Blum and Ebercon, 1981).

TABLE 6. Effect of different salinity treatments on membrane stability index of the studied mango cvs.

Cultivars	Salt concentrations			Means
	0	1000	2000	
Golek	59.68 e	71.77 cde	85.57 bc	72.34 B
Zebda	72.11 cde	80.08 bcd	86.70 b	79.63 AB
Sukkary	79.87 bcd	82.32 bc	79.73 bcd	80.64 AB
Misk	78.39 bcd	79.11 bcd	117.8 a	91.76 A
Hindi Besinnara	63.93 e	66.75 de	71.49 cde	67.39 B
Means	70.80 B	76.01 AB	88.26 A	

Total chlorophyll content

The data shown in Table 7 indicated that the salinity treatment slightly reduced the chlorophyll content of all the studied mango cultivars**.** Hindi had the lowest chlorophyll content, while Misk had the highest chlorophyll content compared with the other cultivars. The decrease may be is due to the activity of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978) as well as damaging to the photosynthetic system (Yasseen, 1983).

TABLE 7. Effect of different salinity treatments on *chlorophyll* content (SPAD value) of the studied mango cvs.

Cultivars	Salt concentrations			Means
	0	1000	2000	
Golek	41.23 b-e	42.03 b-e	37.00 e	40.09 B
Zebda	43.93 bcd	44.40 a-d	40.27 cde	42.87 AB
Suckary	45.00 abc	38.60 de	40.20 cde	41.27 AB
Misk	50.23 a	46.87 ab	42.37 b-e	46.49 A
Hindibesinnara	42.03 b-e	38.60 de	37.80 e	39.48 B
Means	44.49 A	42.10 A	39.53 A	

Sugar content

The data in Table 8 indicated that salinity treatments increased the total sugar content but the differences between the effects of salinity levels were not significant. Accumulation of soluble sugars, proline, and glycine-betaine under stress protects the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment (Gadallah, 1999 and Greenway & Munns,1980).

TABLE 8. Effect of different salinity treatments on sugar content (mg g^{-1} dry weight) of the studied mango cvs.

Cultivars	Salt concentrations			Means
	0	1000	2000	
Golek	0.7300 ab	0.5267 bcd	0.7500 ab	0.6689 A
Zebda	0.6233 a-d	0.8467 a	0.5833 a-d	0.6844 A
Suckary	0.5500 a-d	0.5267 bcd	0.5033 bcd	0.5267 A
Misk	0.6300 a-d	0.6833 abc	0.7167 ab	0.6767 A
Hindi Besinnara	0.3900 cd	0.3667 d	0.6533 a-d	0.4700 A
Means	0.5847 A	0.5900 A	0.6413 A	

Conclusion

It could be concluded, from the obtained results, that Zebda cv. considered as a tolerant mango cultivar to salinity due to its high anti-oxidant potential as well as it could be used on as a salinity tolerant mango rootstock.

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نشاط مضادات الاكسدة لبعض أصناف المانجو النامية تحت ظروف الاجهاد الملحى

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أجريت هذه الدراسة خلال موسم ٢٠١٣ على شتلات نيوسلية عمر سنة لأصناف المانجو التالية جولك، مسك، هندى بسنارة، سكرى، وزبدة وذلك لدراسة نشاط مضادات الأكسدة الإنزيمية وغير الإنزيمية فى المانجو تحت تأثير ظروف الإجهاد الملحى. حيث تم تعريض شتلات أصناف المانجو السابقة الذكر لثلاثة مستويات من الملوحة (صفر ، ١٠٠٠، ٢٠٠٠ ملجم/لتر)، وتم تقدير نشاط إنزيمات البيروكسيديز ، والكتاليز والبولى فينول أكسيديز ، ومعامل ثبات الأغشية الخلوية، ومحتوى الأوراق من كل من الجلوتاثيون المختزل (Reduced glutathione) والإسكوربيت، والسكريات الكلية والكلوروفيل الكلى. أدت المعاملات الملحية إلى تغيير معنوى فى نشاط الإنزيمات المضادة للأكسدة ، ومعامل ثبات الأغشية الخلوية، ومحتوى الأوراق من الإسكوربيت الجلوتاثيون المختزل (Reduced glutathione). حيث أظهرت أوراق شتلات المانجو تحت تأثير الملوحة نشاط أعلى لإنزيم البيروكسيديز ، والبولى فينول أكسديز، بينما انخفض نشاط إنزيم الكتاليز و زاد محتوى الجلوتاثيون المختزل (Reduced glutathione) ومعامل ثبات الأغشية الخلوية، بينما كان تأثير الملوحة أقل على المحتوى من الكلوروفيل والسكريات. وقد كانت الإختلافات واضحة بين الأصناف محل الدراسة فى معظم الصفات التى تم دراستها، وقد أظهر الصنف زبدة قيم أعلى لمستويات مضادات الاكسدة مما يوفر له ميكانيكية مضادة للإجهاد الملحى.