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Abiotic Stress, Antioxidants and Crop Productivity: The Mitigating Role of Exogenous Substances

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Abiotic Stress, Antioxidants and Crop Productivity: The Mitigating Role of Exogenous Substances

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ABSTRACT

A major result of abiotic stress is the excessive production of Reactive Oxygen Species (ROS) which are capable of damaging all types of cellular structures. Whereas well - ordered redox homeostasis act under normal conditions to keep them from accumulating to damaging levels, they are overwhelmed under sub optimal conditions. This review considers the role of plants antioxidant defense system in managing oxidative stress and shows clearly that when confronted with stressful conditions such as drought and salinity the plants antioxidant defense machinery becomes inadequate and must be supported to be able to rise up to the stress challenge. The paper discussed the exogenous application of a variety of substances as a veritable source of such assistance, showing how their supply ameliorate stress effect and enhance crop productivity.

Key words: Oxidative stress, antioxidants, Compatible osmolites, hormones, exogenous application.

List of abbreviations

ROS Reactive Oxygen Species
 O_2^- superoxides
SOD superoxide dismutase
POD peroxidases
CAT catalases
KCZ Ketoconazole
 H_2O_2 hydrogen sulphide

INTRODUCTION

One major avenue employed in dealing with the problem of abiotic stress in agriculture includes the development of improved varieties, bred to over express or down regulate certain stress response traits including the biosynthesis or degradation of hormones, enzymes and other metabolites. Whereas this remains the more desirable option, it sometimes fails to provide the desired alleviation from stress (Verbruggen and Hermans, 2008; Ashraf and Foolad, 2007; Shalata and Neumann, 2001). The other option is the exogenous application of growth enhancers (Ahmed et al, 2010; Hasanuzzaman et al 2014; Ekmekci and Karaman, 2012). Commonly used enhancers include antioxidants (Ekmekci and Karamann, 2012; Azzedine et al 2011), Compatible osmolites (Ahmed et al 2010; Banu et al 2009) and plant growth hormones (Gupta et al 2012).

A major result of abiotic stress is the excessive production of Reactive Oxygen Species (ROS). ROS are normal by - products of aerobic metabolism generated as a result of either energy or electron transfer reactions (Apel and Hirt, 2004; Karuppanapandian et al 2011). They are highly reactive molecules that are capable of causing damage to all cell structures (Gill and Tuteja, 2010). Well - ordered redox homeostasis however keeps them from accumulating to levels where they exert this undesired potential. Under such condition they are believed to play significant role in plant's defense complex as well as in their growth and development, mainly as signaling molecules (Breusegen and Dat, 2006; Gill and Tuteja, 2010). However, under sub optimal conditions such as drought and salinity, they accumulate excessively and overwhelm the plants housekeeping agency that exercises control over them.

The housekeeping agents responsible for restraining the level of ROS from getting to damaging proportion are the antioxidants. These are a diverse group of highly efficient substances that specializes in scavenging ROS. According to Jaleel et al, (2009) they include enzymatic (peroxidases, catalases, superoxide dismutases) and non enzymatic (Ascorbic acid, reduced glutathione, α -tocopherol) antioxidants.

REVIEW

Development of oxidative stress and the loss of crop productivity

One of the major biochemical changes resulting from stress imposition is the excessive production of reactive oxygen species (ROS). This results primarily from stress - induced reduction in stomatal conductance and other biochemical changes that limits ability to fix carbon dioxide, the net consequence of which is that the cell experiences an imbalance in the ratio of NADPH to NADP⁺ due to restricted ability to oxidize NADPH which results in a limited supply of the electron acceptor NADP⁺ (Verbruggen and Hermans, 2008). Electron flow in the electron transport chain is therefore suppressed, causing excess photon energy to be directed into the production of ROS. ROS are capable of damaging all cellular macromolecules. Their activities result in protein oxidation, lipid peroxidation, DNA damage including base deletion, pyrimidine dimers, cross-links, strand breaks and so on with the result that there is reduced protein synthesis, cell membrane destruction and damage to photosynthetic proteins (Gill and Tuteja, 2010). Sofo et al (2015) opines that stress results in enhanced production of hydrogen sulphide (H₂O₂), leading to severe damage to biomolecules due to elevated H₂O₂. Overall, plant growth and development becomes greatly limited with visible symptoms of stunting, senescence, wilting, necroses, yield reduction and even plant death (Fig.1 O. Agada, university Putra Malaysia, ongoing research).

Fig 1: Tomato plant subjected to different levels of water stress showing reduced growth (source: O. Agada, university Putra Malaysia, ongoing research).

R3T1: control (plants watered at 100 % of field capacity)

R3T2: plants watered at 68% of field capacity

R3T3: plants watered at 53 % of field capacity

R3T4: plants watered at 38 % of field capacity

Scavenging role of Antioxidants and its limitations

Plants possess a highly efficient antioxidant system which serves to protect them against oxidative damage. Two groups of antioxidants are found in plants – the enzymatic (catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase etc.) and the non-enzymatic (ascorbic acid, phenolic compounds, alkaloids, glutathione, α-tocopherol) (Gill and Tuteja, 2010). Plants possess a variety of antioxidants dedicated to checking the equally wide array of ROS it generates. Karuppanapandian et al., 2011 maintains that some antioxidants may be more efficient in dealing with some ROS than others (Table 1, Karuppanapandian et al., 2011).

Table 1: Most important enzymatic and non-enzymatic antioxidative systems, their localization and scavenging of reactive oxygen species (ROS) in plant cells

Enzymatic antioxidants	Reaction catalyzed	Enzyme code number	Sub cellular localization
CAT	$2H_2O_2 \rightarrow O_2 + 2H_2O$	1.11.1.6	Per, Gly and Mit
APX	$H_2O_2 + AA \rightarrow 2H_2O + DHA$	1.11.1.11	Cyt, Per, Chl and Mit
GPX	$H_2O_2 + DHA \rightarrow 2H_2O + GSSG$	1.11.1.7	Chl, Cyt, Mit, and ER
SOD	$O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$	1.15.1.1	Cyt, Chl, Per, and Mit
MDHAR	$2MDHA + NADH \rightarrow 2AA + NAD^+$	1.6.5.4	Chl, Mit, and Cyt
DHAR	$DHA + 2GSH \rightarrow AA + GSSG$	1.8.5.1	Chl, Mit, and Cyt
GR	$GSSG + NADPH \rightarrow 2GSH + NADP$	1.6.4.2	Cyt, Chl, and Mit
Non-enzymatic antioxidants			
AA	Substrate for APX. Detoxifies H_2O_2		Chl, Cyt, Mit, Per, Vac, and Apo
GSH	Substrate for various POXs, GSTs and GR. Detoxified H_2O_2 , other hydroperoxidases and toxic compounds		Chl, Cyt, Mit, Per, Vac, and Apo
TOCs	Protects membrane lipids from peroxidation, detoxifies lipid peroxides, and quenching 1O_2		Membranes
CARs	Quench 1O_2 . Photosystem assembly, key components of the light harvesting complex, precursors for abscisic acid (ABA)		Chl, chromoplast, elaioplast, and amyloplast
Flavonoids	Can directly scavenge H_2O_2 and OH		Vac

Source: Karuppanapadian et al., (2011)

Abbreviations: 1O_2 : singlet oxygen; AA: ascorbic acid; Apo: apoplast; APX: ascorbate peroxidase; CARs: carotenoids; CAT: catalase; Cyt: cytosol; Chlo: chloroplast; DHA: dehydroascorbate; DHAR: dehydroascorbate reductase; ER: endoplasmic reticulum; Gly: glyoxisomes; GPX: guaiacol peroxidase; GR: glutathione reductase; GSH: glutathione; GSSG: oxidized glutathione; GSTs: glutathione-S-transferases; H_2O : water; H_2O_2 : hydrogen peroxide; MDHA: monodehydroascorbate; MDHAR: monodehydroascorbate reductase; Mit: mitochondria; O_2 : oxygen; O_2^- : superoxide radical; OH $\cdot$: hydroxyl radical; Per: peroxisomes; POXs: peroxidases; SOD: superoxide dismutase; TOCs: tocopherols; Vac: vacuole.

It has been established that antioxidants are indeed important in maintaining a safe and helpful level of ROS in plants (Shalata and Neumann, 2001; Azzedine et al., 2011; Karuppanapandian et al., 2011). It has also been demonstrated that cellular induction of the antioxidant machinery is important for the protection of plants against stress (Azzedine et al., 2011). Jaleel et al (2009) demonstrated that water stress induced significant increases in the ascorbic acid content of *Withania somnifera*. Cervilla et al (2007) also demonstrated induction of antioxidants under boron toxicity as an important protective strategy (Table 2, Cervilla et al., 2007).

Table 2: Effect of 0.05 mm B (control) and B toxicity (0.5 and 2 mm) on ASA, DHA, total ascorbate concentration and status redox (ASA/Total ascorbate) in leaves of two tomato cultivars ('Kosaco' and 'Josefina')

	ASA (nmol/g FW)		DHA (nmol/g FW)		Total ascorbate (nmol/g FW)		ASA/Total ascorbate (%)	
	'Kosaco'	'Josefina'	'Kosaco'	'Josefina'	'Kosaco'	'Josefina'	'Kosaco'	'Josefina'
0.05 mm	198.94 ± 0.33 [†]	150.14 ± 3.13 ^c	32.29 ± 0.35 ^c	47.03 ± 3.13 ^c	231.23 ± 14.11 ^c	197.18 ± 9.40 ^c	86.01 ± 0.57 ^a	76.10 ± 1.73 ^a
0.50 mm	326.69 ± 16.40 ^b	169.63 ± 6.39 ^b	106.53 ± 16.40 ^b	86.66 ± 6.38 ^b	433.21 ± 18.45 ^b	256.28 ± 9.58 ^b	75.33 ± 3.75 ^b	66.33 ± 2.60 ^b
2.00 mM	379.95 ± 14.35 ^a	258.68 ± 1.06 ^a	536.19 ± 14.35 ^a	112.16 ± 1.04 ^a	916.14 ± 6.15 ^a	370.84 ± 9.40 ^a	41.32 ± 1.45 ^c	69.66 ± 3.33 ^b
P	***§	***	***	***	***	***	***	*
'Kosaco'	301.86 ^{a†}		225.00 ^a		526.86 ^a		67.55 ^a	
'Josefina'	192.82 ^b		81.95 ^b		274.77 ^b		70.66 ^a	
P	***		*		***		ns	
LSD _{0.05}	29.95		115.18		124.95		10.77	

Source: Cervilla et al., (2007)

[†] Values are means ± s.e. ($n = 9$) and differences between means were compared using Duncan's test ($P = 0.05$).

[‡] Values are means ($n = 27$) and differences between means were compared using LSD test ($P = 0.05$).

[§] Levels of significance are represented by * $P < 0.05$, *** $P < 0.001$ and ns (not significant, $P > 0.05$).

Under stress, ROS are over produced and such above threshold levels overwhelm the antioxidant defense machinery, making their protective activities to become inadequate (Karuppanapandian et al., 2011). It has been demonstrated that plants with lower antioxidant capacity experienced greater damage from stress than those with higher capacity (Table 3, Ksouri et al., 2007). Alhdad et al (2013) also reported that antioxidant scavenging activity correlated positively with high antioxidant content. Hoque et al (2007) working with BY-2 cells indicated that salt stress significantly decreased the activities of superoxide dismutase, catalase and peroxidase.

Table 3: Total polyphenol and MDA contents in leaves of two *C. Maritima* accessions irrigated for 28 days with a nutrient solution containing 0, 100, or 400 mM NaCl

NaCl doses	polyphenol contents (mg GAE/ g DW)		MDA contents (mMol /g FW)	
	Jarba	Tabarka	Jarba	Tabarka
C (0 mM)	43±3.75c	42.84±7.67c	4.30±0.24c	4.64±0.29d
S1 (100mM)	66.93±3.51a	37.73±7.83c	1.48±0.22f	7.48±0.15b
S2 (400mM)	56.04±1.13b	31.58±7.85c	5.61±0.07c	12.08±0.02a

Source: Ksouri et al., (2007)

Values (means of three replicates) of each parameter followed by at least one same letter are not significantly different at $p < 0.05$

It follows therefore that plants whose antioxidant machinery has been overwhelmed by excessive ROS production would benefit from the enhancement of its antioxidant profile. One way this has been attempted is via the exogenous application of substances that either enhanced its uptake or biosynthesis.

Exogenous substances and the enhancement of antioxidant defense

A number of studies have shown that exogenous application of certain substances enhances the antioxidant machinery of plants under stress, improving their tolerance and hence productivity. Substances that have been successfully tried include derived or natural antioxidants, growth regulators, osmolytes etc. Ascorbic acid, a non enzymatic antioxidant was shown to increase resistance to salt stress, reduce lipid peroxidation, enhance proline accumulation, improve chlorophyll and carotenoid content, increase leaf area (Azzedine et al., 2011; Shalata and Neumann, 2001);

Hoque et al., (2007) used proline as the source of exogenous application and reported that the cells stress tolerance was enhanced due to a significant reduction in the concentration of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Their work showed that proline was not directly responsible for the reduction of the ROS level. They opined that the influence of proline was due to their ability to enhance plants antioxidant enzyme activity. This finding is corroborated by the work of Ahmed et al (2010) and Banu et al (2009). Also Osman (2015) applied betain and proline as foliar spray on pea plant and found that they enhanced the antioxidant content and activity (Fig. 2, Osman, 2015).

Fig 2: Effect of foliar application of glycine betaine (GB) and proline on SOD (A and B), APX (C and D) and CAT (E and F) activities in pea leaves (A, C and E) and seeds (B, D and F) under drought stress at different growth stages (main of two seasons)

Source: Osman 2015

Jaleel et al (2007) reported that the application of Ketoconazole (KCZ), a growth regulator was able to enhance the antioxidant potential of *Catharanthus roseus* and as a consequence conferred greater tolerance to drought stress. In addition, natural substances obtained as extracts from plants and other organisms have been used with success as sources of exogenous enhancement of antioxidants. A good example is Moringa leaf extract which contain a wide range of growth enhancing and stress ameliorating metabolites. MLE as an exogenous application has been shown to affect different aspects of plant stress response machinery, including the antioxidant system (Rady and Mohammed, 2015; Yasmeen et al 2013, Yasmeen, 2011).

Under stressful conditions plants concentrate most of their resources on the mobilization of the defense machinery rather than on growth and development (Kolbert et al 2012 in Yasmeen et al., 2013). The urgency of survival more or less overrules their productive instinct and so yield is compromised. The enhancement of the antioxidant machinery, by exogenous applications however results in a more robust response to stress and consequently crop productivity. Yasmeen et al., 2013 reported that the modulation of the antioxidant enzyme system by the application of Moringa leaf extract improved wheat performance under saline condition. They reported an 18.5 % increase in grain and kernel yield over the control. Yasmeen et al (2014) also noted that the enhancement of the antioxidant activity was a major reason for the enhancement of tomato productivity. They found that the activities of superoxide dismutase (SOD), peroxide (POD), catalase (CAT), total phenolics in leaves and lycopene contents in fruits were enhanced resulting in fruit yield increase of over 50%. Osman (2015) working with pea plants observed that enhancing their antioxidant yield under drought by the application of betain and proline enhanced assimilate production and translocation to the sink. Green pod yield was 2.72 and 2.63 ton/fed for betain and proline respectively compared to 2.49 ton/fed for control. Jaleel et al., (2007) demonstrated the enhancement of the active principles of the medicinal plant *Cantharanthus roseus* under stress by the exogenous application of ketoconazole. They opined that this effect was due to the ability of ketoconazole to enhance the plants antioxidant potential.

Fig 3: Effects of drought, KCZ and their combination on ajmalicine content of *C. roseus* plants. Values are given as mean $\pm$ S.D. of six samples in each group. Bar values not sharing a common superscript (a–d) differ significantly at $p \leq 0.05$ (DMRT)

Source: Jaleel et al., 2007

CONCLUSIONS

A veritable tool by which plants deal with stress is the deployment of their antioxidant defense machinery. Unfortunately the excessive generation of ROS often overwhelms this system and results in either low crop performance or even death. Fortunately the provision of external help via exogenous application of a variety of substances have been shown to enhance the biosynthesis and activity of this vital defense machinery and hence enable them provide robust response to the stress challenge. This invariably results in productivity enhancement as the exogenous intervention help plant re-channel vital resources diverted for defense to growth and development.

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