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Mechanisms of aluminium tolerance in citrus species: A review

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Abstract

The mechanisms of Aluminium tolerance involved during stress condition in some Citrus species. Aluminum (Al) toxicity is one of the major limitations that inhibit plant growth and development in acidic soils. In acidic soils (pH < 5.0), phototoxic-aluminum (Al³⁺) rapidly inhibits root growth, and subsequently affects water and nutrient uptake in citrus plants. Aluminum when in soluble form, as found in acidic soils that comprise about 40 percent of the world's arable land, is toxic to many crops. Organic acid excretion has been correlated with aluminum tolerance in higher plants. Plants present different degrees of adaptation to aluminium (Al) concentrations in the soil, and the understanding of this characteristic can lead to a viable option for the utilization of acid soils. High temperature and rainfall contribute in turning most of the slope soils acidic in states like Arunachal Pradesh, Assam, Meghalaya, Manipur, Nagaland, Mizoram and Sikkim.

Differential tolerance of plant genotypes to aluminium stress is a more promising approach to increase our understanding of aluminium tolerance in plants. The aluminium cation Al (3+) is toxic to many plants at micromolar concentrations. A range of plant species has evolved mechanisms that enable them to grow on acid soils where toxic concentrations of Al (3+) can limit plant growth. Organic acids play a central role in these aluminium tolerance mechanisms. Some plants detoxify aluminium in the rhizosphere by releasing organic acids that chelate aluminium. Therefore, this review updates the existing knowledge concerning the role of mineral nutrition for alleviating Al toxicity in plants to acid soils.

Keywords: Aluminium, citrus, toxicity, acidic

Introduction

In India, 49 million hectares area is affected by soil acidity of which 25 million hectares have a pH below 5.5 (Bhaumik and Donahue, 1964). Such types of problematic soils are found mainly in the North Eastern region and Western Ghats. About 84%, 77%, 76%, 60%, 57% and 47% soils of Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Sikkim and Tripura respectively have a soil pH less than 5 and considered strongly acidic (Panda, 1998) [32].

Acid soils comprise up to 50% of the world's potentially arable lands. In many acid soils throughout the tropics and subtropics, aluminium (Al)-toxicity is a major factor limiting crop productivity (Kochian *et al.*, 2004). Aluminium (Al) ranks third in abundance among the Earth's crust elements, after oxygen and silicon and is the most abundant metallic element. A large amount of Al is incorporated into aluminosilicate soil minerals, and very small quantities appear in the soluble form, capable of influencing biological systems (May and Nordstrom, 1991) [29]. Moreover, 40±50% of the world's arable soils is acidic which when summed with abundance of Al present in the earth crust leads to Al³⁺ phytotoxicity (Panda *et al.*, 2009) [33]. Hence Al toxicity is considered to be one of the most serious limiting factors for plant growth in acid soils worldwide.

Citrus is an important group of fruits. It is commercially cultivated in more than 50 countries like Brazil, USA, China, Spain, Mexico, Italy, Argentina, Japan, Australia, Greece, Israel, India and South Africa. *Citrus* fruits belongs to the genus *Citrus* of the family Rutaceae, having a basic chromosome number of x=9, 2n=18. In addition to oranges, mandarins, limes, lemons, pummelos and grapefruits, other citrus fruits such as kumquats, calamondins, citrons and many other hybrids are also commercially important. *Citrus* are well known for their refreshing fragrance, thirst quenching ability and providing adequate vitamin C as per recommended dietary allowance (RDA). In addition to ascorbic acid these fruits contain several phytochemicals such as carotenoids (lycopene and β-carotene), limonoides, flavanones (naringin and rutinoides), vitamin-B complex and related nutrients.

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Under Al-stress, sensitive plants display a number of toxicity symptoms depending on the species, variety and/or genotype. Inhibition of cell division in the root tip meristem by Al^{3+} is often reported for many species (Gunsé *et al.*, 2003; Doncheva *et al.*, 2005; Meda and Furlani, 2005) [17, 12, 30]. Increased rigidity of the double helix of DNA caused by accumulated Al^{3+} results in poor cell division in root tip meristem (Meriga *et al.*, 2010) [31]. In fact, in many plants, inhibition of root elongation has been reported within a few minutes time even at micromolar concentrations of Al^{3+} (Matsumoto and Motoda, 2013) [28]. The growth of the plant is affected mainly due to poor uptake of moisture and nutrients by injured root tips (Samac and Tesfaye, 2003; Vitorello *et al.*, 2005) [41, 45]. In some cases, increased susceptibility to drought stress, lodging and nutrient deficiencies are also reported from affected plants (Sun *et al.*, 2010). Under this background crop species which can grow on acidic soils gain increasing attention worldwide. In this context, *Citrus* species are widely recognized as hardy species, which can be cultivated successfully on acidic soils (Jiang *et al.*, 2009) [9]. The analysis of oxidative stress parameters and activities of antioxidative enzymes in sensitive and tolerant cultivars of will thus help in targeting the components of antioxidative defense system associated with Al tolerance. Identification of such tolerance mechanisms would be helpful in breeding programmes for selection of Al tolerant citrus varieties.

1. Effect of Aluminium on plant growth and development

Soil acidity has adverse effects on the growth and development of plants. Aluminium toxicity is an important growth-limiting factor for plants in acid soils below pH 5.0 but can occur at pH levels as high as 5.5. Aluminium (Al) is not regarded as an essential nutrient, but low concentrations can sometimes increase plant growth or induce other desirable effects (Foy, 1983) [14]. Poor crop productivity and soil fertility in acid soils are mainly due to a combination of Al and Mn toxicities and nutrient deficiencies. Among these problems, aluminium toxicity is one of the major limiting factors for crop production on acidic soils. Yang *et al.*, (2015) [22, 47] reported the effects of aluminium toxicity on the growth, photosynthetic activity and chlorophyll content of Eucalyptus clone trees. The results showed that the tested chlorophyll content, net photosynthetic rate, transpiration rate and water use efficiency was apparently inhibited by aluminium. Under uniform Al concentration (4.4 mM), the Al induced limitation to photosynthetic parameters increased with pH, indicating acid stimulation to Al toxicity. They further observed that under constant aluminium concentrations, increased acidity resulted in lower chlorophyll content. Kushwaha *et al.*, (2016) [23] studied the effects of aluminium on twenty cowpea genotypes in relation to soil and plant growth parameters. The levels comprised of 0, 20, 40, 60 ppm. They concluded that the genotypes of cowpea and their aluminium treatments exhibited significant differences for all the characters. Brunner and Sperisen (2013) [5] gave a review about aluminium tolerance in woody plants in which they stated that there are basically two main mechanisms for overcoming the aluminium stressed conditions. The mechanisms of these adaptations can be divided in to those that facilitate the exclusion of Al^{3+} from root cells (exclusion mechanisms) and those that enable plants to tolerate Al^{3+} once it has entered the root and shoot symplast (internal tolerance mechanisms). They also discussed the ecology of woody non-Al accumulator and Al accumulator plants, and present examples

of Al^{3+} adaptations in woody plant populations.

Inostroza-Blancheteau *et al.*, (2011) [20] presented a review regarding molecular and physiological mechanisms of Al toxicity and resistance in higher plants. Advances have been made in understanding some of the underlying strategies that plants use to cope with Al toxicity. Furthermore, we discuss the physiological and molecular responses to Al toxicity, including genes involved in Al resistance that have been identified and characterized in several plant species. The better understanding of these strategies and mechanisms is essential for improving plant performance in acidic, Al-toxic soils. Silva (2012) [43] gave a review about the symptoms of Al toxicity in plants along with the latest finding related to it. Some major mechanisms like root growth inhibition, ROS production, alterations on root cell wall and plasma membrane, nutrient imbalances, callose accumulation and disturbance of cytoplasmic CA^{2+} homeostasis were discussed. The behavior of Al-tolerant and Al-sensitive genotypes under Al was also given.

Chen *et al.*, (2010) [8] reviewed the effects of Al on stomatal conductance, ultra structure, pigments and light absorption, water relations, photochemistry, lipid peroxidation, photosynthetic enzymes, carbohydrates and their relations to the Al-induced photosynthesis inhibition in plant leaves. Al appears to preferentially impair thylakoids and photosynthetic electron transport chain in most plants. In addition to decreasing light absorption by lowering pigment concentration, both energy dissipation and antioxidant systems in Al-stressed leaves are enhanced to protect them from photo-oxidative damage under high light. The amelioration of brassino steroids, boron (B), phosphorus (P) and mycorrhizas on the Al-induced decrease in CO_2 assimilation, as well as some aspects needed to be further studied were also discussed. They emphasised that in addition to decreasing light absorption by lowering pigment concentration, both energy dissipation and antioxidant systems in Al-stressed leaves are enhanced to protect them from photo-oxidative damage under high light. Gill and Tuteja (2010) considered the possible mechanisms that occurs during abiotic stress and further reviewed on how reactive oxygen species that are produced during stress leads to damage of proteins, lipids, carbohydrates and DNA which ultimately results in oxidative stress. They gave an insight to the sites of production of these ROS. They also suggested on the antioxidant defense machinery that protects plants against oxidative stress damages. Horst *et al.*, (2010) [19] showed what role the apoplast plays in Al toxicity and resistance. They emphasised how Al binds to the cell wall particularly to the pectic matrix and to the apoplastic face of the plasma membrane in the most Al-sensitive root zone of the root apex thus impairing apoplastic and symplastic cell functions leading to Al-induced inhibition of root elongation. They opined that protection of the root apoplast appeared to be a prerequisite for Al resistance in both Al-tolerant and Al-accumulating plant species. Their findings suggested that there is an increasing physiological, biochemical and, most recently also, molecular evidence showing that the modification of the binding properties of the root apoplast contributes to Al resistance.

Reyes-Diaz *et al.*, (2009) [39] investigated the short-term aluminium stress on the photochemical Efficiency of Photosystem II in Highbush Blueberry Genotypes. They concluded that among the species used, Brigitta was the best cultivar for use in acid soil followed by Legacy and Blue gold

was the most sensitive. The experiment consisted of different Al concentrations (0, 25, 50, 75, and 100 mM) grown in a greenhouse in hydroponic solutions for 0 to 48 h and were allowed to recover (without Al) over 24 hours. Poschenrieder *et al.*, (2008) [36] reviewed on the current up to date glance into the current developments in the field of Al toxicity and the resistance of plants to such situations. They further gave special emphasis to the root growth and developments as primary targets for Al toxicity, mechanisms of exclusion as well as tolerance of high Al tissue levels. Sharma and Dubey (2007) [42] conducted a study on rice seedlings that were raised in sand cultures containing aluminium concentrations of 80 and 160 μM of Al^{3+} for 5-20 days. They observed a gradual decrease in the root growth as well as hoot growth and the increased production of ROS. It was further stated by them that in order to remediate the effects of these ROS the antioxidative enzymes of SOD, GPX, APX as well as glutathione reductase increased significantly. They concluded that Al toxicity is associated with the induction of oxidative stress which can only be overcome by the mechanisms of these antioxidative enzymes.

Al though excess of metals may produce some common effects on plants in general, there are many cases of specific effects of individual metals on different plants. Emamverdian *et al.*, (2015) wrote a review article about the major role that heavy metals play in living systems including plants. They suggested that the harmful effects of heavy metals leads to the production of several mechanisms that counteracts these harmful effects like augmentation of Reactive Oxygen Species (ROS), formation of chelating metals by like phytochelatins (PCs) or metallothioneins (MTs) metal complex at the intra- and intercellular level, which is followed by the removal of heavy metal ions from sensitive sites, synthesising of non-enzymatic compounds like proline. Another important additive mechanism of plant defense system that they discussed is symbiotic association with arbuscular mycorrhizal (AM) fungi.

There are various mechanisms by which the plants try to combat the Al stress. Barcelo *et al.*, (2002) [3] gave a review on the mechanisms of aluminium toxicity and resistance in plants. They elaborated on three specific mechanisms namely, the threshold for toxicity, hormesis and threshold for tolerance. The most important were the production of root exudates as well as internal detoxification mechanisms as well as the secretion of organic acids by the plants.

2. Effect of aluminium on citrus crop

Li *et al.*, (2016) [25] examined how Al toxicity induced alterations of protein profiles in citrus leaves, and identified some new Al toxicity responsive proteins related to various biological processes. They used seedlings of aluminium tolerant 'Xuegan' (*Citrus sinensis*) and Al intolerant sour pummelo (*Citrus grandis*). They were fertigated for 18 weeks with nutrient solution containing 0 and 1.2 mM $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. Al toxicity induced inhibition of photosynthesis as well as showed a decrease of total soluble protein which only occurred in *C. grandis* leaves, demonstrating that *C. sinensis* had higher Al tolerance than *C. grandis*. They also concluded that Al toxicity responsive proteins related to RNA regulation, protein metabolism, cellular transport and signal transduction might also play key roles in the higher Al tolerance of *C. sinensis*. Citrus belongs to evergreen subtropical fruit trees and is known to be sensitive to Al. Low pH and high Al concentration are the factors contributing to poor citrus

growth and shortened lifespan of trees (Lin and Myhre, 1990) [26]. CO_2 assimilation (Pereira *et al.*, 2003) [35] of citrus have been investigated by a few researchers, very little is known about the effects of Al on the photoprotective system of citrus leaves. Therefore it is a useful plant to analyse the responses of different mechanisms of energy dissipation in photosynthetic metabolism to Al. Jiang *et al.*, (2015) [22] used seedlings of 'Xuegan' (*Citrus sinensis*) and 'Sour pummel' (*Citrus grandis*) and treated them for 18 weeks with nutrient solution containing 0 (control) or 1.2 mM $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (+Al). Thereafter, they studied the Citrus root protein profiles using isobaric tags for relative and absolute quantification (iTRAQ). They studied the molecular mechanisms that were involved in plants to deal with Al-toxicity and to identify differentially expressed proteins involved in Al-tolerance. *C. sinensis* was found to be more tolerant than *C. grandis* roots, thus improving the Al-tolerance of *C. sinensis*.

Raja *et al.*, (2016) [38] gave a thorough review on the Health risk assessment of citrus contaminated with heavy metals and the potential risk of Al and Cu. They concluded that there was no potential risk for children and adult by consuming the studied citrus but as a result of the increased utilization of agricultural inputs (metal based fertilizers and pesticides, sewage sludge and wastewater) by farmers and orchardists, regular periodic monitoring of chemical pollutants content in foodstuffs are recommended for food safety. Aruna kumara *et al.*, (2012) [1] reviewed on how citrus crops cope with aluminium stress. They postulated that due to aluminium (Al) toxicity and low available phosphorous (P) content, crop production in acidic soils is restricted. Inhibition of root elongation, photosynthesis and growth is experienced in citrus also due to Al toxicity. Focusing at toxicity alleviation, interaction between boron (B) and Al as well as phosphorus and Al has been discussed. Al toxicity in citrus could be alleviated by P through increasing immobilization of Al in roots and P level in shoots rather than through increasing organic acid secretion. Despite the species-dependent manner of response to Al toxicity, many commercially important citrus species can be grown successfully in acidic soils.

Chen *et al.*, (2009) [9] found that there were significant differences in changes of organic acid metabolism between roots and leaves of *Citrus grandis* in response to P and Al interactions. They experimented with seedlings of sour pummelo (*Citrus grandis*) which were irrigated daily for 18 weeks with nutrient solution containing four phosphorus (P) levels (50, 100, 250 and 500 μM KH_2PO_4) and two aluminium (Al) levels [0 (-Al) and 1.2 mM $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (+Al)]. The experiment showed that both malate and citrate concentrations in +Al leaves decreased with increasing P supply, but their concentrations in -Al leaves did not change in response to P supply. Thus, proving significantly that the activities of acid metabolizing enzymes in most cases were less affected in roots compared to the leaves by P and Al interactions.

Jiang *et al.*, (2008) [21] conducted an experiment using seedlings of *Citrus grandis* cv Tuyou, which was irrigated daily for 5 months with a nutrient solution containing 0 (control), 0.2, 0.6 or 1.6 mM aluminium (Al) from $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. They showed that shoot growth was more sensitive to Al toxicity than root growth. They found that the leaves of Al-treated plants showed decreased CO_2 assimilation and chlorophyll concentration, yet intercellular CO_2 concentration increased and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity was unchanged.

They postulated that impaired electron transport capacity accompanied by lack of reducing equivalents were the main factors contributing to decreased CO₂ assimilation in Al treated plants.

Chen *et al.*, (2005a) [6] used 'Cleopatra' tangerine (*Citrus reshni* Hort. Ex Tanaka) seedlings and irrigated them daily for 8 weeks with Hoagland's nutrient solution (1/4 strength) containing 0 (control) and 2mM aluminium (Al). They found that leaves from Al-treated plants had a decreased CO₂ assimilation and stomatal conductance, but increased intercellular CO₂ concentrations compared with control leaves. They also found that at a concentration of 2mM Al the leaf area had increased activities of key enzymes in the Calvin cycle, compared with control leaves. They concluded that the reduced rate of CO₂ assimilation in Al-treated leaves was probably caused by a combination of factors such as reduced electron transport rate through PSII, increased closure of PSII reaction centres and increased photorespiration.

Chen *et al.*, (2005b) [7] used 'Cleopatra' tangerine seedlings to study the effects of aluminium on light energy utilization and photoprotective systems in citrus leaves. The seedlings were irrigated with Hoagland's nutrient solution containing Al at a concentration of 0 or 2 mM from Al₂ (SO₄)₃.18H₂O. Thereafter, leaf absorbance, chlorophyll (Chl) fluorescence, Al, pigments, antioxidant enzymes and metabolites were measured on fully expanded leaves. These results corroborate the hypothesis that compared with control leaves, antioxidant systems are up-regulated in Al-treated citrus leaves and protect from photoxidative damage, whereas thermal energy dissipation was decreased. Thus, antioxidant systems are more important than thermal energy dissipation in dissipating excess excitation energy in Al-treated citrus leaves.

Pereira *et al.*, (2003) [35] used five Al concentrations (0, 50, 100, 200, and 400 µmol/L) to study the effects of Al on the growth of 'Rangpur' lime (*Citrus limonia* Osbeck), 'Volkamer' lemon (*Citrus volkameriana* Hort. ex Tan.), 'Sunki' (*Citrus sunki* Hort. ex Tan.) and tangerine rootstocks 'Cleopatra' (*Citrus reshni* Hort. ex Tan.). The plants were grown hydroponically. For all rootstocks, the relative growth rate in terms of plant total fresh matter increased under low and decreased under large Al concentrations. Growth of the shoot, leaf area ratio and leaf weight ratio also decreased for all rootstocks in the presence of Al. The 'Rangpur' lime expressed a decrease of the root system growth, starting from 23 µmol/L of Al. For the remaining rootstocks, this growth reached maximum values at 91 to 117 µmol/L of Al respectively. Taking into consideration all the evaluated characteristics of plant growth, 'Rangpur' lime was the most susceptible to Al.

3. Morphological responses of plants towards Al toxicity

Aluminium toxicity is a potential growth-limiting factor for plants grown in acid soils in many parts of the world. The symptoms of aluminium toxicity are not easily identifiable. Excess Al even induces iron (Fe) deficiency symptoms in rice (*Oryza sativa* L.), sorghum and wheat (Clark *et al.*, 1981, Foy and Fleming, 1982) [15].

Awasthi *et al.*, (2017) [2] conducted an experiment to test the tolerance level of rice varieties to aluminium toxicity and the possible morphological and physiological changes that were involved due to the Al stressed condition. Results show marked difference in growth parameters (relative growth rate, Root tolerance index, fresh and dry weight of root) of rice seedlings due to Al (100 µM) toxicity. Al³⁺ uptake and lipid

peroxidation level also increased concomitantly under Al treatment. Histochemical assay were also performed to explain the uptake of aluminium, loss of membrane integrity and lipid peroxidation, which were found to be more in sensitive genotypes at higher Al concentration. From the genotypes used, Disang was found to be a comparatively tolerant variety whereas Joymati a sensitive variety.

Roy and Bhadra (2014) [40] studied the effects of aluminium (30, 60 and 90 µg/mL) on the seedling root growth, number of primary roots per seedling, seedling shoot length, number of leaves per seedling, seedling fresh weight, and seedling dry weight of rice. Toxic levels of Al in nutrient solution significantly decreased the seedling root growth, number of primary roots, seedling shoot length, and number of leaves per seedling, seedling fresh weight and seedling dry weight. Few genotypes showed longer root length at 30 µg/mL of Al in nutrient solutions when compared with the control. High levels of Al in nutrient solutions were highly toxic for rice seedlings. Based on the root tolerance index, Radhunipagal, Gobindobhog, Badshabhog, Kalobhog, UBKVR-11, UBKVR-16, UBKVR-18, Khasha and IVT4007-B were classified as the tolerant genotypes.

According to Makau *et al.*, (2011) [27] the root elongation was the single most significant index in ranking the genotypes against Al tolerance for crops like maize (*Zea mays*), garden pea (*Pisum sativum*), bean (*Phaseolus vulgaris* L) and cucumber (*Cucumis sativus*).

Panda *et al.*, (2003) [34] used green gram seedlings to study the effects of aluminium phytotoxicity that eventually lead to oxidative stress. They found a uniform decrease in the root and shoot elongations and a significant increase in lipid peroxidation and membrane injury index. Further they found a rapid increase in peroxidase activity, SOD activity and glutathione reductase activities with increasing aluminium concentrations. Both glutathione and ascorbate contents showed a decrease at a higher metal concentration. These results suggested an induction of oxidative stress in developing green gram seedlings under aluminium phytotoxicity.

4. Biochemical responses of plants towards Al toxicity

Aluminum resistance in plants is reliant on antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GPR), as well as non enzymatic molecules of low molecular weight, such as ascorbic acid (AsA), reduced glutathione (GSH) and phenol like compounds such as tocopherols (α-tocopherol), flavonoids, carotenoids (β-carotene), and uric acid (Guo *et al.*, 2007) [18].

Biochemical studies indicate that Al ions have strong affinity for biomembranes (Yamamoto *et al.*, 2003) [46]. This is because the plasma membrane has negative charges, such as phospholipids to which Al binds irreversibly. Aluminium induced quick production of ROS in *Zea mays*, together with rigidification of the cell wall, which could inhibit root development (Jones *et al.*, 2006).

Racchi (2013) [27] briefly gave a review that introduces the formation of reactive oxygen species (ROS) as a result of oxidative stress and the ways in which the antioxidant defense machinery is involved directly or indirectly in ROS scavenging. Major antioxidants, both enzymatic and non-enzymatic, that protect higher plant cells from oxidative stress damage are described. Biochemical and molecular features of the antioxidant enzymes like superoxide dismutase (SOD),

catalase (CAT), and ascorbate peroxidase (APX) are discussed as they play crucial roles in scavenging ROS in the different cell compartments and in response to stress conditions. Among the non-enzymatic defenses, particular attention is paid to ascorbic acid, glutathione, flavonoids, carotenoids and tocopherols. The operation of ROS scavenging systems during the seasonal cycle and specific developmental events, such as fruit ripening and senescence, are discussed in relation to the intense ROS formation during these processes that impact fruit quality. Particular attention was given to *Prunus* and *Citrus* species.

Choudhary *et al.*, (2011) ^[10] studied the effects of five levels of aluminium concentrations (0, 10, 20, 30 and 50 $\mu\text{g ml}^{-1}$ Al) on 32 pigeonpea genotypes by four different methods: hydroponic and sand assays (growth response methods), root re-growth and hematoxylin in root staining. Significant variability was noted for tolerance to aluminium toxicity among the pigeon pea genotypes and the results of all the four screening methods were consistent, suggesting that any one of the four methods could be used for screening purpose. However, in terms of reliable and better precision and short test period, the best method was by the hematoxylin staining at 30 $\mu\text{g ml}^{-1}$ aluminium concentration to discriminate pigeon pea genotypes for aluminium tolerance. Based on the results, most tolerant were found to be IPA 7-10, T 7 and 67 B and most sensitive Bahar, Pusa 9 and Pusa 2002-2 genotypes.

Ahmad *et al.*, (2010) reviewed on how the Reactive oxygen species (ROS) are produced in plants as byproducts during many metabolic reactions, such as photosynthesis and respiration. They emphasised on how oxidative stress occurs when there is a serious imbalance between the production of ROS and antioxidant defense. They opined that the generation of ROS causes rapid cell damage by triggering a chain reaction and how cells have evolved an elaborate system of enzymatic and non-enzymatic antioxidants which help to scavenge these indigenously generated ROS. Various enzymes involved in ROS-scavenging have been manipulated, over expressed or down regulated to add to the present knowledge and understanding the role of the antioxidant systems. Their review sheds light on the manipulation of enzymatic and non-enzymatic antioxidants in plants to enhance the environmental stress tolerance.

Kuo and Kao (2003) ^[24] studied the effects of aluminium on the lipid peroxidation and on the antioxidative enzymes in rice leaves that were treated with AlCl_3 (0-5 mM) at pH 4.0. Under the influence of AlCl_3 they found that the content of malondialdehyde was enhanced but the activity of H_2O_2 was inhibited. The enzymatic activity of SOD was also reduced by AlCl_3 while catalase and glutathione reductase activities were increased. Peroxidase and ascorbate peroxidase activities were only increased after prolonged treatments. This proves that Al treatments can cause oxidative damage, which in turn leads to lipid peroxidation.

You *et al.*, 2014 ^[48] isolated fifty-three and thirty-nine differentially expressed protein spots by the use of 2-dimensional electrophoresis from Mn-toxic *Citrus sinensis* and *Citrus grandis* roots, respectively. Mn-toxicity-induced changes in protein profiles which greatly differed between the two species. *C. sinensis* conferred more resistance to Mn toxicity than *C. grandis*.

Similar results were obtained by Oh *et al.*, (2013) whereby they studied the proteomic changes of wheat seedlings under Al stress and identified the proteins that are responsible for Al stress in wheat roots. They used a 2-dimensional

electrophoresis and found that a total of 47 proteins were expressed under Al stress. Nineteen proteins were increased whereas 28 proteins were significantly decreased. They helped in identifying the defence responsive proteins with morphological and physiological state under Al stress.

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