American Journal of Bio Science
2021; 9(6): 210-216
http://www.sciencepublishinggroup.com/j/ajbio
doi: 10.11648/j.ajbio.20210906.16
ISSN: 2330-0159 (Print); ISSN: 2330-0167 (Online)

Bio-regulators: Silicon, Salicylic Acid, Ascorbic Acid Improve Salt Tolerance in Cucumber (*Cucumis sativus* L.)

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To cite this article:

Mostapha Maach, Mustapha Akodad, Abdelmajid Moumen, Ali Skalli, Hanane Ait Hmeid, Hicham Gueddari, Mourad Baghour. Bio-regulators: Silicon, Salicylic Acid, Ascorbic Acid Improve Salt Tolerance in Cucumber (*Cucumis sativus* L.). *American Journal of BioScience*. Vol. 9, No. 6, 2021, pp. 210-216. doi: 10.11648/j.ajbio.20210906.16

Received: February 22, 2021; **Accepted**: March 22, 2021; **Published**: December 29, 2021

Abstract: Soil salinity is currently considered as a major environmental problem. This issue is accentuated by climate change, especially in arid and semi-arid regions. In these areas, drought and high level of salt in soils and irrigated waters are the main abiotic factors limiting plant growth and productivity. On the other hand, rapid population growth and reduction of arable land are major factors that could affect food security. Regarding salt tolerance, most crop plants show sensitivity to high salt levels, but it differs strongly between species and slightly between cultivars within the same species. Cucumber (*Cucumis sativus* L.) is an important economic crop and is sensitive to salinity. To enhance cucumber performance and resistance to high levels of NaCl, different strategies can be employed, In recent decades, an increased research interest has been devoted to develop new strategies to overcome the deleterious effects of salinity on cucumber plants by using molecular markers and genetic transformation as tools to generate salinity-tolerant genotypes together with the implementation of some cultural techniques. Among the other strategies currently being used is the exogenous application of biostimulants and bioregulators. In this regard, and in order to improve salt tolerance of cucumber plants, we have discussed here the effect of foliar application of bio-regulators such as silicon, salicylic acid, and ascorbic acid on plant metabolism and yield.

Keywords: Salinity, Cucumber, Silicone, Salicylic Acid, Ascorbic Acid

1. Introduction

Salinity is one of the main abiotic stresses in plant agriculture worldwide. It affects a large part of the world's land surface and is a major threat to agricultural production. Most plants are constantly subjected to a range of environmental stresses, such as salt, drought, and cold [1]. In this regard, almost 20 percent of the cultivated area and about half of the irrigated land in the world are affected by salinity. High soil salinity reduces plant growth and thus global food production [2]. It is also one of the most serious constraints affecting agricultural production [3]. Under conditions of salt stress, excessive concentrations of Na^+ decrease essential nutrients such as K^+ and Ca^{2+} in plants [4, 5]. In addition, certain high levels of salinity can cause some ionic and osmotic disorders which will lead to a decrease in turgidity, membrane damage, and inhibition of water and essential ion absorption, metabolic disorders are manifested by altered levels of growth regulators, enzymatic inhibition, and also metabolic dysfunction, including photosynthesis which will eventually result in plant death [6, 7].

Salt stress is characterized, in most cases, by two stresses mainly at the plant tissue level: one is osmotic stress caused by relatively high concentrations of soil solutes that disrupt the homeostasis of water potential, and the other is ion- specific stress resulting from excessive accumulation of Na^+ and Cl^- and altered ratios Na^+/Na^+ [8, 9].

One of the most effective ways to overcome salinity problems is the introduction of salt-tolerant crops. At present, selection for salinity tolerance of crops has generally been limited by the lack of reliable breeding characteristics. Multiple genes appear to act in concert to increase salinity tolerance, and some proteins involved in protection against salinity stress have also been recognized. However, the development of methods and strategies to improve the deleterious effects of salt stress on plants has received considerable attention in recent years. In this respect, and in this chapter we will mention some products (bio-regulators) with applications commonly used in agriculture to improve salt tolerance in cucumbers (*Cucumis sativus* L), namely silicon, salicylic acid, and ascorbic acid.

1.1. Origin and Classification

The cucumber (*Cucumis sativus* L.), belongs to the family Cucurbitaceae, and comprises 30 species divided into two groups according to the number of chromosomes [10]. It's considered to be native to Asia, as the wild cucumber exists in India and a closely related species is found in the eastern Himalayas [11]. Cucumber (*Cucumis sativus* L.) is one of the oldest cultivated vegetables that has been known historically for more than five thousand years and probably originated in India. From India, the plant seems to have spread eastwards into China and westwards into Asia Minor, North Africa, and Southern Europe long before written history. Cucumber is a thermophilic and frost-sensitive plant species, which grows best at temperatures above 20°C; as a result, it is cultivated in almost all countries with temperate climates.

1.2. Nutritional Value of the Cucumber

The table below 1 shows main energy and mineral values in the cucumber fruit. In 100g of edible portion of cucumber you will find a significant amount of the following elements, Vitamin A (45 IU), Vitamin C (12mg), Magnesium (15mg), and Phosphorus (24mg).

Table 1. *Nutritional value in cucumber [12].*

Edible portion (per 100g)	
Water	96 g
Protein	0,6 g
Fat	0,1 g
Carbohydrates	2,2 g
Vitamin A	45 UI
Vitamin Bl	0,03 mg
Vitamin B2	0,02 mg
Vitamin C	12 mg
Niacin	0,3 mg
Valcium	12 mg
Iron	0,3 mg
Magnesium	15 mg
Phosphorus	24 mg

2. Improvement Salt Stress Tolerance in Cucumber (*Cucumis sativis* L) by Using Bio-regulators Described Below

2.1. Definition

Silicon (Si) is a quantitatively important inorganic constituent of higher plants, but it is almost absent from the main scientific publications on higher plants. (Si) is the second most common element in the soil after oxygen and is a beneficial element for plants, especially under adverse environmental conditions [13]. The earth's crust is made up of 27.61170 of the Silicon, which comes in a wide variety of forms and stabilities. In a simplistic model, Si is found at each intermediate stage in a gradual transition between molecular solubility (silicic acid), homogeneously dispersed colloids (hydrosols), non-rigid gels (hydrogels), and rigid gels (xerogels) [14]. The boundaries of this continuum are not clear-cut, and the entities are only arbitrarily definable. The application of silicon has been shown to be a promising method for reducing the adverse effects of salt stress on plants [4].

2.2. Silicon Improves Salt Stress in Cucumber (Cucumis sativis L)

Silicon considers being one of the most important and also most possible mechanisms that reduce the activity of toxic metal ions in the environment. The concentration of Si in soil solutions varies in ~~the~~ range of 0.1-0.6 mM, and changes little over the whole range of physiological pH values. When culture solutions with much higher concentrations are used, polymerization and precipitation are of concern [15].

2.3. Improving Cucumber (Cucumis sativus L) Under Salt Stress Using Silicon

To face this problem mentioned in the introduction, there will be some strategies to improve and adapt the salt stress of plants, among them, the application of exogenous substances can be a promising solution that could save on the one hand time and on the other hand the necessary labor force.

So, the key to this problem will be presented in the application of silicon, the second most common element in the soil [13]. It can be accumulating in different ways, also in different varieties of plant species. Plants can be classified as high, intermediate and non-accumulators according to their modes of silicon uptake (active, passive, and rejector [3].

Silicon has been shown and also considered beneficial in lowering some of the risks of environmental stresses, including biotic stresses such as plant diseases and pest damage [16, 17], also abiotic stresses such as salinity, drought, frost, and finally heavy metal toxicity [18, 19].

Silicon plays an important role in mitigating the damage caused by salt stress, which has been widely published in various crops, namely, wheat, sorghum, barley and soybean. However, his application can be an alternative approach to increase agricultural production in saline soils.

In this chapter, we have focused our study on cucumber (*Cucumis sativus* L.), which belongs to the most important vegetable group and also the most sensitive to salt stress. Furthermore, cucumbers are characterized by low silicon accumulation due to a lower transport density [20]. According to [21], cucumber is among the horticultural crops important that may be more sensitive to salinity As a result, [21], showed that bio-regulator silicon has a considerable role to play in mitigating the rate of salt toxicity in cucumber plants by increasing the activity of key antioxidant enzymes and thereby decreasing oxidative membrane damage. In addition, earlier work by [21] also suggested that the silicon-mediated salt tolerance of cucumbers has been attributed to reduced oxidative stress. Nevertheless, has not yet cleared whether this is an action of silicon or simply a consequence of stress reduction. In this respect [22], suggest that silicon improves the salt tolerance of cucumber plants (*Cucumis sativus* L.), mainly by improving water absorption, especially in the root path, and the positive regulation of aquaporin gene expression may partly contribute to increased water absorption. Silicon application reduced both Na^+ and Cl^- accumulations in the roots under the effect of salt stress.

In addition, silicon will regulate the osmotic adjustment in which plants respond to saline stress by osmotic adjustment, typically by increasing concentrations of solutes such as proline and soluble sugars to adjust the osmotic potential for better water absorption [23]. As a result of the osmotic adjustment, cells can recover their volume and cell turgidity in a few hours. However, the rate of cell elongation is very low, resulting in a change in cell size [24-27]. This whole procedure necessarily depends on a phenomenon that depends on the genotype's mechanism of absorption of water by plants thanks to silicon under salt stress. [28], reported that the application of Silicon has an important effect on the decrease of transport of ions from Na^+ and in parallel improves the ratio K^+/Na^+ in salt-stressed wheat. However, the decrease in accumulation of Na^+ due to silicon is not always the case [29, 3], suggesting the involvement of other mechanisms. Recently, a number of studies have been carried out on the attenuating effect of silicon on salinity induced osmotic stress and found that the addition of silicon improved the water status of the plant, as in tomatoes [29], maize, sorghum, grass, and cucumber.

3. Salicylic Acid Improves Salt Stress in Cucumbers (*Cucumis sativis* L)

3.1. Definition

Salicylic acid belongs to an extraordinarily diverse group of plant phenols generally defined as substances with an aromatic ring bearing a hydroxyl or its functional derivative. The first author who introduced and gave a general presentation for salicylic acid was Raffaele Piria in 1838 [30], who said that the name salicylic acid (SA), coming from the Latin word Salix, a willow tree, was given to this active ingredient. During the 19th century SA and other salicylates, mainly methyl esters and glucosides which are easily convertible to SA, were isolated from various plants, including spirea and wintergreen. The first commercial production of synthetic SA started in Germany in 1874. Aspirin, the trade name for acetylsalicylic acid, was introduced by the Bayer company in 1898 and quickly became one of the world's best-selling drugs. Aspirin was as effective as SA and caused much less irritation to the human digestive system.

Salicylic acid free and very soluble in polar organic solvents and moderately soluble in water, it is a crystalline powder that melts at 157-l59°C. The pH of a saturated aqueous solution of SA is 2.4. SA fluoresces at 412 nm when excited at 301 nm, and this property can be used to detect this compound in a number of facility systems [31]. The presence of SA in plants, long suggested, has been confirmed by several investigators using modern analytical techniques [31-34]. An exhaustive study of SA in the leaves and reproductive structures of 34 agronomically important species confirmed the ubiquity of this compound in plants. Rice, crabgrass, green foxtail, barley, and soybean had SA levels above 1 μg/g fresh weight.

3.2. Pathway and Biosynthesis of Salicylic Acid (SA) in Plant

Salicylic acid (SA) is an important signal molecule in plants, two routes of SA biosynthesis have been proposed in plants. The most important mechanism for the formation of benzoic acids in plants is the degradation of the side chain of cinnamic acids [35], which are important intermediates in the shikimic acid pathway. Therefore, a priori, SA (ortho-hydroxybenzoic acid) could be considered as a derivative of cinnamic acid. The conversion of cinnamic acid to sulphuric acid will probably be done by one of the two routes described in the figure below (Figure 1).

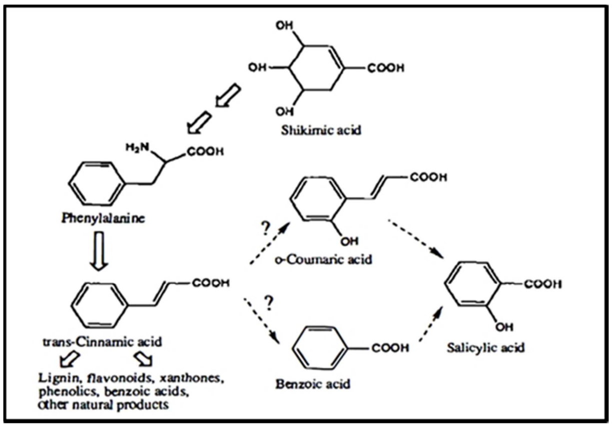

Figure 1. *Proposed pathway for the biosynthesis of salicylic acid in plants [36].*

3.3. Salicylic Acid Improves the Quality of Cucumbers Under salt Stress

The cucumber (*Cucumis sativus* L.) is one of the most important horticultural crops in the world. In addition, it is one of the main commercial agricultural crops in many countries, but it is very sensitive to salt, water, and temperature stresses. It is considered to be a market garden crop that is cultivated worldwide. Cucumber growth and yield are reduced by salinity stress [37]. The demand for food is a major global problem worldwide. Soil salinity, drought, soil erosion and other climate changes are reducing food production in arid and semi-arid areas.

Salinity is one of the main obstacles to declining agricultural productivity, and the production of salt-tolerant crops has been given high priority in sustainable agricultural research [38]. It reduces plant growth and yield and interferes with many biochemical processes such as photosynthesis, respiration, synthesis of phytohormones, homeostasis of reactive oxygen species (ROS), and antioxidant systems. In addition, the water deficit is becoming a serious and significant problem in many countries of the world. Cucumber is one of the most important horticultural crops and cucumber and other cucurbits have a root system and high sensitivity to water deficit stress. Cucumber and other Cucurbits have a high transpiration rate and sensitivity to water deficit stress [39]. It is well known that drought stress increases the production of oxygen species (ROS) in different cell organelles. However, salinity stress produces reactive oxygen species (ROS) in plant cells, which can be damaged if the ROS are not rapidly produced. SOD, POD, and CAT in the detoxification of ROS by catalyzing the exchange of free O_2^- to O_2 and H_2O^2. Reactive oxygen species are more numerous in plants exposed to saline stress than controls. ROS includes both radical (superoxide radical, hydroxyl radical and alkoxy radical), and non-radical (hydrogen peroxide and singlet oxygen) forms, which damage chlorophyll, proteins, DNA, lipids, and other macromolecules. Several reports have suggested that changes in unsaturated fatty acid content can improve plant tolerance to salinity [40]. Thus, these ROS negatively affect different biological and physiological processes and death will occur if stress is prolonged [41, 42]. Ascorbic acid (AsA) is ~~a~~ low molecular weight but powerful antioxidant. In this respect, various studies have shown that salinity increases the ROS trapping enzymes in cucumber. [43], were able to prove that AsA can be considered appropriate and affordable plant bio-regulators to overcome the negative and destructive effects of water stress on the cucumber plant. Exogenously applied SA and Kin induced TOD and CAT activity in saline soil conditions has been reported previously for wheat, mung bean [4] and tomato. POD is one of the main antioxidant enzymes that contribute to protection against salt-induced oxidation and stress in cucumber plants.

In addition, AsA participates in a wide range of plant processes, namely photosynthesis and cell expansion, and also increases plant resistance to various abiotic stresses. Among the different bio-regulators already tested on various plant species, gamma aminobutyric acid (GABA) has emerged as a promising multi-protective agent with the capacity to rapidly increase plant tolerance to various abiotic stresses such as water accumulation, high temperatures, heat, shocks, salt and water deficit stresses. In plants, GABA is involved in a multitude of physiological functions such as growth regulation and tolerance to various abiotics stresses [44].

Research by these groups of researchers [4, 45] shows that the stimulation of salt tolerance through seed pre-treatment and foliar application of salicylic acid and kinetin have been well reported for many crop species. However, the mechanism of improvement due to seed pre-treatment with salicylic acid and kinetin is not yet fully understood. In addition, salinity stress can alter the hormonal balance in plants, so hormonal homeostasis is a possible mechanism for the salinisation of cytokinin and salicylic acid [4]. The site of salicylic acid application could contribute to a mechanism for the regulation of physiological processes, such as stoma closure, ion transport and absorption, membrane permeability and photosynthesis [4]. Concerning cytokinin is involved in some growth strategies and thus physiological processes such as cell division, apical dominance, lateral root initiation, stomatics and vascular behavior and development under abiotic stress.

Another study carried out by a few researchers showed that the experimental pre-treatment of seeds with salicylic acid improved the salinity tolerance of cucumber genotypes. The beneficial effects of seed pre-treatment with SA on plant biomass and physiological responses have been reported in a number of species grown under abiotic stresses [39]. SA has enhanced the defense mechanism against various in plant species [39]. SA also plays an important role in growth and development [4, 5].

4. Improvement Salt Tolerance in Cucumbers (*Cucumis sativis* L) by Ascorbic acid Foliar Application

4.1. Definition

Ascorbic acid (AsA), as an antioxidant, is defined as a small water-soluble molecule that acts as a primary substrate in the cyclic detoxification and neutralization of superoxide radicals and singlet oxygen. In addition, it is very effective in improving plant biomass [46].

4.2. Ascorbic Acid Improves Salt Stress in Cucumber (Cucumis sativis L)

Water deficit is becoming a serious and significant problem in many countries of the world [42]. Cucumber is one of the most important horticultural crops, cucumbers and other cucurbits have a fibrous root system and are very sensitive to water stress [39].

Salt stress can affect plant growth and development directly through its potential toxic effects, and indirectly through its osmotic effects [47]. Recently, several studies have reported that salinity also causes increased production of reactive oxygen species, which may themselves lead to secondary signals. ROS are highly reactive in the absence of any protective mechanism. They can severely disrupt normal metabolism by oxidative damage to essential membrane lipids, proteins and pigments [48]. To recover ROS, [49] showed that plants synthesize different types of defense systems composed of non-enzymatic antioxidants such as ascorbic acid and enzymatic antioxidants such as catalase (CAT), peroxidase (POD), ascorbate peroxidase (AP) and glutathione reductase (GR). Scavenging system has the potential to extinguish ROS in stress-tolerant plants. Osmotic adjustment is the cellular response to reduced turgidity. The cytosolic and organellar machinery of glycophytes and halophytes is equally sensitive to Na^+ and Cl^-; osmotic adjustment is therefore achieved in these compartments by the accumulation of compatible osmolytes and osmo-protectors [50].

However, Na^+ and Cl^- are energy efficient osmolytes for osmotic adjustment and are compartmentalized in the vacuole to minimize cytotoxicity. Many oxidants, such as the hydroxyl radical, contain an unpaired electron and are therefore highly reactive and harmful to plants at the molecular level. Ascorbate plays a central role in photosynthesis, as is implied by its high concentration in chloroplasts. To deal with this problem, ascorbic acid (AsA) is involved in the regulation of many critical biological processes such as photo-inhibition and cell elongation ([51]. Ascorbic acid is also involved in the cell cycle and many other important enzymatic reactions (e.g. ethylene biosynthesis). In this chapter we have been able to prove that AsA can be considered as suitable and affordable plant bio-regulators to overcome the negative and destructive effects of salt and water stress on the cucumber plant.

According to the data and studies provided by Liang et al. [52], growing cucumbers under water stress conditions had a significant negative effect on the physiological condition of the plant and also resulted in a decrease in the quality and quantity characteristics of the cucumber plant. Secondly, water stress can be an equally serious trade problem for cucumber growers. Therefore, the use of cheap, available and affordable plant-based bio-regulators can be considered as a better and appropriate solution to this problem. But different crops may have different responses to the use of different plant bio-regulators in their cultivation process [52]. Thus, another study conducted by [43], demonstrated that foliar application of AsA can be considered among the main bio-regulators to mitigate the effects of and physiological responses to drought stress damage and effects on morphological, physiological, and biochemical properties.

5. Conclusion

Salinity is becoming a major constraint to crop production, and it is becoming severe with climate change; which could have an impact on food security. Currently, bio-regulators such as silicon, salicylic acid, and ascorbic acid play essential roles in abiotic stresses tolerance such as drought and salinity. These three bio-regulators are among relevant and affordable plant bio-regulators to overcome the negative and destructive effects of salt and drought stress in cucumber plant by enhancing the osmolytes the osmolytes accumulation and improving osmotic adjustment. Besides, they are extremely effective at low concentrations; on the other hand, they are very beneficial for the health of the environment through their low use in terms of quantity applied in the field.

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