

# Caffeic acid helps to mitigate adverse effects of soil salinity and other abiotic stresses in legumes

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## Abstract

Leguminous crops are beneficial components of sustainable crops production. Biotic and abiotic stresses especially salinity is a wide spread environmental problem. It is more profound in the irrigated areas, where the underground water is brackish. Plant vegetative and reproductive growth is adversely affect by salinity and other abiotic stresses like drought and presence of heavy metals etc. Certain organic compounds which are produced at cellular level in plants help in reducing the effect of different stresses through increasing the nutrients uptake efficiency, antioxidants activity and by decreasing the toxicity of ions. Caffeic acid is actively involved in the synthesis of lignin in plants. Increasing the thickness of cell wall and improving plants defense mechanism become active against any stress like ion toxicity and heavy metals contamination. Superoxide radical binds due to use of caffeic acid, thus decreasing the stress of salinity to minimize lipoxygenase function. The ferulic acid is produced through caffeic acid mobilization by o-methyl transferase. Exogenous application of caffeic acid is a good enough option against different stresses like salinity, drought and heavy metals.

## Key words

Nodulation, N-fixation, Antioxidant, Secondary metabolites, Drought, Heavy metals

## Introduction

The leguminous crops play a remarkable role in sustainable crops production. They convert atmosphere nitrogen (N) into the form which is usable by the plants[1]. Soil nutrients status especially N economy improves, if legumes are a part of cropping scenario. Symbiotic relationship of legumes and bacteria helps in this context. The bacterial genera like Rhizobium, Sinorhizobium, Mesorhizobium, and Bradyrhizobium etc., are given special consideration for their role in nodulation and N fixing[2]. Leguminous crops provide the soil with an environmentally safe source of N in comparison with the prevailing use of mineral fertilizers[3]. Certain biotic and abiotic stresses are observed to cause adverse effects on viable symbiotic associations. Stress declines the leguminous crops productivity and their ability of N fixation[4]. Main stressful factors include salinity, drought, heavy metals issues etc.,[5].

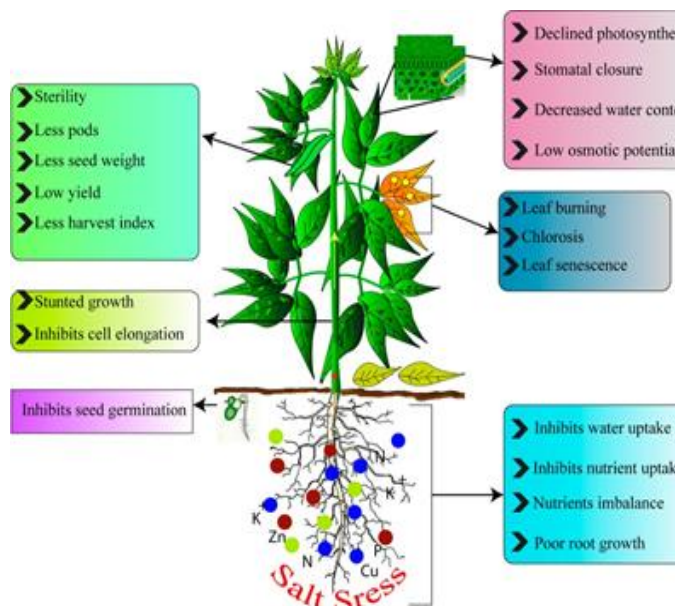
## Soil Salinity and Leguminous Crops

Accumulation of excessive soluble salts in soil is called soil salinity .In Pakistan, salt affected area is 6 mha.It is reported that

70 of tube-well water is brackish in Pakistan. Mostly the underground water contains much more soluble salts. Salt affected area distribution in the Punjab and Sindh provinces is described as 53% and 15.6%, respectively. These area subjected to desertification and abjection. Due to salinity problem the water potential becomes low in soil and plants and consequently the physiological and biochemical processes are affected by various mechanisms. This is how soil salinity exerts negative effects on plants growth and productivity. Legumes show more sensitivity during salinity stress especially at the developmental and seedling stage. Growth process of different legumes like faba bean [6],lentil [7], mung bean [8] and soybean[9] due to salinity has been observed to be affected badly . The reduction in yield of certain legumes at various level of salinity (soil EC) is showed in Table1.

Table 1: Reduction in yield of certain legumes at various level of salinity

Crop	Soil EC	Loss in yield (%)	Reference
Faba bean	6.6 dSm <sup>-1</sup>	50 %	[11]
Mung bean	8.0 dSm <sup>-1</sup>	60 %	[10]
Soybean	7.0 dSm <sup>-1</sup>	46 %	[12]



**Figure 1: Behavior of Plants during Salinity Stress**

Plants have developed defense mechanisms against the external environmental variations. The secondary metabolites produced in plants are important in this regard. These metabolites support the plants to maintain the functioning of their internal system and make them adoptable accordingly to counteract the effects produced due to various environmental stresses. The organic compounds (secondary metabolites) are produced via certain biosynthetic pathways. Production of organic metabolites is a basic tool to strengthen the adoptive response system in plants. These organic compounds are associated with plant maturity, growth and increasing resistance in it against the adverse effects of harsh and unfavorable environment [13].

Phenolic compounds are essential metabolites, including flavonoids and phenylpropanoids, synthesized by plants. An important phenylpropanoid is caffeic acid (3, 4-dihydroxycinnamic acid), present in plants. Caffeic acid is a primary precursor for the formation of lignin in plants. It is associated with the turgor pressure, water flux, growth, regulation of cell expansions and phototropism[14]. Caffeic acid, and its by-products play an important role in plants against biotic and abiotic stresses including UV light, drought, low and high temperature stress, salinity stress and heavy metals stress etc. For example, the caffeic acid accumulation under fungal attack prevents the plant from fungal disease like brown rot[15].

It has been reported that caffeic acid increases the resistance against diseases/pathogens in apple plants [16]. The exogenous addition of caffeic acid as 500 micro gram ml<sup>-1</sup> inhibits the *saccharomyces* and *fusarium* species growth [17]. The plants under stresses (biotic and abiotic) accumulate the caffeic acid [18,19,20,21] and is reported that during salinity stress the level of salt tolerance increases in plants due to the accumulation of

caffeic acid. Caffeic acid is a powerful antioxidant that increase the plant stress resistance. It also oxidizes the reactive oxygen species (ROS), thus, scavenging the plant from free radicals[22,23].

### Caffeic Acid Biosynthesis

Caffeic acid is synthesized from phenylalanine. As a first step the cinnamate is produced from phenylalanine in the presence of phenylalanine ammonia-lyase (PAL) enzyme[24,25]. Some other various by products are produced by methylation, dehydration and hydroxylation reactions that regulate the internal physiology of plant under different stresses. In the second step, from cinnamate, p-coumarate is synthesized in the presence of cinnamate 4-hydroxylase (C4H). In some microbes and plants, p-coumarate is also synthesized directly from L-tyrosine in the presence of ammonia-lyase (TAL) enzyme [26,27].

Finally, caffeic acid is produced from p-coumarate by hydroxylation. Caffeic acid is further used in the formation of lignin and some organic compounds associated with plant stress tolerance [28,29,23]. Plant's physiological processes alter in stress condition due to production of reactive oxygen species (ROS) [30]. These ROS are harmful and sharp for plant organelles and membranes. Reactive oxygen species oxidize and disintegrate cell structure [31]. The common ROS are hydrogen peroxide, singlet oxygen mediated hydroxyl radicals and superoxide radicals etc.[32,33,34]. The mitochondria and chloroplasts are main sources of ROS which contain more energy actions and are associated with a more flux of electrons [35,36]

Plants have a system to destroy the ROS. This mechanism is associated with various enzymatic and non-enzymatic antioxidants[37]. Several studies showed that the caffeic acid was an antioxidant which played an important role in plants physiology as well as pharmacology. Caffeic acid restricts the lipoxygenase activity to prevent the cell membrane and organelles from being oxidized [38]. The caffeic acid and its derivatives like rosmarinic acid, ferulic acid and chlorogenic acid are distributed in plants. When cellular physiology of plants is disturbed due to release of free radicals. It is neutralized and stabilized by caffeic acid derivatives that have ability to donate the hydrogen[23]. Caffeic acid plays an important role in the scavenging of alkoxy group generated due to break down of metal ions in hydro peroxide enriched methyl linoleate [39].

### Caffeic Acid Metabolism

1. O-methyltransferase change the methyl set to caffeic acid and transfer into ferulic acid [40].
2. CoA ligase breaks the synthesis of feruloyl-CoA from ferulic acid [41].
3. Caffeoyl-CoA is produced by mix up of CoA in caffeic acid through CoA ligase[42].
4. Chlorogenic acid is formed by the addition of caffeic acid in quinic acid[28].
5. Cichoric acid is also produced from caffeic acid in plants.
6. Sinapic acid is formed by the successive caffeic acid methylation [43].

### Caffeic Acid Functions under Various Stressful Conditions

Species of plants	Stress	Function OfCaffeic Acid	Reference
<i>Helianthus annuus</i>	Stress of Radiation Stresses of Salinity Deficiency of nitrogen	Enhanced activity of antioxidant ,lignifications increased and pattern of growth change	[44]
<i>Solanum tuberosum</i>	Drought Stress	Minimum addition of ROS, Enhanced the activity of chloroplast and mitochondrial resulted in lesser Stress Load	[45]
<i>Zea mays</i>	Drought	Antioxidants function increased	[46]
<i>Citrus unshiu</i>	Drought stress	Shoot and root growth increased	[47]
<i>Glycine max</i>	Salinity Stress	Antioxidant function increased by improved the ROS scavenging capacity	[19]

### Symbiotic relationships with plants

Certain microbes present in soil develop mutualistic relationships with their specific host plants species and improve the plants growth and development. These microbes include fungi,

actinomycetes and bacteria etc [48]. The most important and widely explored microorganism that mutualistically get attached to their host plants are bacteria. Among soil bacteria, rhizobia play very important role to develop symbiotic relationship with leguminous plants. They cause nodulation and fix atmospheric nitrogen into ammoniacal form [49]. Caffeic acid and its derivatives have been reported to be involved in the function of leghaemoglobin and nitrogen fixation in nodules of roots[50,20].

### Exogenous Application of Caffeic Acid and Symbiosis

Addition of caffeic acid under stress condition increases the functioning of nitrogen fixing rhizobium [20]. Caffeic acid is an antioxidant which helps at enhance the enzymatic level in the fixation of nitrogen. Exogenous addition of caffeic acid raises the carbohydrates and protein quantity in nodules[50,51,52] and was reported that caffeic acid had exhibited significant effects on the arbuscular mycorrhizal (AM) relationship with plants.

The losses in crops yield are about 50 to 70% due to different abiotic stresses. Caffeic acid helps in developing the plant defense mechanism under different stresses and makes the plant enable to withstand the harsh environmental conditions [53].

### Caffeic acid and Salinity

The 30% of the world of crop production is lost due to salinity stress. Under salinity stress, plant physiological processes are disturbed due to absorption of harmful ions (sodium and chloride) in excessive quantity. Addition of these toxic ions produce ROS. The metabolic processes in plants in connection with respiration, photosynthesis, cell division etc., are disturbed. These ROS destroy the cell membrane, organelles structure and bio-molecules like DNA and RNA, when produced in high more quantity[35].

Caffeic acid is known as a strong antioxidant that oxidizes the ROS in plant cells[18,19] was reported that caffeic acid had ability to shield the plants from superoxide radicals. Caffeic acid suppresses the activity of lipoxygenase from peroxidation of lipid to protect the cell organelles [22]. Plant nodulation has been improved under salinity stress by exogenous use of caffeic acid. It was reported that caffeic acid had produced nitric oxide which reduced the salinity stress on nodulation by the mechanism which included cyclic guanosine monophosphate mediated signaling coupled with nitric oxide signaling to capture the ROS [20]. They concluded that addition of caffeic acid decreases the stress of salinity to show binding with superoxide radicals.

### Caffeic Acid and Drought

Drought stress declines the potential of yield of the crops up to about 54 to 84% .The plant vegetative and reproductive growth is affected by the shortage of water because water is necessary at every stage of plant growth and seed germination [54]. Plant physiology under drought condition is managed by caffeic acid and its derivatives in mesophyll cells as high energy molecules are absorbed by caffeic acid under drought stress .In this mechanism o-methyltransferase catalyzes the caffeic acid by methylation and produce ferulic acid [40]. During drought stress, the photosynthetic activities are controlled by the existence of more concentration of caffeic acid and ferulic acid [55].

### Caffeic Acid and Heavy Metals

Increasing the population day by day and expansion in the

industries have become a big threat for the agriculture in this modern age. These ever growing industries and expanding municipalities are main sources of contamination of heavy metal. Plant growth is affected in various aspects by the heavy metals. It was reported that plants growth is restricted and the biomass decreases due to the presence of heavy metals in elevated concentration [56]. Caffeic acid shields the cell from damaging action of heavy metals by production of lignin in cell wall and increasing its resistance against physical obstacles [57].

## Conclusion and Recommendations

Effects of salinity hamper the vegetative growth of legumes crops and their developments through modifying the nutritional imbalance, the hormones complicated associations, osmotic influence and the high level of specific ions. It has been reported that caffeic acid and its byproducts develop the plant mechanism against the abiotic stresses (salinity, drought, heavy metals). Caffeic acid minimizes the lipoxygenase function and protects the membrane of cell organelles from lipid peroxidation. It is concluded that the use of caffeic acid show positive effect during salinity and other abiotic stresses to oxidize the ROS and bind the superoxide radicals. However to understand the secondary metabolites and role of caffeic acid against different salinity stresses, experimentation are yet necessary.

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