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Effect of Ascorbic Acid on the Wilting and Resistance of Capsicum Annum L. Plant

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Abstract
The change from reversible to permanent wilting in whole red pepper(*Capsicum annum*) followed by a very harsh salt stress of root exposure to 200 mM solution of NaCl.It was noticed that a seed or seedling present in saline soil was wilted very quickly, but if replaced to non-saline soil within 6 hours then seed start recovery however if the duration is increase and it reaches to 9 hour, then the salt stress make it plastic *i-e* remained wilt and died but the addition of antioxidant (0.5mM ascorbic acid) to the root medium and during at the time when the plant is in salt stress condition, it recover the stress almost 50% of wilted seedling others solution which containing organic solution they does not effective like anti-oxidants (vitamin C), The salt severely damage the root, stem and leaves by accumulation in them.
Keywords: Red pepper, ascorbic acid,wilting.

Introduction

Negative plant response to salinity depend on the toxic effect of salt and also on condition as well as the duration of the actual stress the plants strain (response to salt stress, is variable from growth inhibition and accelerated leaf senescence under a normal stress. While in severe stress permanent wilting of shooting occur the process underlying these different responses to the adverse effect on salinity is still far from clear [6], [4]. This report investigates that fast change from reversible to permanent wilting in Capsicum annum seedling when face to high level of NaCl salinity. One condition was that salt changed cellular storage of damaging active O2 species might be involved as we know about per oxidation that free oxygen can damage the lipids protein, DNA that why the active oxygen species can damage essential membrane lipid as well protein and nucleic acid [3] &[7]Level Active oxygen which is present in plants, when the condition is normal and suitable for growth, than the plant produce antioxidant which prevent the work of antioxidant and that is called protective antioxidants. However, when the condition is not suitable the productivity antioxidants become low and don't stop the peroxidation [10] & Park,[8]. There may be anti oxidation enzyme has been reported. Their report also fallows another technique which would be to increase the cellular level enzyme substrate and that is vitamin C (ascorbic acid). As we know that vitamin is water soluble so if we give the vitamin C from the outer source, so that act as primary substrate in the cyclic pathway for enzyme detoxification of hydrogen per oxide, by the addition of this it act directly to neutralize nascent oxygen (atomic O2 which is highly reactive)
This report investigates the following facts.
A. Red pepper seedling face permanent wilting when it grow in salt stress area or soil and is associated with salt increase in cellular level of damaging active oxygen species.
B. Whenever we add ascorbic acid (vitamin c) which is additional to the root of the seedling, so it decreases the buildup of active oxygen which decreases the peroxidation. So the role of ascorbic acid is so important.

Material and Methods

Red pepper seed was germinated in without light for 2days and then for their growth the polystyrene floats with the root held in aerated 0.1 straight modified hog land solution plus MCaCl2 for the purpose to maintain the activity and work of calcium at an adequate level. The chamber i-e growth chamber was at 27± 2 C0 with 12 hrs a day and 150 µmol S-1M-2 light (power intensity). The humidity is also variable during days and night as well as i.e. the day 45% and at night 65%. Before treatment of salt 12-16 uniformed seedling were placed in incubator also with their root in 4.01 of nutrient minerals solution contain different nutrients probability with the addition of freshly prepared 0.6mMascorbic acid for 22hr when this duration is wind up then the seedling were again transferred to fresh nutrients solution ± 200mM NaCl ± 0.3 mm Ascorbic acid. NaCl at 200mm rapidly changed completely wilting of shoot. Where the solution of polyethylene glycol (PEG) is also used to activate the osmotic effect of 200 mM NaCl.

Ascorbic acid (vitamin c) the effect of external supply on the tissue organization of Ascorbic acid was determined. The seedling which is germinated were incubated with or without 0.6mM for 22 hrs and then by the additional 9 hrs with or the absence of 200 mMNaCl. After rising 0.6 mM CaCl2 stem, leaves and root they are freezed by liquid nitrogen and ground mortor ground tissue then mixed with9ml of 9% TCA and when the supernatant obtained after centrifugation in 1600gm for 13—16mm at 3c was analyzed [9].

The compression rate of lipids were tested redetermine the level of malondialdehyde in 2 gm of leaf, root and stem. The product of lipids peroxidation is malondialdehyde which was then examined by thiobarbitraic acid.When extra Ascorbic acid or NaCl is added to the extraction medium so when this is add did not affect the measurement without addition of reactive TBA. Three separate experiment which include 8 or more plant per treatment result for root stem and leaves in each mean. The effect of Ascorbic acid which is possible on the accumulation of sodium in the stem of salinized seedling. Intact seedling were raised 0.6mM CaCl2 and 3 cm is exiced from the base of the stem when dried by oven for 68hrs at 71C0 the powder weighted and incubate in 8 ml 0.3 NaCl for 44 hrs , the filtration and determination of contents of ion by ICP test.

Results

The ascorbic acid increases the capacity of seeding to recover from salt stress.Root when expose to severe salt stress (200 mMNaCl) and that cause rapidly and complete wilting with the visible collapse of stem and leaves, resistance was tested by determine the recovery of wilted red pepper seedling when it transfer to non-saline medium the recovery was observed in 60 mints, but the seedling show recovery because that was placed for 6 hrs or 5 hrs with saline to non-saline medium.

However when the seed is placed in saline medium for longer time i.e. (8 to 9 hrs.) resulted will be in progressive decrease in recovery thus after 9 hrs. Seedling remained wilted and later died (0% survival) despite the transfer back to non-saline condition. If we make the distinguish between osmotic and toxic effect the seedling treatment to similar using a non-penetrating polyethylene glycole instead of NaCl. Treatment of the seedling with polyethylene glycol and contrast to the NaCl. Seedling to which we provided polyethylene glycol solution at 1.35 mpa osmotic potential wills completely wilted.Most importantly the addition of ascorbic acid (vitamin C) to the root increase the capacity of survival treated with 200 mM NaCl for 7,8,9 hrs. Thus seedling treated for 9 hrs. With 200 mM NaCl alone doesn't showed any fruitful result and 0% of recovery however c.50% of seedling treated for 9 hrs. With 200mM NaCl and 0.4mM of ascorbic acid by the mixture of these showed repaired recovery after return to non-saline medium. Chemically analysis showed that root addition of ascorbic acid(0.4mM) for 33hrs (24+9hrs) increase seedling level from 2.4 ±0.2 µmmol g^{-1} FW to 3.1±0.3 µmol g^{-1} FW in salinized seedling and form 2.3±0.2 µmol g^{-1} FW to 2.3±0.2µmol g^{-1} FW to 3.1±0.2µmol g^{-1} FW in non-saline seedling.So we make a comparison on the protective effect of ascorbic acid on the survival and that of small organic compound without know anti-oxidant activate when we add glucose, glycine, beatine, leucine or proline to root each at 0.4 mM have very little and sometime no effect on seedling survival of 200 mM NaCl treatment for 9 hrs.So the addition of ascorbic acid makes it,s survival complete.

One of the expected result of stress changed celluler build up of active oxygen species increase the peroxidation. The assay of celluler accumulation of lipids per-oxidation products if we provide thiobarbituric acid reactive substance (TBARS). Salt stress change progressive increase in accumulation of TABRAS in root, stem,and leave of salt stressed seedling as 6n, 9n.

Treatment	TBRAS (µmol g-1 FW)		
	1 hNaCl	5-6 hr NaCl	7-9 hr NaCl
Root	555±60	812±47	1014+48
Root+Ascorbic acid	385±5	628±11	510+42
Stem	200±17	325±22	690+35
Stem+Ascorbic acid	212±21	316±35	465+13
Leaf	360±18	560±20	846+15
Leaf+Ascorbic acid	365±15	424±13	547+22

Effect of Different Salt Stress on the *Capsicum annuum*.

Discussion

The wilted Red Pepper (*Capsicum anum*) seedling showed an awesome ability for shoot recovery up to 6n of severe salt stress treatment with 200Mm NaCl. HoweverGreen way et al 1980 observed a same capacity for shoot recovery in *Red pepper* plant (seedlings) in a day one or more cycle daily cycle of wilting by Air drying for half an hour and then back the root to aqueous media [5], But the loss of the ability to recover from salt stress and change to permanent wilting, does not appear. The results showtransparency that complete and permanent wilting of 100% of the seedling change by the 9h of salt treatment has relation to uptake and toxicity of NaCl But a suitable treatment of seedling for 9h with a solution of non penetrating PEG 500 in place of NaCl did not

change permanent wilting.The external supply of Ascorbic acid (vitamin c) help in increase tissue and cell growth levels in Red pepper seedling. As show in the previous report showing plant uptake of external ascorbic acid [1]. The ascorbic acid (vitamin c) addition increased the percentage of seedling of *Capsicum annum* able to survive the toxic effect of a 9h expose to NaCl. With this if we use organic carbon sources without direct antioxidants activity. The remarkable protective effect of external ascorbic acid is appeared like its antioxidants activity.

Several result show that the salt induced transition to permanent wilting. A few attempts indicate that the salt induced transition to complete wilting occurs in plants were related with enhance in the cellular activity of damaging active oxygen species (AOS). AOS and the effect of ascorbic acid on the seedling survival were associated with partial inhibition of these increases.

A) The level of TBARS in leaf, root, stem and these parameters which show in the experiment, their tissue increased also their development increase when the plant is in stress condition.

B) Providing external ascorbic acid is constantly change and almost reduced salt induced accumulation of TBARS and also reduces the seedling death because the TBARS also reduced stress at some suitable level.

When the TBARS is accumulated in roots, stem, leaves and other effected part of the plant which is salinized treated with stopping growth effect of ascorbic acid was appear after 6h of NaCl treatment. All the suffer seedling have the capacity for rapid recovery i-e that tissue which is treated with the additional ascorbic acid is still alive. Although the stopping growth and change in growth effect of ascorbic acid on lipid breakdown and increased seedling survival appear to be related. But the real circumstances is not clear know one condition was that additional ascorbic acid would stop stress change increase in the leakage of essential electrolyte follow that damage which is caused by peroxidation (Blokhina, et al 2003). However the ascorbic acid which is add or introduced not stop or increase the leakage of electrolyte from the root of that Red pepper plant. which is salt effected , and also do not help in accumulation of NaCl in the different parts. Finally the face is that leaves and roots were produced by the seedling which have got recovery from 9hr of salt treatment with ascorbic acid which is suggests that the ascorbic acid which is add may effect that tissue which have the ability of cell division in the salt stressed root and shoot tissue, while the low ascorbic acid may have been touch with mitotic quiescence in the root dividing cell and conversely treatment of with exogenous ascorbic acid was touch with an increase from 33%-53% in the normal root and shoot emergence from somatic embryo (Tonic2000). So the above report show that root treatment with the ascorbic acid which was exogenous can increase the ability of red pepper plant to survive under very harsh condition i.e. when the plant is kept 9hr in the salt stress which almost lethal for that the plant increase its resistance capacity is associated with antioxidant activity of ascorbic acid.

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