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Effects of salt-stress on root and shoot growth in avocado

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I'll be talking at the beginning of the presentation about the effect of salt stress on root and shoot growth in avocado, and mainly present results of controlled experiments. I'll then shift over to Miriam Zilberstaine to show some of our field results.

Avocado is known, as Steve wonderfully reviewed for us this morning, as the most sensitive fruit tree to salinity. We have the data to demonstrate that the threshold level for salinity is lower than for any other crop plants, and that the slope, the reduction in the growth, as affected by increased salinity level is very high.

Surprisingly, we have very little data about the mechanisms of the salt effect in the plants. We are going to present some first indication and demonstrate that the salinity processes of sensitivity to the stress in avocado might be a little bit different than in many other plants. This may suggest the reason for the increased sensitivity to salinity for avocado.

One of the things that is well known and which is one of the enigmas in salinity research, is that, although the first organ of the plant that is exposed to salinity is the root system, when we check to see what is first plant organ affected by salinity and what is most affected by salinity, we find that it is actually shoot growth that is more affected in most plants, and not root growth.

We have very little information about the effect of salinity on the roots in avocado. I was very happy about the discussion this morning which focused so much on the root, which we'll use as a nice introduction to the work that we are doing.

Some information in the literature suggests that avocado roots might play an important role in the tree response to salinity. For one thing, there was a study by Steinhardt and co-workers that demonstrated, as in many plants, under salinity, there is a reduced capacity for trees to take up water from the soil.[1] In avocado, however, it is at much greater levels than what we see in other crop plants.

In that study which was summarized in 1992, it was found, and this was mentioned before, that the response to salinity under field conditions is usually initiated after five to seven years of salinization. And this may also suggest that the effect of salinity might occur via the roots, or there is an important role to the roots, more so than in other plants, because it takes time for the root system to be affected.

The question that we decided to evaluate several years ago is to see whether the extreme sensitivity of the avocado to salinity results from some extreme or some unique root response. In general, the root system of avocado is very unique; it is different than

[1] Steinhardt, R., D. Kalmar, E. Lahav, Y. Shalhevet, and A. Naor. 1987. Proc. Intl. Conf. Utah. Page 61.

many other plants. It is very shallow, as was mentioned before, and in most of our experiments in Israel and as others found around the world, we found that the active parts of the roots are at the maximum depth of about forty or forty-five centimeters. This was mentioned before, and we will show some data to that effect as well. In addition, the branching level of the roots is very low, which means that there is very little root area as well, which minimizes the ability to take up water, and possibly also the ability to take up nutrients.

The objective of the project and the data in this presentation are from our study which evaluated the avocado root to salinity. I will present data from two studies. First, how does the root respond to salinity in comparison to the shoot? Secondly, we conducted some initial studies to understand the mechanism of ion relations for avocado root growth in response to salinity stress.

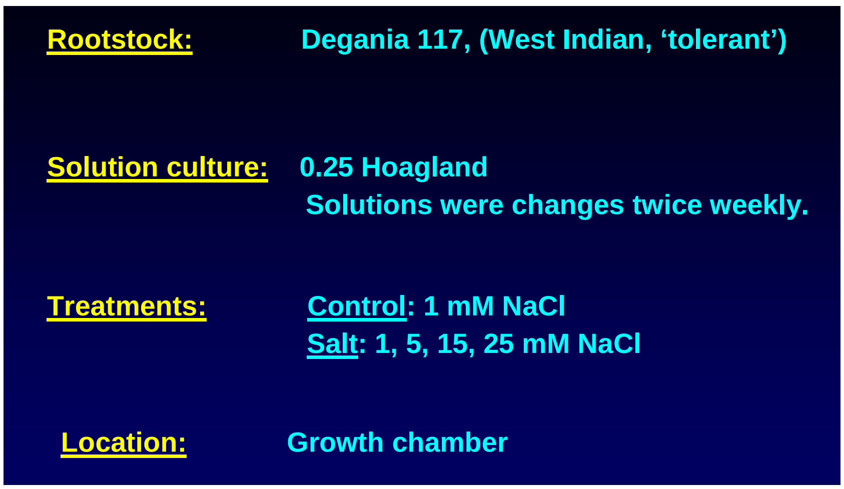

Figure 1: *Parameters of the controlled experiment using solution culture.*

The first study that we did was using Degania 117, a West Indian rootstock (Figure 1). In Israel it is considered to be relatively salt tolerant. The experiments were conducted in solution cultures, and the salinity levels for the control were kept at 1 mM sodium chloride. We then elevated the salt level to 1, 5, 15, or 25 mM because we had to first identify at what salt concentration the root and shoot growth are affected. At the beginning, these were growth chamber experiments since we evaluated shoot and root growth; we wanted to make sure that we were working under controlled conditions.

And I want to start almost with the end of this talk by showing that we were absolutely shocked to see the responses of the roots to salinity. For some yet unclear reason, the control plant in (Figure 2) was grown under 1 mM sodium chloride, and the other two treatments were 15 mM NaCl, and 25 mM NaCl. Taking into account the fact that these plants were transplanted, they were germinated in soil, and then moved to the different salt conditions, there's very little growth that actually occurred under 25 mM.

From this picture one can already see that there was a dramatic inhibition of root growth under salinity stress. When we look at the shoots, which we will look at in more detail, we see that the salinity effect on the shoot is similar. Already from this image it is possible to see that the avocado behaves differently in response to salinity than most known crop plants.

Figure 2: *Responses of seedling Degania 117 roots to various salinity levels (1, 15 and 25 mM NaCl) in a controlled experiment using solution culture.*

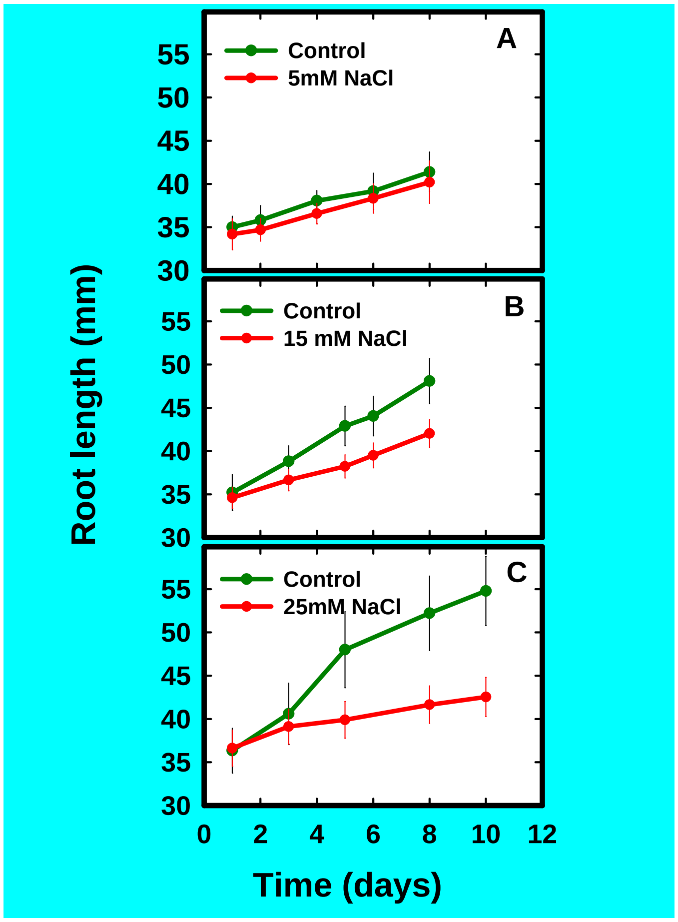

Figure 3: *Effect of salinity on root elongation in seedling Degania 117 over time with various salt concentrations (5, 15 and 25 mM NaCl).*

We measured which parameters in the shoots and in the roots were affected. What I would like to show here is the root length, or the increase of root length under different treatments (Figure 3). We compared 1 mM the control to 5 mM, and found no effect. We're looking at root length versus time. When we compared 1 mM to 15 mM, we found out that there was a reduction in root growth. Comparing 1 mM to 25 mM, we found out a much larger reduction in root growth. So the threshold for salinity effect on the root elongation falls somewhere between 5 and 15 mM sodium chloride, and a very drastic reduction occurs at 25 mM.

When we calculated rates of root elongation, we found out that at 15 mM sodium chloride root elongation was reduced by 43%. This is an incredible percentage. At 25 mM sodium chloride, there was a 75% reduction.

When we looked at what happened

to shoot growth, in the 25 mM sodium chloride concentration, leaf production was reduced by 15% only. Where root elongation was reduced by 75%, shoot elongation was only reduced by 15%. Thus, this is an incredibly unique crop. Figure 4 shows shoot growth under the three treatments 5 mM, 15 mM, and 25 mM, with very similar appearance. We see a slight reduction in growth in the higher salinity levels.

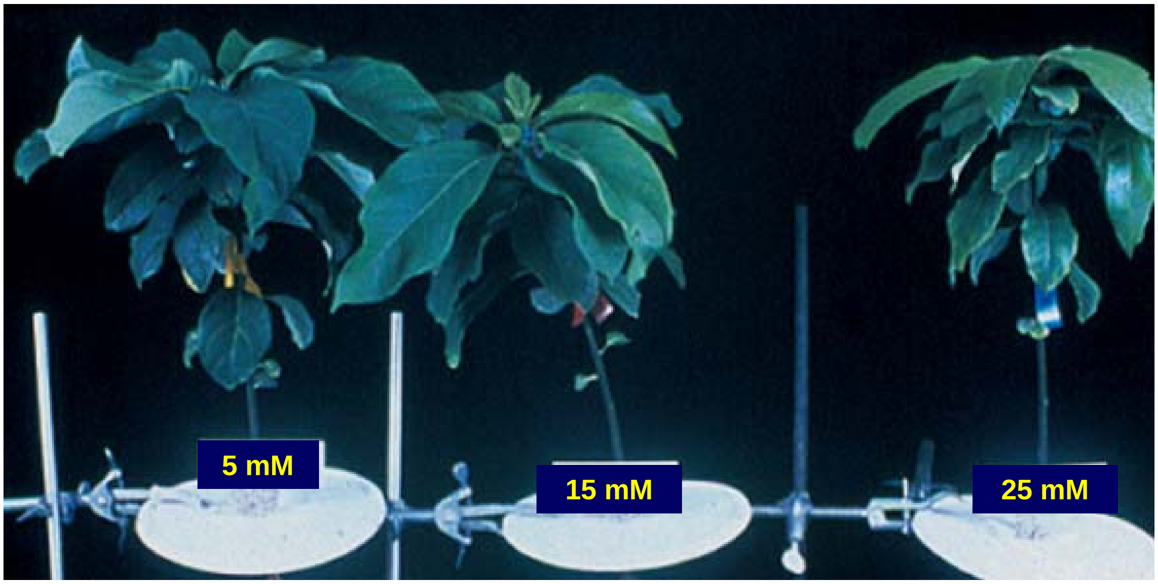

Figure 4: *Responses of shoot growth of seedling Degania 117 to various salinity levels (5, 15 and 25 mM NaCl) in a controlled experiment using solution culture.*

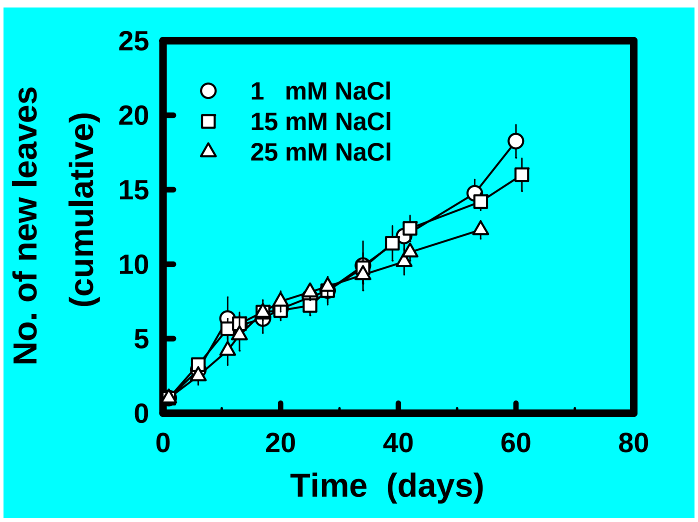

Figure 5: *Shoot growth of seedling Degania 117 was reduced 12% at the higher salt concentration (25 mM NaCl).*

We looked at whether leaf initiation is being affected under saline conditions (Figure 5). This is the cumulative number of leaves that were initiated on the plant in 5, 15, and 25 mM. We found that in the 15 mM treatment there was no significant difference in leaf appearance. But in the 25 mM NaCl treatment there was a 12% reduction, a small reduction in leaf growth.

When we looked at the total generation of leaf biomass on the plant (Figure 6), here also there was a reduction under salinity, but a small reduction under salinity. Under 15 mM sodium chloride it was only a 10% reduction in total leaf biomass production. Under 25 mM sodium chloride, there was 20% reduction in leaf biomass production. Again, there was relatively small reduction in biomass in comparison to the very large reduction of root volume.

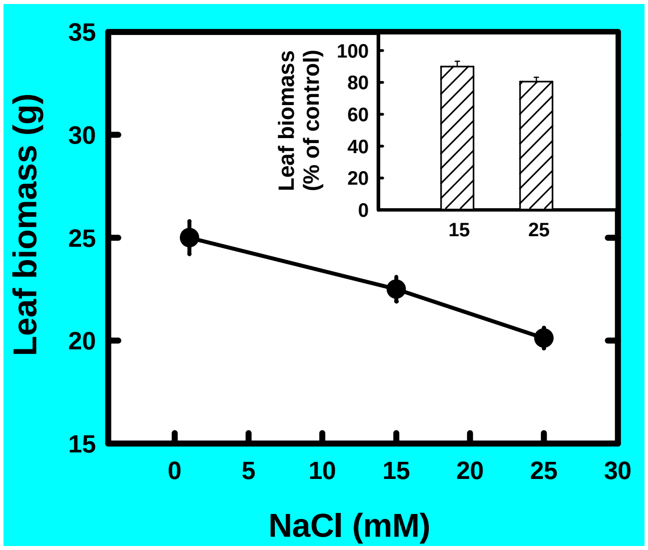

Figure 6: *Leaf biomass production was reduced in seedling Degania 117 plants by 10% (15 mM NaCl) or by 20% (25 mM NaCl).*

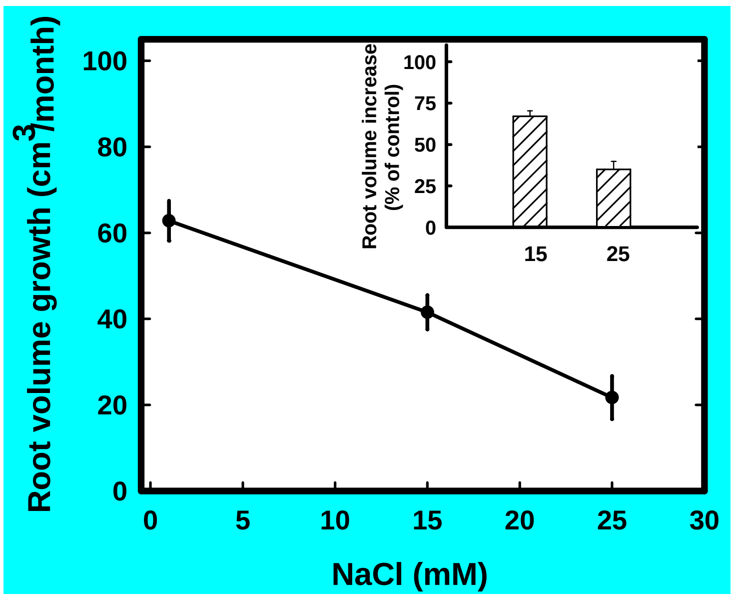

Figure 7: *Root volume was reduced in seedling Degania 117 plants by 10% (15 mM NaCl) or by 20% (25 mM NaCl).*

These are the root volume results. This is an unfolding experiment. These are the representative results from different experiments in which we tested to see whether the phenomenon is repetitive. This was to check root volume growth. We were concerned that in the first experiment we only measured elongation growth, and may have missed the effect on root branching and root thickness.

We evaluated the overall root volume growth (Figure 7), and again we saw that in 15 mM sodium chloride there was a 33% reduction in root volume growth, and 65% reduction under 25 mM. So this result is highly repetitive, and represents all phases of root development: root volume, root weight, root elongation, and root branching as well.

We knew that the Degania 117 as a rootstock is very salt tolerant, and we were curious to know what happened with other rootstocks? So we've looked at Schmidt, which is a Mexican race seedling rootstock (Table 1). It is sensitive to salinity. We found that the responses to salinity were similar to Degania 117 in the sense that the root growth is, again, much more sensitive to salinity than shoot growth.

Since all these experiments were carried in solution culture, and since we were dealing with root growth, the next step was, of course, to check what happened in plants that were grown in soil. So, we've moved to plants grown in containers for the first time. And the first experiment we've conducted was with Degania 117, and the salinization duration was for 60 days. We received very similar results to what we found in solution culture.

Table 1. *The reduction in root and shoot growth in the Schmidt, a Mexican race seedling rootstock in response to saline solution culture.*

	Reduction as a percentage of the control treatment		
Saline treatment	Root elongation	Root volume	Leaf biomass
15 mM NaCl	-	35%	12%
25 mM NaCl	65%	60%	24%

We then conducted a much larger experiment, comparing seven rootstocks, under a salinization duration of 3 years. The idea was to check what happened with several years of salinization to a wider varieties of ungrafted rootstocks. It is important to point out that the rootstocks that were selected are what Miriam Zilberstaine usually referred to as the "team". These are the best rootstocks that we have in terms of salinity tolerance that came out of Dr. Avraham Ben Ya'acov's selection project. I know that VC 803, VC 804, and VC 256 are also known for Phytophthora tolerance, and some of them are being tested in California as well. The containers were 50 liters containers, in a screen house and the rootstock age was four years. The salinization duration was 3 years, and the salinity level in the control was 250 milligram chloride per liter and the salinity level was 750 milligram chloride per liter.

Figure 8. *Parameters of field study using ungrafted seedling rootstock material.*

I would first like to demonstrate is what happened to shoot growth. We found that branch weight to be a good representative of growth of biomass production and new branches for comparison between different rootstocks.

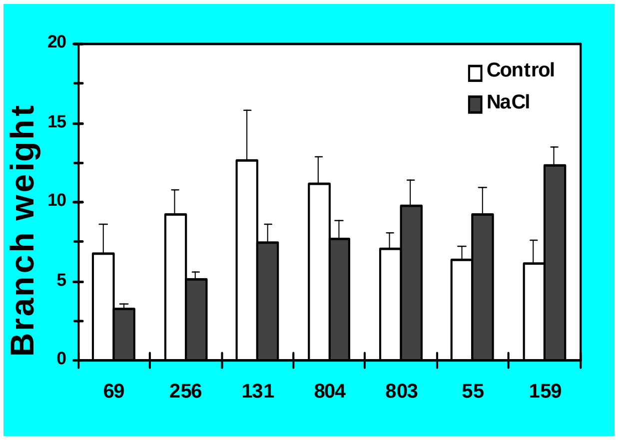

Figure 9. *Growth of seven ungrafted seedling rootstocks under saline conditions. Control was 250 mg Cl/L vs. the salinized trees, 750 mg Cl/L.*

When we look at these seven rootstocks (Figure 9), the black is for salinity and the white is for control, first of all, we see that there is huge variability in response to salinity even among those West Indian "team" best salt tolerant rootstocks. For example, we can see that the four rootstocks on the left, the growth was reduced under salinity, but, amazingly, the three rootstocks on the right the growth of the shoot actually increased under salinity. We arranged the rootstocks in this figure based on their tolerance to salinity. The most sensitive ones are on the left and the more salt tolerant ones are on the right-hand side.

The way we evaluated root growth was by cutting plexiglass windows inside the containers, and looked at the angles the roots grew along the windows. We took measurements every couple of days, marking the growth with different colors (Figures 10, 11 and 12) and then we digitized the images and we calculated the root elongation rates. These are representative results from the data (Figure 13). We looked at the elongation of the root tips, root elongation rate, again with the different rootstocks. They were arranged according to the same sensitivity levels that we found from the shoot data.

Figure 10. *Image 1 of root growth.*

Figure 11. *Image 2 of root growth (after several days).*

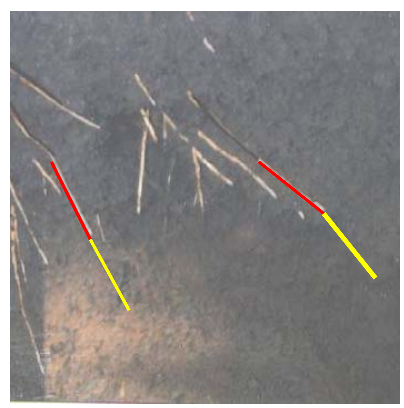

Figure 12. *Image 3 of root growth (after several more days).*

The two important questions were:

1. To see whether the root growth is indeed more sensitive to salinity than the shoot growth. Our results demonstrated exactly this. In all the rootstocks, the sensitivity of the root growth to salinity was slightly higher than the sensitivity of the shoot growth

(Figure 9 vs. Figure 13). This again confirms the results from the solution cultures that the avocado is very unique in this respect.

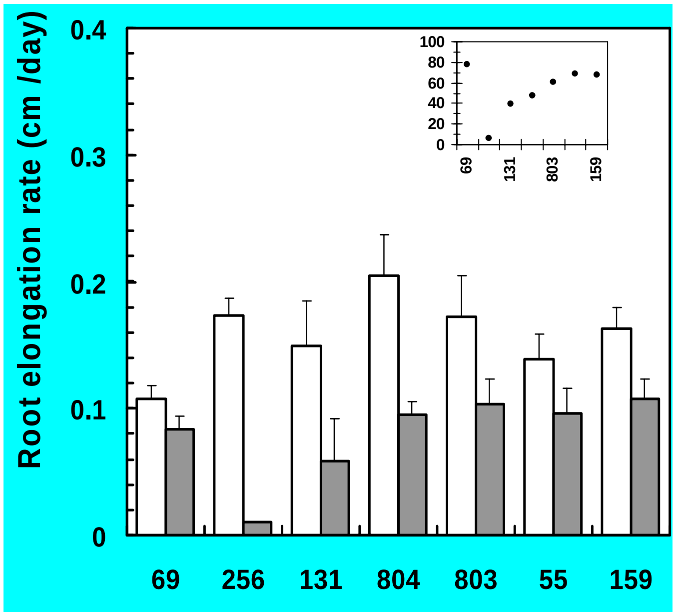

Figure 13. *Root elongation rate of seven ungrafted seedling rootstocks following salinization (250 mg Cl/L vs. 750 mg Cl/L).*

2. If we exclude the first rootstock, VC 69, and we look at the rest of the rootstocks, we see that the extent of sensitivity increases from left to right. So that means that the sensitivity of the rootstock to salinity, if we exclude VC 69, is similar to the sensitivity of the shoot. Therefore, it is possible that the inhibition of root growth highly correlates to the inhibition of shoot growth.

I want to show two slides (Figures 14, 15) which are not directly related to salinity, but are important because they connect us to the field on the one hand, and also demonstrate that avocado roots are not only sensitive to salinity but to other soil conditions as well. These are results of experiments conducted with grafted trees in the field. We dug a trench along the rows, and between the rows. We washed some of the soil's surface to expose several millimeters or centimeters of the roots, and we collected the images of what the root profiles looked like.

I only want to show the presentation from depth of 5-35 cm of the soil, because this is where we find the bulk of the root system. Below 40 cm there is only a small amount of roots. We show a comparison between roots of plants which were irrigated with tap water and roots of plants which were irrigated with recycled water. It is very easy to see, that if we look at the profile, in the tap water we see a very large number of white active root tips growing, whereas in the recycled water we see a lot of black roots, which does mean that there is a lot less root growth.

Figure 14 is from Hamaapil where a surface drip irrigation project was conducted using 'Hass' grafted on seedling VC51 rootstock. Figure 15 is from Ein-Hachoresh which is heavy soil, but we had identical images from the much lighter soil at Hamaapil as well. It is important to note that in this specific case, there is no difference in the salinity of the two types of waters. Therefore, we're not talking about salinity, but of some other factor, possibly boron, possibly the effect of the soil. Since growers are talking about switching to irrigating with recycled water, this is an important issue that has to be discussed later; it definitely has to be looked at.

I would like to show some initial results where we try to focus, or at least begin to look at what is the basis for the increased sensitivity of the avocado roots to salinity. What we are looking at, and Steve Grattan and others have already mentioned, that under salinity it's not only the toxicity of sodium and chloride that reduces growth, but also the effect of ion imbalance, such as, possibly reduction in uptake of potassium or calcium. We try to see whether the effect of salinity on growth is related to the ion concentration in the tissue.

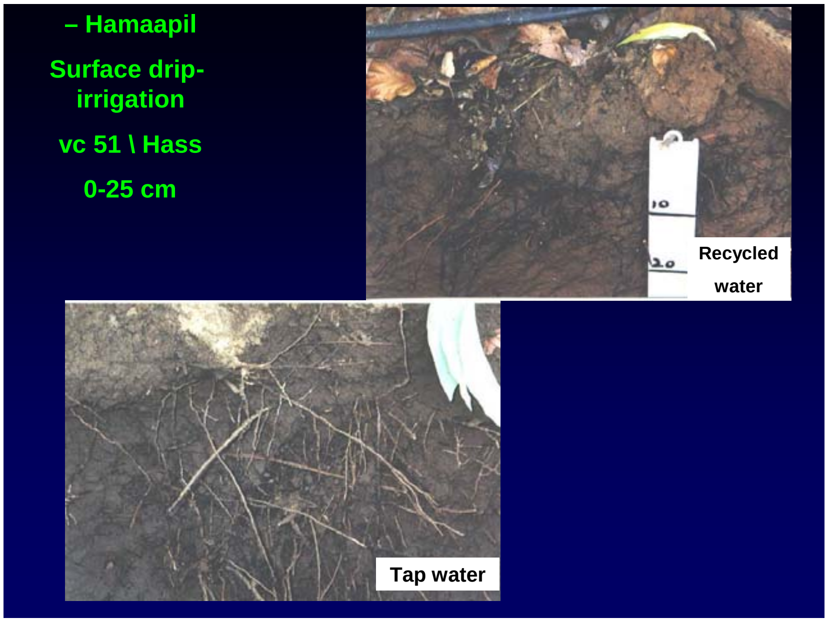

Figure 14: *Comparison of avocado roots (VC 51 seedling rootstock) watered with tap water vs. recycled water at Hamaapil.*

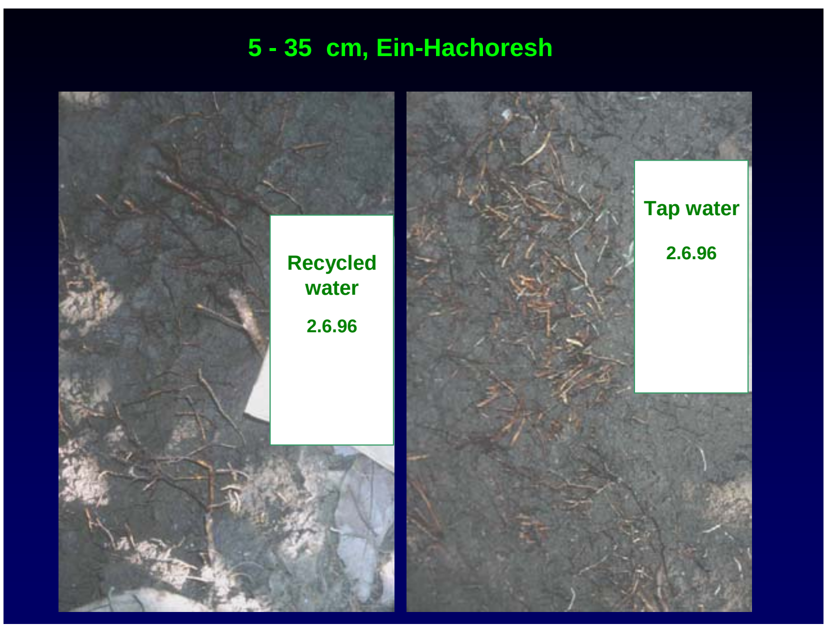

Figure 15: *Comparison of roots watered with tap water and recycled water at Ein-Hachoresh.*

I don't want to go into this entire complicated figure (Figure 16). I just want to show the results for four elements, sodium, chloride, nitrogen, and phosphorus. We look at the extent of change in the ion concentration due to salinity as related to a change in growth, i.e. growth reduction in the roots. Data that is more to the left side on the x-axis means more growth inhibition, and where the percentage is higher than 100% that will mean accumulation.

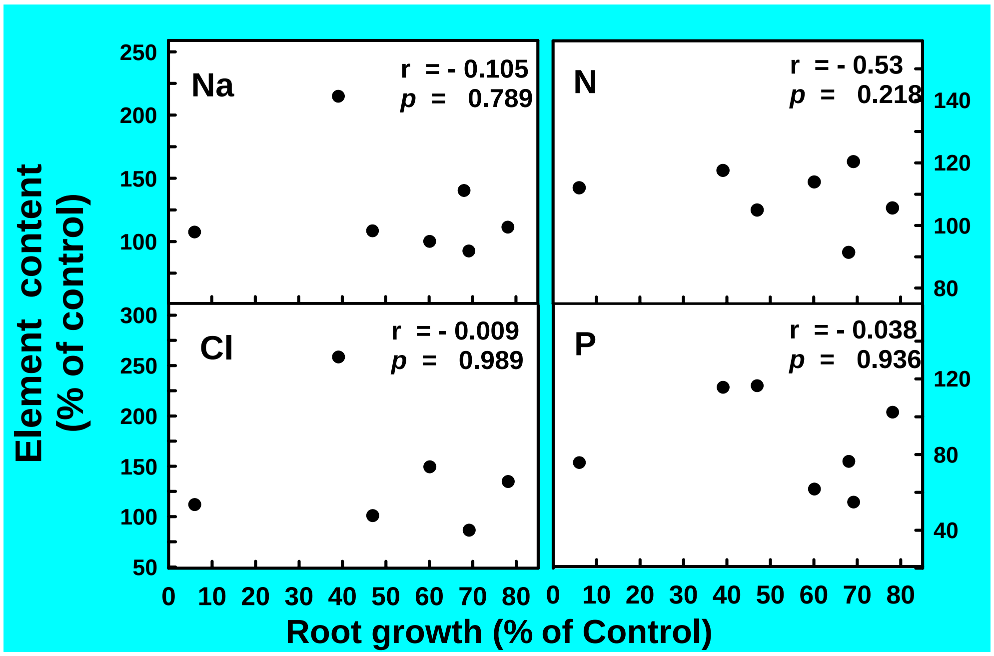

Figure 16: *Element content correlated to root growth for sodium, chloride, nitrogen, and phosphorus of various seedling rootstocks exposed to either 250 mg Na/L (control) or 750 mg Na/L. Refer to Figure 13 for relative root growth rates.*

For example, for sodium, consider the sensitivity of the rootstocks to sodium concentration. If we ask the question of whether this occurred because sodium accumulation, in order for this to occur will have to see a positive slope of the data that is as root growth in the treated plants was less inhibited as compared to the control there would be a greater accumulation of sodium in the roots. In general, we found negative results for almost everything, that our values are very low.

Also for calcium, magnesium, potassium, iron, and zinc the only thing that was probable, and again the data was very scattered and the "p" value was very high manganese (Figure 17).

And when we look for the kind of changes in the shoots (Figures 18, 19) we asked if there is a correlation between the changes in mineral contents in the different seven ungrafted rootstocks? Does it relate to the sensitivity of shoot growth to salinity? We also found that possibly sodium accumulation in the tissue might be related to growth reduction, but that changes in sodium accumulation were not very high. We're only talking about 20% increase. And also, surprisingly, magnesium; calcium probably not, but possibly magnesium could be involved in shoot growth reduction.

That's a question that might have to be addressed in future research. In general I would say that it does not seem to be that, other than possibly sodium accumulation in the leaves. It does not seem likely that salinity has affected growth in the different rootstocks by affecting the nutritional concentrations in various plant organs.

To summarize, I just want to point out that we found out that root growth is more sensitive to salinity than shoot growth. In the root, possibly manganese is involved in growth inhibition and in the shoot possibly sodium or magnesium.

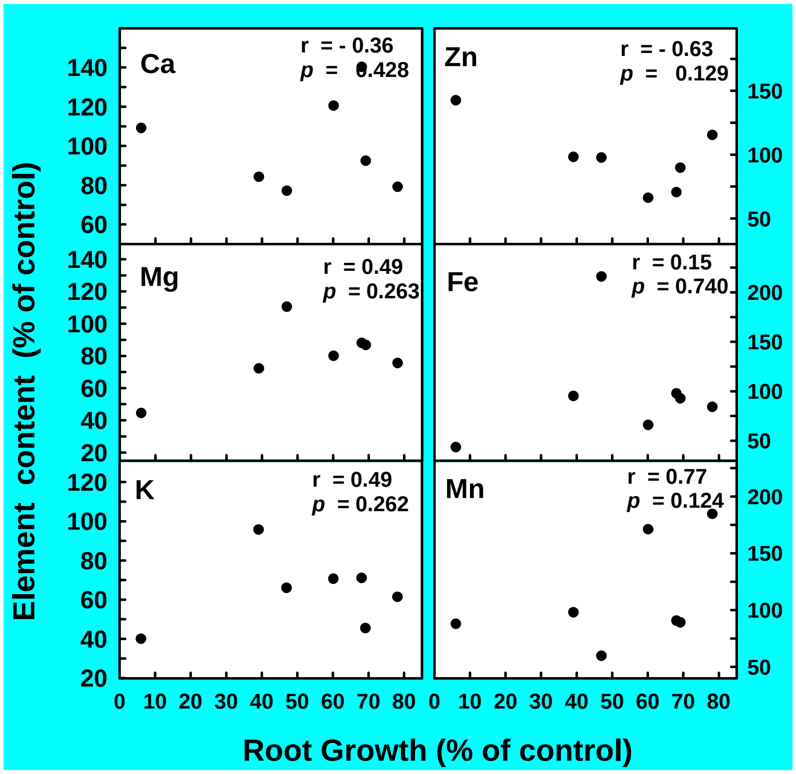

Figure 17. *Element content correlated to root growth for calcium, magnesium, potassium, iron, zinc, and manganese of various seedling rootstocks exposed to either 250 mg Na/L (control) or 750 mg Na/L. Refer to Figure 13 for relative root growth rates.*

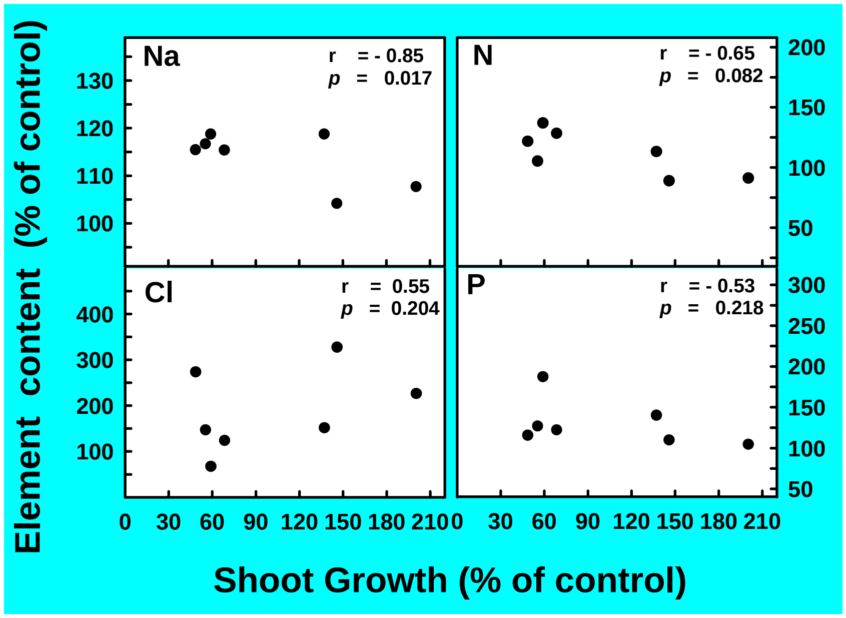

Figure 8 *Element content correlated to young shoot growth for sodium, chloride, nitrogen, and phosphorus of various seedling rootstocks exposed to either 250 mg Na/L (control) or 750 mg Na/L. Refer to Figure 9 for relative shoot growth rates.*

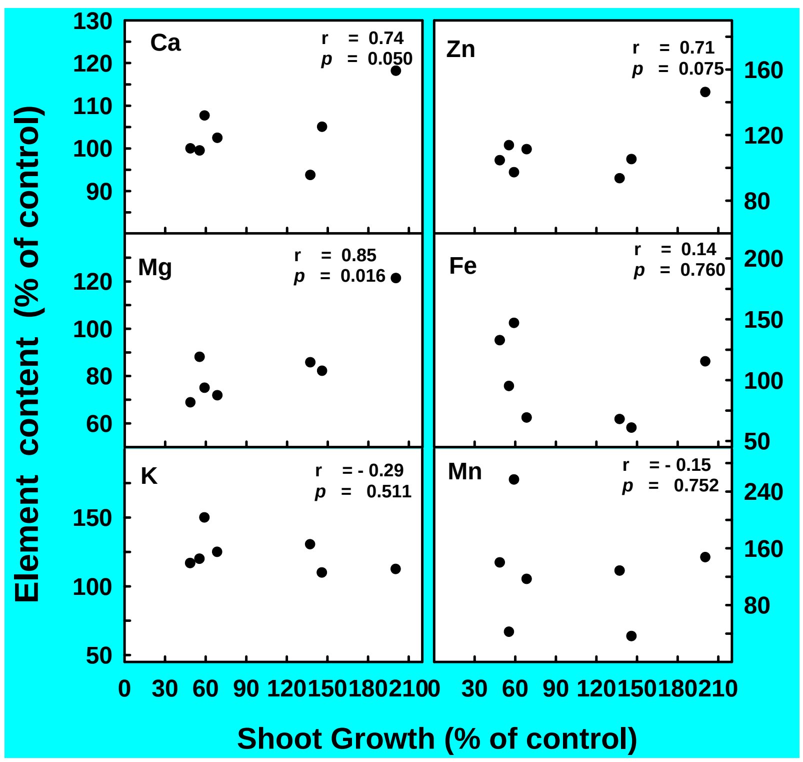

Figure 19. *Element content correlated to young shoot growth for calcium, magnesium, potassium, iron, zinc, and manganese of various seedling rootstocks exposed to either 250 mg Na/L (control) or 750 mg Na/L. Refer to Figure 9 for relative shoot growth rates.*