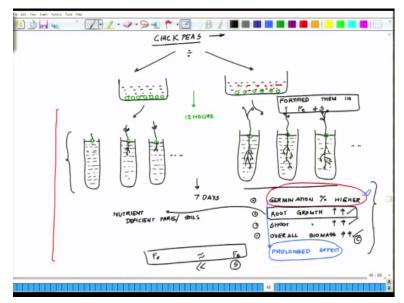
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Lecture-20 Nano-Pyrite Field Trial with Spinach and its Mechanism Details

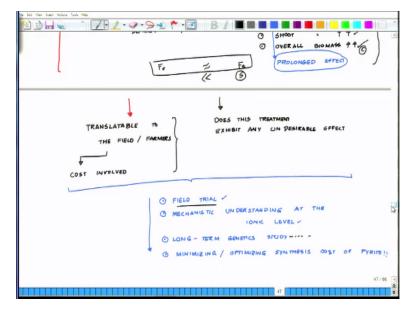
So, in the last class we talked about the laboratory trails of testing the seed stimulant effect of iron pyrite, where we use chickpea as a model question to exhibit the effect. So these are the conclusion what we do germination percentage goes higher.

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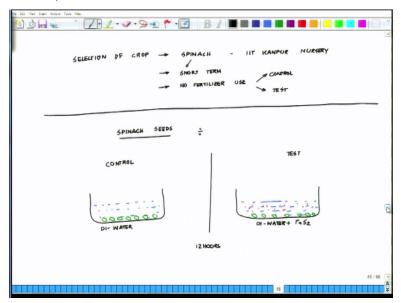
Root growth is higher, shoot growth is higher and overall biomass goes up and sulfur percentage those plants were higher. But iron remain constant from here when we talk about overall biomass got higher it means your carbon assimilation is higher.

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Now from here we talked about the how to translate this technology to the field, so is it translatable to the field or to the formers but it cost involve at does this treatment has any undesirable effect. And in order to address we need to have a field trail, mechanistic understanding of ionic level and long-term genetics study which will of course and the minimizing or optimizing synthesis phosphor-pyrite.

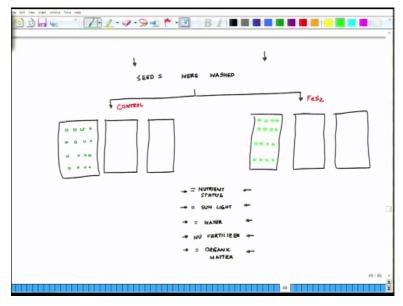
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So, the selection of crop which was made was spinach and it is a short term crop and no fertilizer was used. So, the way it was done was again you take the spinach seeds just the way chickpeas where taken, again these were divided into 2 categories control and test and those were treated

overnight in water DI water de-ionized water. And DI water+FeS2 suspension okay you are having the FeS2 suspension, here you are having the seeds.

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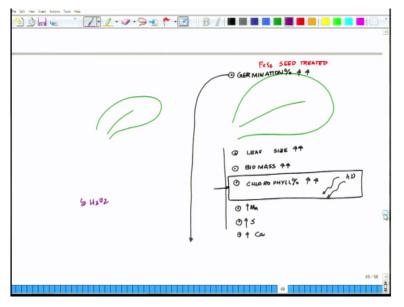


Now after 12 hours of treatment next morning, so you both the treatment in the night next morning seeds were washed thoroughly. And now already your field is prepared what you do on those plots. So you select 3 plots for control or 3 or 4 plots for control and 3 or 4 plots for test, same condition, same soil testing values everything same. So, they are getting one has to keep in mind that both of them have equal nutrient status okay.

Then equal sunlight, equal water and as we decided no fertilizers whatever organic matter is there, so equal organic matter okay. So everything was kept the same distributes it in a way, now to do you all those treated seeds shown at a distance similarly so here you having the FeS2 or pyrite treated seeds and here you are having the control seeds beside here treated seeds exactly the same way all the reputation.

Now the crop was allow to stand there for like 60, 70 days before the first harvest takes place okay which is essentially whenever you grown a spinach after 2 months we take the first cut sometime people does it with 50 days, sometimes 40 days depending on but it varies. But you keep it constant ok and what was observed is here are the results which emerged out of this study.

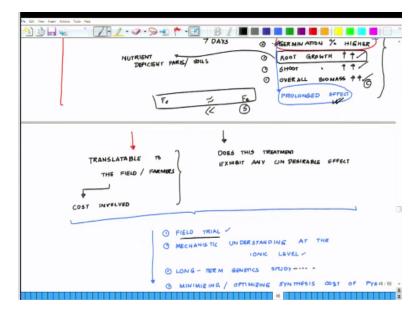
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The leaf, see the leaf size of these one they are bigger than the control significantly bigger leaf size FeS2 seed treated okay. So, leaf size goes up the first thing which was observed, it is a very interesting observation to see the leaf size is grown up. Second the overall biomass went up, next one of the very interesting observation which was made was these leafs chlorophyll percentage goes up.

Now when you talk about the chlorophyll percentage going up that essentially means these plants which are seed treated with iron pyrite has higher ability to harvest sunlight. So they are harvesting more sunlight, so the third thing apart from it manganese goes up, in sync with a previous result sulfur goes up as compare to the control okay. Now that showed that this is a long-term effect, so the effect if you remember in the last class I was telling.

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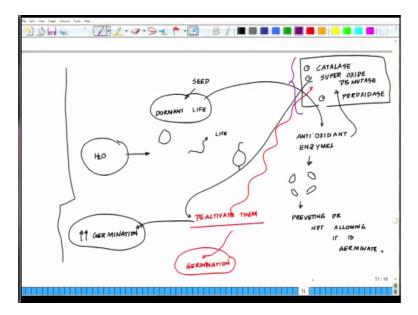


Like go back to last class is this effect a prolonged effect or is it just concentrated to germination say apparently look like that this effect is a prolonged effect. This effect is not only germination, so of course before get into that, so basically the germination part I did not talk about. So, the germination assay clearly say germination percentage is significantly higher on the FeS2 treated as in sync with a chickpea results.

So leaf size went up, your biomass went up, your chlorophyll percentage went up, manganese went up, sulfur went up, your calcium went up which was very interesting. So, nutritionally they are much more fortified with some of these compounds manganese, sulfur, calcium. And as we have already discussed when before performing the test, the nutrients status was kept same, sunlight was same, water was same, no fertilizer or it just grown in organic.

But about equal amount of organic matter, so what is that which is changing the fate of these seed treatment and the fate so much so that this effect is so prolonged that like you know I mean 60 days the effect is being seen and more stunning was this part, chlorophyll percentage, how that goes up. It means there is something very unique which is happening that first let us try to answer how the germination percentage is probably going up, will come slowly with each one of these effects.

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So, what really a seed is if you look at it, a seed is a dormant life okay, this dormant life when it gets water it spanks into life. In other word it germinates and you know something like this and we all take germinated seeds those who all are health conscious they do it every morning. Now what water does, there are 2 questions here, 1 how the seed maintains it dormant life and how water changes the dormant.

Because you know that seeds are kept in dark you cannot keep seeds in moist place because they will go bad or they will terminate and then they will go back or they will get a fungus attack. So, when we talk about a dormant life what it is that makes that life because it is a dry seed you know if you see a seed unlike when that means lot imagination wanted and the very moment you put it, it is going to you know give out acid if it is a self assemble machine.

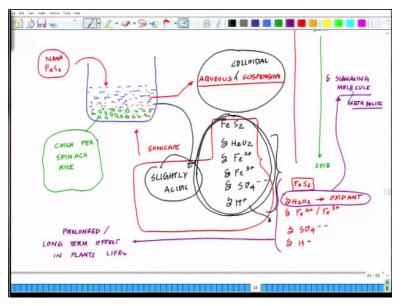
The machine will act whenever it will gets its trigger and the trigger is water, now the question is very fundamental question is what keep the seeds, what does not allow the seed to germinate. So, it has been observed over a years that there are series of antioxidant enzymes present in the system of the seed which antioxidant enzymes which creates a kind of a fold inside the seed preventing it antioxidant enzyme, preventing or not allowing it to germinate.

So by this same logic if the antioxidant enzymes, so antioxidant enzymes as you know in the biological system there are 3 major antioxidant enzymes you have catalyst, super oxide

dismutase or in short they call SOD peroxidase. So these antioxidant enzymes prevents the seeds from germination but then by the same token if you could deactivate them somewhere other ever mechanism to deactivate them.

Then the seeds should move towards germination with the logic if something which is preventing the germination if you could deactivate that switch it will lead to germination. One of the studies back in 1976 exactly showed this result that by deactivating the antioxidant enzyme you enhance the germination or you use the substrate.

So, how you deactivate them, have to have something like you know you may use say catalyze in-vitro, you use a superoxide dismutase inhibitor or peroxidase inhibitor or something okay. So this is the first catch which triggered us to explore this pathway, these iron pyrite aqueous suspension.



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I will take you back to that very first slide where I showed this. told you that I will come back to this one, does this milieu does something of that kind of the stuff. Because as I told you your major player is FeS2 fine but then the challenge is with these once H2O2 which is an oxidant okay, you have Fe2+, Fe3+ very trace amounts so. Then you have So4- and then you have higher quantity of protons, who is playing the role among all this.

Now most likelihood the one where we put our guess was this one, the first one which who is doing something interesting. Because hydrogen peroxide is an antioxidant molecule as well as it is in a trace, trace amount, it is a profound signaling molecule and it is a very powerful metabolite, how to test what hydrogen peroxide is doing. So, this was the juncture where the field trail is showing a great result.

But this needs further explanation that what that trace amount of H2O2 could do, how that possibly could breach or how that possibly could break the antioxidant molecule bridge, you know get into the system and do something which we never taught of and that is a matter of fact no one taught of an antioxidant nanoparticle suspension which is not getting inside the seed just by a surface reaction could do something way more profound than one can think of okay.

So our journey will continue and this line that how this trace amount of H2O2 and trace amounts of these molecules brings about a prolonged or a long term effect in plants life okay, thank you.