Trials in Maize, reactivating dormant genes using high doses of Salicylic acid and Charcoal (DRAFT)

Recent literature suggest that the split from a common ancestor between rice and the ancestor of maize happened some 45 to 60 million years ago. Maize was formed from teosinte as a common ancestor. Sorghum apparently split from the common ancestor with maize sometimes between 16.6 and 11.9 million years ago.

If the environment is overcharging the abilities of the gene set, then the plant seems simply to split up in different lines of development (??), although keeping the, during evolution silenced gene sets, or inactivated subprograms.

Now and then, trigger events, like stress or chemical influence, or not compatible gene sets in hybridizations, cause those silenced subprograms to activate and express themselves causing to see, what's hiding in their past.

These so called trash genes, with apparently no function, have still a function and can be switched on or off as genetic expression If we find out the trigger mechanism, and as well if we find out their ancient function and the use of their reactivation in an actual environment, we might be able to get much higher productivities per ha, then by raising densities.

In the example of Maize, I want to show, when using Salicylic acid in high doses together with bio char, we have the possibility to get a glimpse of what reactivation could bring us as a useful gene expression in future.

All activities in plants are subject to a very rigorous energy management that is based on a continuous, updated balance. Different trigger values in stored energy, availability of energy as short or long term storage, experience based forecast behavior etc. leads to what we call, trigger to act.

Weather patterns, root system size, water content of the rhizo sphere, temperature/ days accumulated etc. are indirect or direct values that lead to the decision making to go one step further and flower, or to add flowers or abort, to initiate seed filling or to stop and abort seeds, to start de mantling of leafs, or start senescence etc.

Those trigger values or decisions causing parameters in ancient and different environmental sets had different values and therefore, changing the environmental conditions, had to be silenced, or where needed, had to override the existing system, with different trigger values.
In the last centuries, we introduced an additional change, or parameter, the plants never had to deal with, high-density monoculture planting and breeding.

The original plant, some 10.000 years ago, was a multi flower plant with multiple but small cobs. The cobs were small with low number of single flowers per cob. Expressions even show, that ancient plants had in every axle a complete copy of the Masculine-feminine combination. (See photo 1) what we call a breeding failure today.

This feature is still preserved in rice and sorghum.

Photo 1: Masculine flower in one of the axles together with the feminine flower (cob)

In the last 10.000 years, one of the main breeding and selection criteria where apical dominance of the cobs, which means, that the first cob at the plant gets served first, attracting all nutrients and suppressing the development of the other cobs from the following axels downward.

I think this criterion was adopted because in prior to industrial fertilization times, resources for the plants were scarce; apical dominance produced bigger single cobs with a higher rate of survival and good seed harvest, then the multi cob variants.

If you now apply sufficient fertilizer and improve the root system and add a trigger substance, the plants again decide for a multi cob per axle/shank, and multi cob per plant system, activating gene sets, which had been silenced through breeding and selection.

The fertilization alone may increase productivity by more seeds per cob or higher 1000 corn weight; but for changes in the gene expression, an additional trigger for full activations is needed.
In this case high doses of salicylic acid together with charcoal where used. (69 gram of SA in 100 liters H_2O / ha at day 15 and 253 grams of SA in 100 liter H_2O/ ha at day 35; charcoal in stripes from the original land clearing with doses of 136 to 150 metric tons /ha)

Photo 2: Root system size increase caused through application of high doses of Salicylic acid in the char coal stripes
Roots at day 23 after germination, already in stage V 8.

Photo 3: Root system is 50 cm in diameter and reaches down with heavily branched secondary roots to more then 45 cm. (roots at day 23 after germination)

Photo 4: Intensive secondary root system in every internode’s with excessive branching, caused by the Salicylic acid application on char coal stripes. At day 23, already 4 levels of visible above the soil internodes had developed secondary, branching roots.

Salicylic acid application has several functions in maize. First it induces a chain of self defense mechanism (SAR or self induced resistance) to engage, were H2O2 is produced, to burn off attacking hyphens from fungi as well as to fend of attacking bacteria. (H2O2 Serves as well as an internal short/long distance communication tool)

In second place, cells, which have been invaded already by hyphae, are ordered to die, (instant death) to avoid, that the fungi continues feeding on the plant saps.

Third, around the exterior limit of the dead cells, a wall of lignin is build, to avoid further penetration of the hyphae into neighboring cells. (Light brown haloes around necrotic spots)

As a second line of programs, to compensate possible damage, the plant induces rapid root and leaf area development, as well increases chlorophyll concentration per active leaf area, to capture more solar energy and produce more photosynthates. (Chlorophyll A and B)

The maize in question, after 30 days already was at a development stage, maize normally has when 45 days old. V 12 and 4 inter nodes fully developed into adventitious roots with heavy branching.

Photo 5: After 30 days already in development stage V12

Photo 6: Stage V 12, big leaf surface, high chlorophyll concentration

At day 49 (normally at day 63 to day 65) flowering started. The viability of pollen in commercial maize is in the range of 7 to 10 days in this case the pollen availability was extended to 23 days, (the viability of the female receptors has a window of 4 to 6 days), and with the female receptors in our case the window was 16 days. (Due to de phased development of the different cobs at the plants)

As mentioned before, apical dominance is the mayor feature of modern hybrids. Applying Salicylic acid, apical dominance could be neutralized and in some cases reverted. See Photos 7,8,9

Photo7: neutralized apical dominance, 3 female flowers flowering at the same time with a de phasing of possible up to 4 days between the different cobs from top to bottom (top ending to be receptive in 2 days, bottom starting to be receptive since 2 days)

Photo 8: reversed apical dominance, second cop flowers first and attracts in the further development all nutrients (second cop continues towards flowering when first cob stops being receptive)

Photo 9: second cob flowers first, reversal of apical dominance through high doses of Salicylic acid

The effect of neutralizing the apical dominance is not only affecting positively the single cobs per axle production, it also reactivates an other ancient program that still is working in rice and sorghum, the replication of the whole architecture of the plant in every axle. Photo 10, 11,12,13,14

Photo10: multiple pollinated cobs per axle/shank

Photo 11: multiple cobs per axle

Photo 12: multiple cobs per axle on the same shank

Photo 12: reversed apical dominance in the same axle/same shank, second cob is more developed then the main, former apical one

Photo 13: multiple flowers in same axle/on same shank

Photo 14: multiple flowers in one axle/same shank

After 70 days 35% of the plants had 3 developed and pollinated cobs, 47.8 % had 2 developed and pollinated cobs and 10.7 % had 1 cob. A few plants (6.5%) had between 4 and 7 cobs developed and pollinated. In the 2 and 3 cob fractions several non pollinated cobs where visible, but did not develop due to lack of pollen in time.

In commercial grown corn the average number per plant is between 0.9 to 1,3 cobs per plant. (Depends on variety/ hybrid, nutrition, density and soil quality).

The Salicylic acid treated plants had an average of 4.2 flowers per plant and 2.3 cobs developed per plant. To get all flowers pollinated, it needs a wider pollination window and a better timing and dosage of the applied Salicylic acid.

Taking one plant, cutting it up we can show what is happening exactly in the different axles.

Photo 15: several cobs formed in one axle/shank, several axles/shanks with several cobs per plant

Photo16: same plant in the laboratory

Photo17: same plant after removing all leaf shows 5 fully pollinated cobs, 2 in the first axle, if counting only the first 4 cobs, totaling 2300 seeds produced per plant

Photo18: Normal, commercially grown cob without Salicylic acid (same variety)

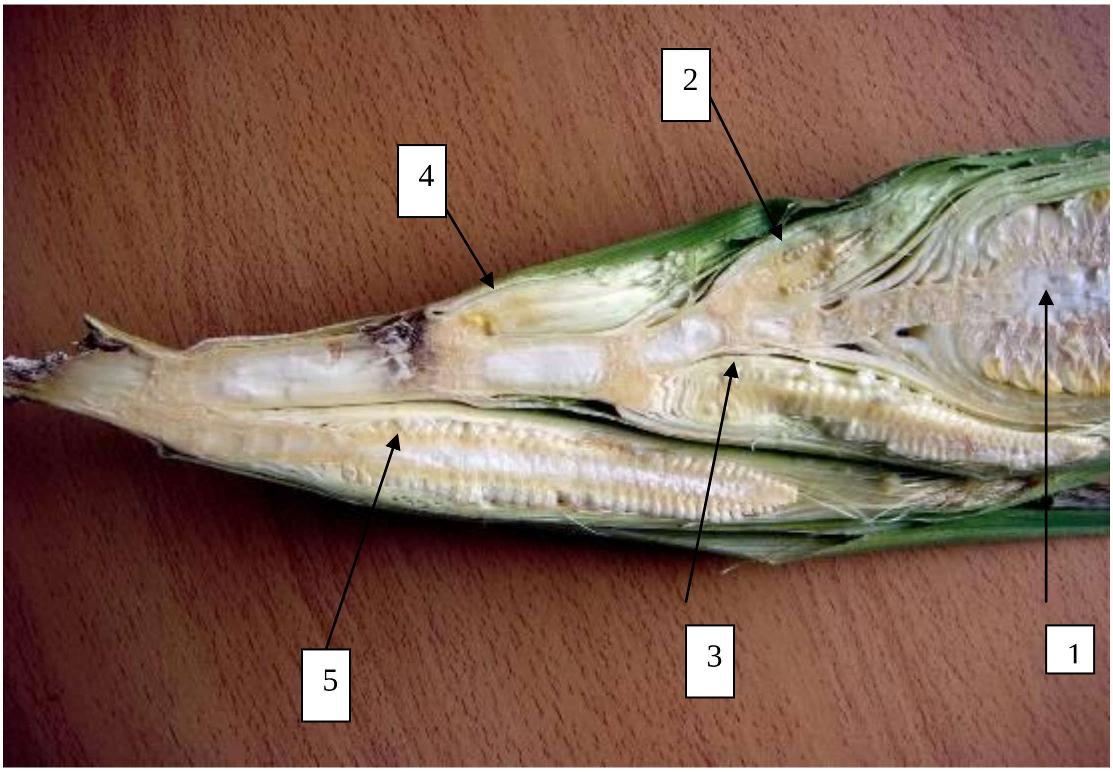


Photo 19: with applied salicylic acid grown plant, showing 5 cobs in different stages of development growing on the same shank in the same axle

Cob number 3 and 4 are better developed because this is the outer side where the salicylic acid was applied, although systemic, there might be concentration differences in side the plant.

If all cobs, when developing, encounter viable pollen, then pollination will happen and the cobs will develop. The plants showed no sign of lack of nutrients in those, which managed to pollinate.
The same as in photo 19 in one axle can happen, as you see in photo 21, in several axles.

Photo 20: Comparison of non-applied and salicylic acid applied plants

Photo 21: Salicylic acid applied plant with multiple cobs per shank and axle

As the window of viable pollen, due to a severe drought during the whole flowering period was somehow reduced and as well a severe attack of silk eating caterpillars occurred, some of the cobs had poor pollination.

This in future could be overcome by complimentary irrigation during critical moments and a better insect monitoring- and control- system in place.

As well pollination could be improved planting several rows in between, with an 5 to 8 days delay between the additional inter planted rows, left unseeded at initial planting. (Additional trials showed good results on that)

The question arising is, if there is an actual communication between plants in place which signal the state of reproductive development and therefore changes the receptivity of pollen donator/ receptor?

Photo 22: multiple cob formation in one shank and in several axles at the same plant

Photo 23: different types of cobs produced

As the seeds used where from a hybrid, the cobs produced from left to the right are the following.

1) 2 recessive cobs, Salicylic acid applied but still with 456 and 486 seeds per cob (in total 6% recessive plants; in commercial seeds up to 8% recessive plants are common)

2) 2 well developed true hybrids (positive heterosis) with 704 and 720 seeds per cob, Salicylic acid applied

3) Number 5 in the row is a Salicylic acid applied sample where apical dominance was reverted, total 872 seeds, due to failure in pollination

4) Number 6 is Salicylic acid applied, 3 cobs per shank, with apical dominance and total 1936 seeds in one axle (still some pollination failures)

5) Number 7 is Salicylic acid applied, 3 cobs per shank, with 1329 seeds in one axle (still some pollination failure)

6) Number 8 is Salicylic acid applied, 3 cobs in one axle, 5 cobs per plant, counting only the 3 most developed cobs with 2000 seeds per plant (fully developed, could reach over 4000 seeds per plant); all plants /cobs where cut prematurely.

Photo 24: comparison of commercially grown and salicylic acid grown maize

Commercial grown maize cobs have a seed count per cob in between 350 to 500 seeds in average 450 seeds per cob.

Applying Salicylic acid and fertilization, the seed count per cob is much higher and the 1000 seed weight as well is higher (3500 seed per kg in commercial cobs and 2560 seeds per kg in SA applied cobs)

At the end what counts, are the seeds per plant produced under same density and fertilizer regime.

Raising density, would bring a smaller increase in total weight, than rising seed per plant produced, at same density and seed weight.

The actual efforts of the seed producing companies are raising plant densities, and plant resistance to insects and herbicides.
That’s the wrong way to go, because we are already overshadowing the plants at the actual densities using overlapping rhizo sphere, therefore raising densities wont do much good.
If raising densities is affordable, looking at the support of the plant structure with needed minerals and energy, then raising the number of cobs per plant should be as well.

Charcoal and its possible function in soil:

Annual crops take up about 80% of their needed minerals during the first 35 days of their development. The soil should be aggregate forming and contain around 60% of their field capacity in humidity to allow an uninterrupted water film over the whole surface, still permitting penetration of air in between the aggregates.
The uninterrupted water film serves as main carrier for dissolved minerals, which are gradient and electric field driven towards the root surface.
Uptake of minerals in the vicinity of the roots (1,5 mm distance around the roots), maintain the nutrient concentration gradient between soil particles and roots.

Bio char has huge amount of functional groups and/or negatively charged sites on the surface, as well during the charring process a good amount of humic and fulvic acid like structures with an aromatic backbone, which move, solved by water, into the surrounding soil. These humic and fulvic acid like structures are capable to neutralize free, plant toxic aluminum and chelate a wide range of minerals.
The volatiles, plus the degrading organic compounds in the soil deliver the needed CO_2 which liberates the H^+ from water to interchange the captured positive charged cations from the Cation Interchange Sites on top of the char and on top of clay particles.
The desired high CEC of the char, together with the rapid delivery of H^+ to release minerals into the solute, avoid leaching losses but as well satisfy the mineral demand of the plant. This is called a high mineral replenishment potential, in case some of the solute is lost by short time, heavy rainfall, leaching into deeper soil layers.
One of the functions of the char in soil is, to deliver minerals in the needed quantity and quality just in time at the lowest possible energy expenditure for the plants.
If timely delays in quantity and quality of the minerals occur, then the plants have to exudate acetic and citric and oxalic acid and sugars to pay a somehow higher energy price for mining the needed minerals. If some of the minerals are lacking, the growth gets stunted, and the development delayed.

The offer of a high mineral replenishment potential alone is not improving plant growth over some 20 to 30 %, you need to stimulate the root development, leaf development, the solar energy capture and storage system, to take full advantage of the increased nutritional capacity of the soil.
This requires some form of communication with the plants to make the plants act.
This communication has to be capable to override experience based decision structures and reactivate desired programs
Here, trigger substances like butenolides (smoke chemicals, $C_4H_4O_2$, $C_8H_6O_3$) or salicylic acid ($C_7H_6O_3$), or ascorbic acid ($C_6H_8O_6$) or chitin/chitosan ($C_8H_{12}NO_5$), benzoic acid, amino acids or peptides and others come into play, causing an immune response in plants, triggering defense, and onset of compensatory actions.
The mere reaction of plants to the presence of char in soil indicates, that plants had dealt over several millions of years with char as an essential ingredient in soil.
This includes as well, that plants have something like “awareness” about char being present in soil. Awareness logically includes, that there is a sensing system, which gives data about presence (spatial orientation?), quality (age?) and quantity of char present.

Photo 25: To the left and right, the stripes which are higher, are with 150 t charcoal per ha, in the middle the stripe is without charcoal, both stripes have essentially the same mineral content according to the soil analyses (day 15) and have the same dose of Salicylic acid applied

Photo 26: Same spot with 20 days of growth

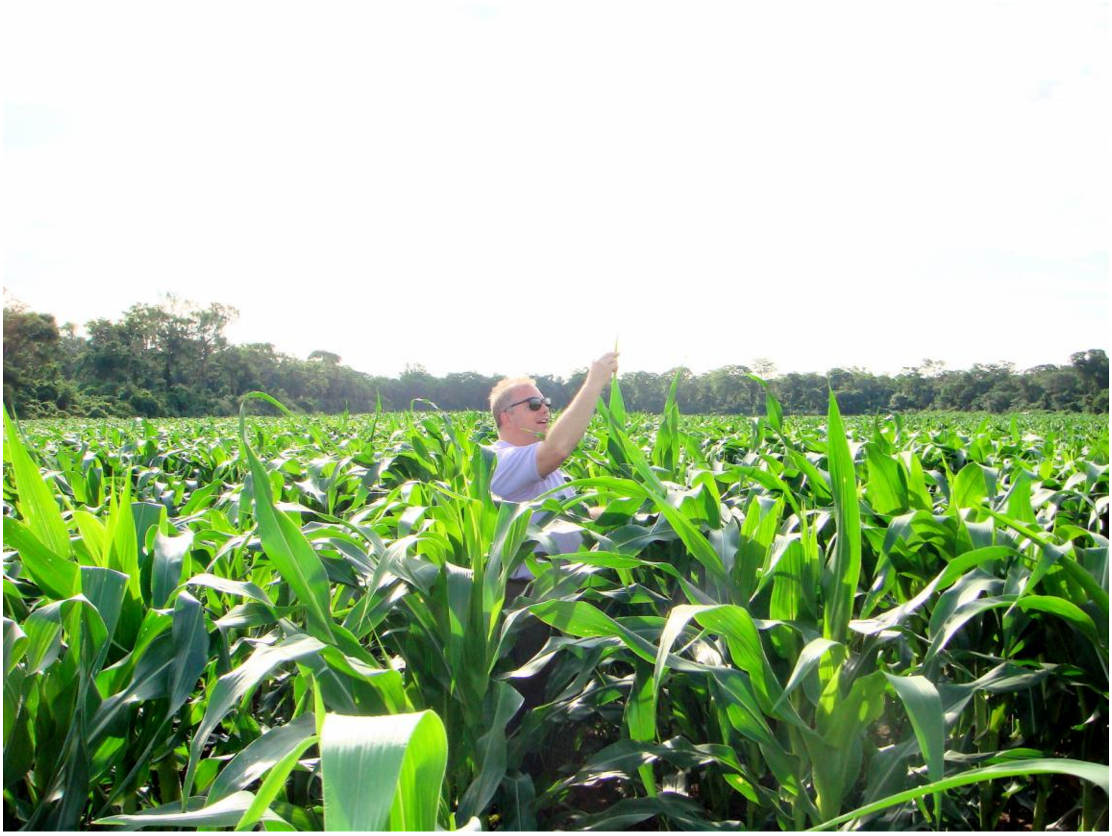


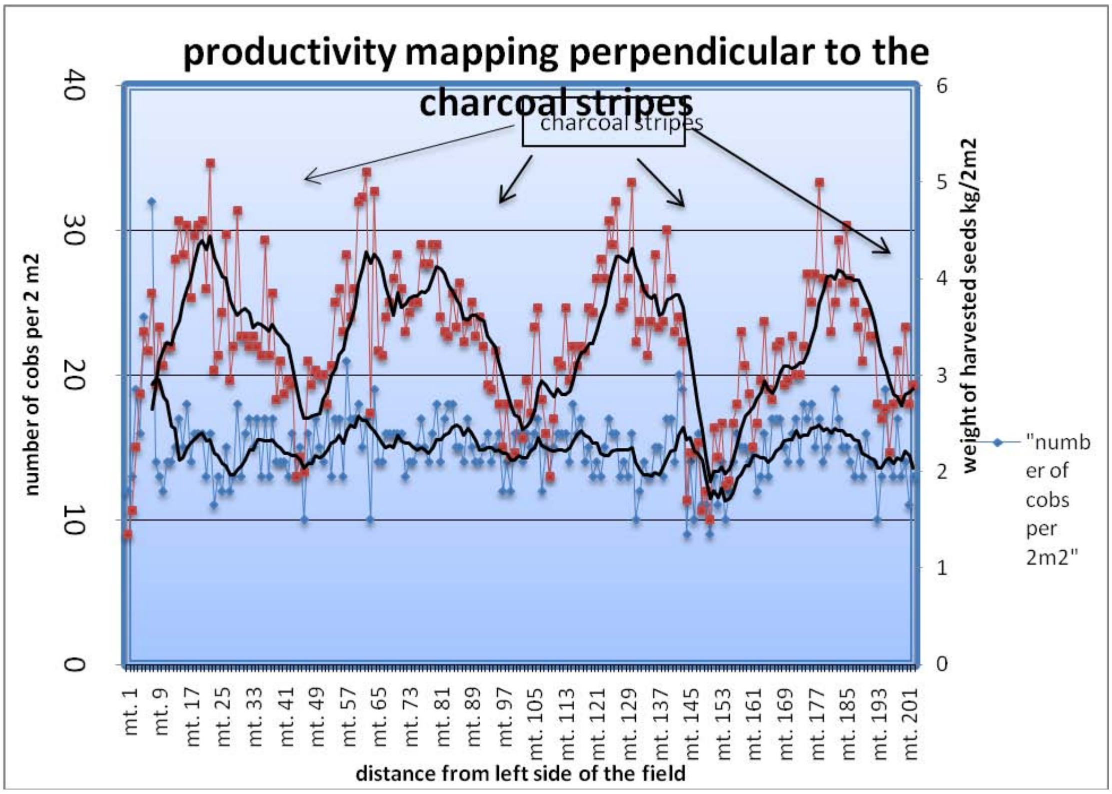
Photo 27: Same field, same day, some 10 meters to the left in the middle of the charcoal stripe

The sensing could be indirect via reactions or changes in the soil biota surrounding the roots or being present in the rhizo-sphere, or direct via direct contact with char, or substances, released by char.

The question to ask now is: what advantages does presence of char in soil indicate to plants?
Presence of char in an given soil would indicate, that there has been a sudden minor or major change at the surface of the soil which, as a consequence would include an open, not overshadowed area, more access to sun and rain, elimination of competence, relocation of a huge amount of plant available minerals on top of the soil (ashes of burned biomass), partial or total elimination of diseases or disease causing spores, fungi, bacteria, insects etc.; more partially burned biomass and roots decaying; open channels in the soil from decayed roots etc.
This would suggest that plants as well are capable to reason consequences of presence of bio char and include those consequences as accumulated experience into their programs.

Another more simplistic explanation could be, that char is interfering with the exudates and is adsorbing, desorbing, absorbing substances like plant hormones

(Auxin etc.) from the surroundings of the roots. This as well would cause an immediate change of the programs inside the plants, root development would be different, apical dominance would be eliminated or suppressed, management of the reproductive system would be different etc.
Applying the right mineral composition and huge reserves of NPK has a very limited growth increase response. Starter level application of NPK brings about 4.5 tons of seeds per ha. Higher level N additions (up to 120 kg per ha in split applications additional to the starter application of NPK) rises productivity up to 7.5 tons per ha. The char stripes from the forest clearing, now in soil since 18 years bring, measuring productivity in 1meter steps perpendicular to the charcoal stripes, (see picture 28) some 12 tons seed per ha. Adding salicylic acid to the whole area (charcoal and non charcoal) rise productivity of the whole area to 17 tons of seed per ha.(better response in Char applied area)



Graph 28: The graph shows an up to 250 % increase of total seed weight per 2m2 in the charcoal stripe areas (without Salicylic acid) of the field. Without Salicylic acid there is no remarkable difference in cobs per area but more seeds per cob and higher seed weight per 1000 seeds.

A third pathway of information transfer and resulting action would be the presence of char or fire caused substances. Looking at possible information carrier or signaling substances between C1 to C25 with all possible combinations of H1 to H24 and O1 to O9 and some additional substances with Cl and N included, we look at several thousand substances, which probably are produced in small quantities during burning of biomass and during continuous oxidation of charcoal in soil.

Summary of the observations:

Higher mineral content of available minerals in soil (fertilization) increases productivity from 4.5 tons to 7 tons per ha.

Apical dominance is still in place. No multiple cob formation visible; window of pollination did not change.

Where bio char is in soil (between 136 to 150 metric tons per ha after 18 years) soil analyses show no significant differences in mineral content but productivity is up to 250 % higher then in the surrounding non charcoal containing soil. (Same fertilization, same water regime, same soil)

Adding salicylic acid as a foliar application in non-charcoal areas increases seed weight, seed per cob and overall expression of plants. No change of apical dominance or multi cob formation.

Combining charcoal and salicylic acid changes the quality of the response. Together, apical dominance is neutralized or reverted, multi cob formation per shank and multiple axles with several cobs per shank are occurring. Seed weight is increased, root size is dramatically increased, and chlorophyll concentration in leaves is increased. Window for pollinating as well increases. More drought and cold resistance is observed.

This suggests, that in combination, charcoal and salicylic acid are capable to override the memory induced growth limitations (experience based limited reaction to growth stimulus through ad libidum mineral availability) as well have a different quality of influence on the soil biota and there activity towards the plants (stimulus of N uptake, stimulus of mineral uptake, direct action of plant hormone like substances on root development and plant development etc.)

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