TERRA **EARTH**

SIMBIOSIS: PLANT – BIOTA - SOIL

To make it easier to model plant survival in drought conditions, let's draw some analogies with space travelers. Imagine that Ilon Mask's spaceship with limited supplies of food and energy landed on the distant planet of Mars and desperate travelers began to colonize the distant and hostile world.

In addition to natural courage and devotion they have a very limited supply of energy and food on board the spacecraft and even less time to spend in the atmosphere of an alien distant planet.

It is clear that the energy supply of such seeds is very limited. To simplify calculations, we will fix it at approximately 1 gram of weight per 20 seeds of winter wheat (you can check - exactly 1 $q \pm 3\%$, no more and no less). It is simple and convenient for further calculations, you will see it for yourself!

Approximately the same chances of surviving in the semi-desert of the planet called the Earth have the wheat seeds which were sown in super-hard, not friendly to them soil by a modern farmer.

So the seeds have already been sown and begin to germinate. In a few days we have both primary roots and a young pale sprout. It should be stressed that both the top and the root consume only the energy of the seed because, as agronomic science shows, for the first 10-12 days, plant development is accomplished only at the expense of the mother-seed. Both roots and sprouts are only derived from carbon reserves of the grain itself in the form of starch and complex sugars, fats and amino acids. To grow quickly in an alien environment, the root needs soil microbiota. To attract it from the soil, the young plant roots begin secreting tasty nutrients, the so-called exudates - mucous substances based on exopolysaccharides, amino acids, organic acids, vitamins, and so on.

Due to this, different species of microbiota are concentrated in the root area, thus creating a biological film mainly from bacteria around the root. Bacterial colonies are bound by a so-called matrix created by these exudates. 97% of the film is moisture, 0.2-0.4% - microorganisms and 2.6- 2.8% - exudates. And the more unfavorable are

the conditions (soil drought, depleted soil, microbiota killed by chemicals, high salt content, etc.), the more exudates the plants produce, thus losing its initial seed energy and adapting to difficult survival conditions (similar to what I. Mask can expect).

In a relatively short time, a tubular formation is formed around each root (we will call it the "bacterial cover" for simplicity), which now reliably protects the root from phytopathogens, depriving them of food, i.e. "tasty" (for microbiota) complex sugars (exopolysaccharides), and releasing harmful (for foreigners) anti-nutrient compounds. In other words, it acts as a natural antibiotic.

Now the young root feels reliably protected as the bacterial film adheres securely to the root with its inner side and to the soil with its outer side, thus attracting precious moisture and all organic compounds, including straw residues, insoluble phosphorus, soil potassium, etc., which are actively digested by the microbiota of the bacterial cover¹, turning into a water-soluble form, and are actively absorbed by the root surface. Thus a so-called symbiosis arises, i.e. the combination of plant – microbiota – soil.

It is clear that the larger the surface and branching of the young root, the faster the capture of the surrounding space occurs, or the colonization of a distant planet if we use the analogy. It is on this surface that complex biochemical processes of digesting soil nutrients and organic residues of previous year plants take place. Usually, the root itself does not take these components as food, because they are in insoluble solid form. And here numerous "mercenaries" come to the aid: the inhabitants of the Earth in the form of microorganisms called fungi, bacteria, micromycetes, algae. By digesting solid organic matter around the root, they convert it into water-soluble, "tasty" root compounds that enter the plant through the root surface. Obviously, the more numerous are such helpers, the easier the root adapts to the environment. The grateful aliens (plants) respond to the local aborigines (microbiota) with generous gifts (root secretions, i.e. exudates, complex jelly-like secretions in the form of sugars). Accordingly, the number of helpers in the form of microbiota is constantly growing along with the root surface and the whole plant.

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PREFACE, OR WHAT PLANT MUMMIES TESTIFY TO

Proceeding from the existing technics, the author and his colleagues conducted dozens of experiments on 7-12-day plants sprouting from seeds and concluded that they are ineffective, which is especially noticeable in the context of the global climate change. In his opinion, since the colonization of the surrounding soil by plant is performed not by a dry root (by the way, most classic methods strongly recommend to carefully measure roots by placing them side-by-side) but by a living plant and, accordingly, a living root which is 70-85% filled not with « liquid « (which respected scientific methods recommend to dry) but with living liquid substance with hundreds of physical, chemical and biological components. And it is this substance, not the dry roots, that must be carefully examined. If we draw an analogy with medicine, it turns out that instead of studying a living person (by the way, who also contains 70-75% of «liquid»), modern methods

developed decades ago recommend to study dried body, including incorruptible relics and Egyptian mummies!

Besides, when studying young growing plants, the weight of dried young plants greater than in the control is traditionally considered a positive result.

According to the author, it is hard to agree with this approach, because a large green mass that grows, takes energy from seeds, limiting the germination of the root. In drought such a plant dies quickly.

And most importantly, what should always be relied on: in such experiments the weight of dried 7-12-dayold plants cannot be greater than the weight of the grains from which these plants have grown, which makes the existing traditional approaches not very promising, especially in drought.

How does the plant react to drought and is it possible to help the plant survive and avoid damage to the owner? All this will be discussed below.

THE PRICE OF ANOTHER PLANET COLONIZATION

In modern microbiology it is customary to speak of a «bacterial film», but, in the author's view, the concept of «film» is more associated with a film on the

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teeth, i.e. a flat surface. The term «cover» echoes well with the well-known «cover» at the end of the root.

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It is important to note that the soil adhering to the bacterial cover also accumulates moisture and in case of soil drought makes it possible for the young plant to survive till significant rainfall.

The colonization of the maximum possible soil volume in order to develop long roots requires the consumption of the seed energy in the form of root exudates. And this is a significant loss of grain weight, about 15-30% (!), which is especially appreciable in drought conditions. The main consumption of exudates occurs in the area of the bacterial "cover", where bacterial mucus can reduce the resistance to the movement of the germinating root deep into the soil.

It is important to note that the bacterial film, or cover is the only multifunctional organism that depends, on the one hand, on the plant itself, on the other hand, it is guided by external danger signals from chemical fertilizers, heavy metal pollution, acid rain and, perhaps most importantly today - from soil overheating and lack of moisture. Such signals are transmitted to receptors and biofilm control centers by means of signaling molecules which are the carriers of information about danger. Accordingly, the plant, and ultimately the grain, loses

1 In modern microbiology it is customary to speak of a "bacterial film", but, in the author's view, the concept of "film" is more associated with a film on the teeth, i.e. a flat surface. The term "cover" echoes well with the well-known "cover" at the end of the root. additional resources to respond

adequately to the danger. In case of drought, root secretions (exudates) increase in order to accumulate additional moisture in the plant, especially in its root. Today all farmers know that the more branched and deeper the root system is, the more likely the plant will survive drought.

DROUGHT IMPACT MODELING

To confirm this and determine the amount of exudates, i.e. the seed energy loss required to adapt to the environment, we will make an experiment on couching 20 winter wheat seeds weighing 1 gram placed in 4 cups (5 pcs in each). The cups were filled with sterile sand. All in all, 6 experiments were conducted. In the first experiments (1, 2, 3) drought was modeled and sand was moistened by 40% of the lowest soil moisture content. Four repetitions of experiments without additional watering at a room temperature of $+$ 28 C caused in a few days a lack of moisture, i.e. drought. In the next three experiments (4, 5, 6) the plants were optimally watered, the sand was moistened by 75% of the lowest soil moisture content (see Fig. 1).

Selected plants together with sand are weighed and entered in table 1 under the letter M_{1} , then the sand is washed off and the remnants of moisture on the plants are carefully removed with paper towels (see Fig. 3). The weight in grams is entered in the table under the letter $M₂$, listing all subsequent measurements for 20 plants³. Dried to atmospheric moisture (12.5%) plants are entered in table 1 under the letter M_3 . Then dry roots m_1 , sprouts $m₂$, seed residues m3 are carefully separated (see Fig. 4 and Table 2). The initial weight of 20 grains before sowing is listed in table 1 under the letter M_{0} .

I. Drought simulation simulation set

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Simultaneously with the control, which shows the natural reaction to drought of untreated wheat seeds, an experiment was carried out on inoculating seeds with a biological product of complex action containing natural (native) bacteria hardened by high temperature in a nutrient medium based on domestic organic soils (patent UA 11960

). Hardening microorganisms by high temperature according to a specially invented algorithm leads to the appearance of alarm signaling molecules (SM) in the form of biological compounds, complex sugars: dextrins, lignins, poly- and monosaccharides, which attract soil microbiota to the root area, thus increasing the size of the bacterial cover, as well as bacteria decomposition products, for example, hydrogen sulfide H2S and other stress-related compounds, which increases the bacterial protection of plants. To prove that signaling molecules (SM) can affect the behavior of a plant under thermal stress, the soil was sprayed with the same biological product at the rate of 2 l/ha per 200 liters of liquid after planting the seeds in the soil, that is, the biological film on the soil surface was located at a distance of $4\div 5$ cm from the sown seeds.

THUS THE SCHEME OF THE EXPERIMENTS WAS AS FOLLOWS:

II. Optimal moisture (75% of the lowest soil moisture content)

Experiment 4. Control. Non-treated seeds Experiment 5. Inoculation of seeds with a biologi-

- cal product at the rate of 2 l/t per 10 liters of working solution
- Experiment 6. Spraying the soil surface with a biological product after sowing seeds in the soil at a rate of 2 l/ha per 200 liters of water.

After 10 days the sprouted plants were carefully separated from the soil, 10 best ones were selected in each experiment and the sticky sand2 was cautiously shaken off. The photo of such plants with the remains of sticking sand are shown in fig. 2.

It is interesting to note that without such mechanical interference it is almost impossible to separate the remains of sticky sand. This is especially true of the first three drought simulations.

same way. Such experiments should always be conducted by the same person or by a special mechanism using vibration.

3 By doing this, we wanted to simplify comparisons with the control where 20 seeds weigh about 1 g.

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grains, i.e. how many times this product increases the weight of grain within 10 days of experiment, which is the purpose of agronomy as a science in general.

5. Coefficient K, is relative increase in the root lateral surface

 $K_4 = \left(\sqrt[3]{m_2/m_1}\right)^2$ m_2/m_1 , \int_{0}^{2} (7)

where m_1 and m_2 are root weights in the experiment and control, respectively¹.

These calculations are listed in table 1 and 2.

Analyzing the results of table 1 and 2, the following conclusions can be drawn:

- 3. The studied products have a positive effect on increasing the relative weight of the root system, i.e. the plant drought tolerance coefficient K1, increasing it by 10-12% (see Table 2, column 7). This promotes the plant better survival during drought.
- 4. Accordingly, the biologic product efficiency coefficient R also increases in drought by 21 and 30% and by 5 and 19% under favorable conditions, respectively (Fig. 7).
- 5. This can be explained by the fact that in the control variant the loss of exudates reaches 20.1% of the initial grain weight. At the same time, inoculation of seeds with a complex proprebiotic helps to form a bacterial cover with lower energy consumption of the seed itself (16.8 and 13.7%), which increases its root system (Table 2, column 4) in drought and coefficients of moisture retention $K₂$ and drought toleranceK..
- 1. The weight of sticky sand M1 in drought is almost 2 times greater than at optimal humidity (see Table 1, column 3). The studied experimental products increase the effect of soil adhesion by $16 \div 25\%$ in drought and by 8÷20% with sufficient humidity, i.e. have a positive effect on moisture accumulation around the roots (see Table 1, column 4).
- 2. The studied products also have a positive effect on increasing the weight of wet plants M2 (Fig. 5, 6) and, accordingly, the plants moisture retention coefficient K2, increasing it by 17-20% in drought and by 4-17% under favorable seed

germination conditions (see Table 1, column 7).

which shows how much water the plant has accumulated in preparation for drought. That is, how many times its dry weight has increased compared to the weight of the wet plant.

3. Coefficient K₂ is relative seed energy consumption for producing exudates Δm:

4. Coefficient R is the effectiveness of the biological product used for seed treatment

At the same time, the studied biological products have little effect on the loss of exudates under favorable conditions, which is quite logical. In this case, the studied biological products act as conventional biological stimulators of plant growth, and not as anti-drought stressors.

It is clear that the plants in experiments 1, 2 and 3 under arid conditions released significantly more exudates, which is confirmed by the weight and density of the sticking sand. As can be seen from table 1, its weight is almost 2 times greater than that in similar experiments 4, 5, 6 with sufficient moisture. In the case of continued drought, the outer surface of sticky sand is hardened, retaining moisture.

Now it is not difficult to identify the exudates Δm as the difference between the initial weight M_o of dry seeds and sprouted dried plants $M₂$. That is

 $\Delta m = M_0 - M_3,$ (1) or $\Delta m = M_0 - (m_1 + m_2 + m_3)$ (2)

where m1, m2, m3 are dry weight of green vegetative part, or sprout, grain, or rather its remains, and roots.

Note that the last formula (2) gives a more accurate result because in the area of the grain, due to adhesive exudates, sand sticks so tightly that it can be removed only by mechanical separation of the grain from the root4.

To assess the plant's ability to withstand thermal shock and lack of moisture, the following criteria are introduced:

1. Coefficient K, is drought tolerance coefficient:

$$
K_1 = m_3 / m_1
$$
 (3)

as the ratio of root weight to sprout weight.

It is clear that the greater the K1, the more developed the root in relation to the sprouts is.

2. Coefficient K, is water retention coefficient

$$
K_2 = M_2 / M_3 \tag{4}
$$

$$
K_3 = \Delta m / M_0 \bullet 100\%,
$$
 (5)

compared with seed dry weight M_{0} .

$$
R = M_2 / M_0 \tag{6}
$$

which shows how the product promotes the forma-

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3) Loss of exudates reduces the weight of dried sprouted plants by 12-25%, while increasing the weight of wet plants by 20-50%, i.e. the moisture retention coefficient of plants as the ratio of wet and dry plant weights, which also has an effect on plant survival.

PRACTICAL **CONCLUSIONS**

4) Mucous exudates form a bacterial cover (bacterial film) around the roots which increases during drought. The cover, together with the soil adhering to it, creates an additional supply of moisture, increasing the chances of the plant to survive drought.

1. 1. In order to prepare the seeds for extreme survival conditions during drought, it is advisable to do it in advance by inoculating seeds with combined proprebiotics, bacterial metabolites and signaling molecules (alarm signals for thermal stress). The seeds prepared in this way will survive drought much better and preserve their natural potential for future harvests.

N_o

1 2 $\overline{2}$ $\overline{4}$ ϵ

6 1,051 36,65 120 7,92 0,926 7,5 119 8,6 117 0,125 11,8

 $\overline{7}$

EXUDATES AND DROUGHT

20,1

1 2 3 4 5 6

20,1

1 2 3 4 5 6

To guide the reader's eye, the results are presented in Fig. 5 and 6 in the form of diagrams.

Based on the above typical experiment and having conducted dozens of similar studies, it can be argued that:

1) The plant increases the secretion of root exudates in response to lack of moisture.

2) Additional loss of exudates reduces the biological potential of the plant, inhibiting its development. At the same time, additional exudates during the drought primarily accelerate the growth of the root system, inhibiting the growth of vegetative mass (in fractional or percentage terms). This increases the drought tolerance of plants as the ratio of the roots and the vegetative part weight, i.e. the plant chance to survive drought.

PIC. 6. Weight of seeds and dry plants after 10-day sprouting (20pcs)

2. It is also extremely important to spray the soil surface with biological products similar to the ones considered after sowing seeds ahead of drought.

ON THE ROLE OF THE AGRONOMIC SHOVEL

It should be noted that conducting similar experiments on plant survival in global warming over the past three years, the author used an approach similar to the above. The obtained results often differed significantly, but always allowed to logically appreciate the plant's energy loss for exudates production and prove or disprove the effectiveness of a biological product as a means of counteracting drought by accelerating the growth of the root system and bacterial cover around it.

We emphasize that the practical implementation of this approach and application of a new class of biological products to thousands of hectares of different crops have shown that an agronomist should not look at the sown field from a car window or with a drone camera trying to come to a conclusion as to the product effectiveness but should take a shovel, get out of the car and dig up the roots of plants, because it is them that would testify to the success or failure (in addition, it will also show the soil condition, which cannot be done by the drone!).

Replacement of wild wheat varieties with artificially bred synthetic varieties led to significant changes in both the drought tolerance coefficient and the moisture retention coefficient. Accordingly, the losses of exudates also differed, being higher in the more drought-adapted wild varieties.

There was also a large dependence of microbiota activity on magnetic storms, i.e. the sun activity. Accordingly, plants also responded adequately to magnetic phenomena, releasing more or less exudates.