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Mechanisms of tolerance of nodule bacteria and host plants to drought stress

Abstract

Agricultural production is highly susceptible to environmental changes and is directly affected by both favorable and unfavorable climate changes. Global climate change, as well as other biotic and abiotic stressors, represents a major challenge for agricultural production today. Extreme conditions such as floods, droughts, and temperature extremes, which are increasingly common today, directly affect plant life as well as soil microorganisms. Some of the consequences of global climate change are impaired seed germination, inhibition of the photosynthesis process, poor nutrient availability, a decrease in the number of microorganisms in the soil, and changes in the gene expression of plants and microorganisms. Thus, global climate change poses a particular challenge in the production of high-quality food and increases the demand for new types of crops that must meet the criteria of high productivity.

Keywords: *nodule bacteria, climate change, drought, tolerance, adaptation mechanisms*

Introduction

Drought is one of the most harmful abiotic stresses that have a negative impact on modern agricultural production (Zhang et al., 2016). Drought causes water stress in crops, thus becoming a limiting factor in the growth and productivity of numerous plant crops (Rampino et al., 2007). Soil water deficits can be persistent in climatic zones characterized by poor water availability or intermittent water supply during the growing season. Therefore, drought is a major environmental stress that affects plant growth and development as well as soil nodule bacteria, thus disrupting their water status. Water deficits negatively affect the growth and development of host plants, resulting in poor or non-existent establishment of symbiosis between nodule bacteria and legumes.

Drought disrupts physiological processes such as photosynthesis, respiration, intake of nutrients, but also affects the overall metabolism of nodule bacteria as well as the process of

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symbiotic nitrogen fixation (Paul et al., 2017). In drought conditions, gas exchange is disrupted, resulting in excessive production of reactive oxygen species (*ROS*) in both host plants and nodule bacteria, which leads to the development of oxidative stress (Osmolovskaya et al., 2018).

The survival of rhizobia and their colonization of the host plant is of great importance for the formation of healthy nodules, which results in a better supply of nitrogen to the plant, since the nodule bacteria carry out the process of symbiotic nitrogen fixation in the formed nodules (Egamberdieva et al., 2017). In a symbiotic relationship, the process of nitrogen fixation is strictly linked to the physiological state of the host plant, i.e. not a single highly efficient strain can achieve its full capacity for nitrogen fixation if limiting soil factors (salinity, insufficient moisture, unfavorable pH, plant diseases and inadequate photosynthesis) affect the host plant (Kabbadj et al., 2017).

Water influences soil microbial dynamics as a resource, solvent, and transport medium. Each of these modes is important in regulating the function of nodule bacteria, as they interact to drive complex microbial and biogeochemical responses (Paul et al., 2017). The availability of water as a resource depends on the energy state, or its water potential. If more energy is available, water as a resource is more available (Schimel, 2018). For nodule bacteria, water potential is controlled by their survival and function. Because the microorganisms are small and in close contact with their environment, there is nothing between them and the soil except their cell membrane and wall. Therefore, it is essential that their water potential is in equilibrium with the water potential of the soil around them (Paul et al., 2017).

As the soil dries out, the soil water potential decreases, which leads to changes in the cells of the nodule bacteria. In order to avoid losing water to the environment and dehydrating, the cells of the microorganisms begin to accumulate dissolved substances to reduce their internal water potential and cause it to equalize with the soil water potential (Wallenstein and Weintraub, 2008). As the cellular water potential of the microorganisms then decreases, this can lead to reduced cell turgor, which is equivalent to wilting in the host plant. The loss of turgor leads to numerous changes in the physiological functions of the microorganisms, which results in a decrease in the dynamics of metabolism. Ultimately, as the water potential in the cells of the microorganisms decreases, cellular functions deteriorate, causing the death of the microorganisms (Schimel, 2018).

Since water is necessary for many biomolecular processes, including protein stability, various interactions between enzymes and substrates, as well as for maintaining the overall cellular structure of nodule bacteria and host plants, the paper will provide an overview of adaptation mechanisms of nodule bacteria and host plants to drought stress.

Adaptation mechanisms of nodule bacteria to drought

Nodule bacteria have developed several different mechanisms that enable them to survive more easily in drought conditions. The adaptation mechanisms of rhizobia that enable them to survive in drought conditions are dormancy, exopolysaccharide (EPS) and biofilm production, molecular adaptation, cell membrane adaptation, osmolyte accumulation and metabolic adaptations (Lebre et al., 2017).

Dormancy

As a result of unfavorable abiotic factors such as drought, nodule bacteria enter a reversible form of dormancy in which the cells go into an inert state of metabolic activity. Dormancy is considered an energetically demanding process, since the return of bacterial cells to an active form will require a lot of resources and energy (Rittershausi et al., 2013).

When a nodule bacterial cell enters dormancy, its growth stops, metabolic activity decreases, and the production of numerous macromolecules decreases. At that point, the cells begin to accumulate carbon, most often in the form of trehalose sugar, glycogen, and triglycerides (Lebre et al., 2017). Dormancy is thus considered an important survival strategy for nodule bacteria under various stressful environmental conditions, especially under conditions of stress caused by a lack of sufficient water. The mechanism itself enables the long-term survival of soil microorganisms but also ensures the preservation of biodiversity, since "dormant" bacterial cells become protected reservoirs of genetic diversity (Bär et al., 2002).

Exopolysaccharide production and biofilm formation

Exopolysaccharides are the most important part of the extracellular matrix, accounting for 40-95% of bacterial weight (Marczak et al., 2017). It is an integral part of the glycocalyx and participates in the formation of biofilm. Biofilms are found in various ecological environments as dominant structures of communities in which microorganisms coexist (Besemer, 2015). Biofilm formation is necessarily associated with the survival of nodule bacteria in soils with low water content (Wu et al., 2019). Biofilm composition plays a significant role in the regulation and improvement of various metabolic and physiological functions within the bacterial community. The main component of the biofilm is water, which accounts for 97%, while the remaining 3% is made up of bacteria (Singh and Chauhan, 2017).

Within the biofilm, water ensures the flow of nutrients, microorganisms and oxygen. Such a hydrated environment enables better connection with hydrogen bonds, which minimizes the possibility of drying out (Vurukonda et al., 2016).

Synthesized exopolysaccharides by nodule bacteria play an important role in increasing the tolerance of microorganisms to drought thanks to their hygroscopic nature, i.e. the ability to absorb moisture from the environment. Also, the hydrophilic properties of the synthesized exopolysaccharide contribute to a faster rate of water absorption (Castellane et al., 2015).

According to Naseem et al. (2018), microorganisms that have the ability to synthesize exopolysaccharides can lead to the alleviation of drought stress in the soil. The synthesized exopolysaccharides thus affect the soil structure and lead to its improvement, thus directly increasing the resistance of numerous legumes to drought. Research conducted by Wilpiszesk et al. (2019) showed that microorganisms that have the ability to synthesize exopolysaccharides lead to the formation of microaggregates in a higher percentage. Thus, soils with a higher content of smaller aggregates contain more nutrients for microorganisms and plants in an accessible form, than larger aggregates would contain (Grover et al., 2011).

Molecular adaptation

In addition to the symbiotic genes that are crucial for the interaction between nodule bacteria and legumes, the genome of nodule bacteria also contains other genes that are important for the life cycle of rhizobia, such as stress-induced *genes.* Stress-induced genes such as *ots* AB, *gro* EL, *clp* B, *rpo* H play such an important role in the tolerance of nodule bacteria to different environmental conditions (Silva et al., 2017).

The survival of nodule bacteria in adverse conditions such as drought requires the ability to recognize changes in the environment, the ability to express genes in new environmental conditions and ultimately the overall adaptation of bacterial physiology to adverse environmental conditions. The response to heat stress has been well studied in many bacteria, including nodule bacteria (Silva et al., 2017). Heat shock proteins (HSPs) are a group of proteins that

can be found in every cell of a living organism. Various types of stress, including drought, lead to the induction of the expression of genes called heat stress protein genes. They play a very important role in protecting cells from protein denaturation caused by temperature changes.

Today, the development of highly efficient nodule bacteria strains used for pre-sowing inoculation of legumes should not neglect their tolerance to various types of stress (Silva et al., 2017). If nodule bacteria are not able to overcome various abiotic changes in the environment, their application in the inoculation of legumes will not have a great effect (Igiehon and Babalola, 2018).

In many agricultural soils, there are indigenous populations of nodule bacteria that may be very inefficient for host plant growth and nitrogen fixation, but may be very well adapted to adverse soil conditions such as reduced water availability. Therefore, investigating the molecular basis of nodule bacteria's stress tolerance is a first and fundamental step towards improving highly efficient nodule bacteria for pre-sowing legume inoculation.

Cell membrane adaptation

The cell membrane represents the main barrier between the intracellular space and the external environment. It is strongly influenced by changes in the external availability of water. Root nodule bacteria that are exposed to extreme fluctuations in water status usually have changes in the composition of phospholipids and fatty acids of the cell membrane. This includes an increased proportion of unsaturated fatty acids. It is believed that the increase in the proportion of unsaturated fatty acids in the bacterial cell membrane plays an important role in protecting root nodule bacteria cells from desiccation (Lebre et al., 2017).

Osmolyte accumulation

Under conditions of water scarcity, nodule bacteria cells accumulate intracellularly small molecules called osmolytes. They establish osmotic balance and enable continued protein function at low water potential, which ultimately enables the survival of nodule bacteria cells under conditions of water scarcity, or drought (Kibido et al., 2019).

Osmolytes, such as trehalose and glycine betaine, are considered part of a general adaptive strategy for survival in drought conditions of most bacteria, including nodule bacteria. They are low molecular weight compounds that can accumulate from the environment or be synthesized within bacterial cells. Osmolytes thus inhibit membrane changes associated with water loss from cells and a decrease in intracellular fluidity (Vriezen et al., 2007).

Metabolic adaptations

The loss of water from the cells of root nodule bacteria creates a large metabolic load for the cell itself, because there is a change in the kinetics of metabolic pathways, a change in the rate of energy generation, as well as a change in the growth rate of bacterial cells. In this state, nodular bacteria most often switch from anabolic to catabolic metabolism.

The balance between anabolic and catabolic metabolism is quite complex. In drought conditions, all glucose is redirected to the synthesis of trehalose, while the oxidation of fatty acids becomes an effective alternative source of energy for the cell (Finn et al., 2013).

Also, genes involved in glycogen metabolism are highly expressed in nodule bacteria cells exposed to drought, as intracellular glycogen helps restore the volume of nodule bacteria cells (Sanko-Sawczenko et al., 2019). Metabolic adaptation may thus greatly contribute to the ability of nodule bacteria cells to adapt to drought stress (Vurukonda, 2016).

The impact of drought on the growth and development of host plants

Any abiotic factor that exceeds the optimal value limit for plant growth and development represents stress. During the growing season, plants are exposed to various stressful environmental conditions that adversely affect their growth, development, grain quality and yield. Plants cannot avoid adverse conditions, but some varieties show greater and some less resistance. Stress tolerance is the ability of a plant to adapt to sudden changes if they occur and not be too damaged. There are several types of stress in plants, such as water stress, temperature stress, stress caused by increased salt concentration. A significant problem in plant cultivation is water stress, or drought, and of all the extreme weather events caused by climate change (Rampino et al., 2007).

Drought is the main and most common cause of reduced yields of the most important agricultural crops both in the world and in the Republic of Croatia. Under the influence of climate change, drought represents a major problem in the world, causing inhibition of plant growth and a decrease in economic productivity, especially in arid and semi-arid areas (Wilhite and Pulwarty, 2017).

Drought adversely affects grain filling, causing maximum yield reduction. Depending on the host plant, lack of soil water at the time of grain filling can reduce grain yield in more sensitive varieties by 40-60%.

Photosynthetic efficiency of soybeans under drought conditions

In higher plants, the function and organization of the photosynthetic apparatus changes in response to abiotic stress. The most common osmotic stress is high temperature, drought, increased salinity, and low soil pH. They lead to changes in physiological, biochemical, and molecular processes in the plant (Poljak et al., 1998). As a result of osmotic stress, the loss of photosynthetic pigments often occurs, so one of the first responses of the plant to unfavorable environmental conditions is reported to be a decrease in the rate of photosynthesis (Josipović, 2019).

An indicator of the state of the photosynthetic apparatus is the measurement of chlorophyll fluorescence. The effect of drought on the photosynthetic apparatus begins with the closure of stomata in moderate drought, while metabolic and structural changes are caused by long-term drought. In drought conditions, stomata close and gas exchange is limited. If intensive drought is present for a long period of time, there is a significant disruption in metabolism and changes in cell structures. Since the water balance in the plant is determined by transpiration and absorption of water from the soil, if the transpiration rate is higher than absorption, there is a decrease in cell turgor and it begins to decline. The cell volume begins to decrease, and all this together leads to an increase in the concentration of cell components. As a result, there is an increase in osmotic and a decrease in water potential, which causes a halt in the photosynthesis process (Fathi and Tari, 2016). In conditions of mild to moderate drought, plants close their stomata, while in severe drought, metabolic processes are inhibited. The accumulation of small molecular weight organic substances called osmolytes begins. In case of long-term drought, osmolytes bind to cellular proteins with hydrogen bonds and thus protect proteins from denaturation by replacing water (Kereša et al., 2008).

Plants often experience an accumulation of free radicals in drought conditions, the most significant of which are reactive oxygen species caused by free radicals, causing oxidative stress. The resulting free radicals often lead to membrane damage, protein denaturation and numerous changes in the DNA molecule, thus causing major damage to plant cells, which can ultimately lead to reduced yields.

Mechanisms of plant drought tolerance

The complex trait of drought tolerance is described in the work of Kereš et al. (2008), where drought tolerance of genotypes is viewed from the breeding perspective as the ability to maintain high yields regardless of adverse environmental conditions. They also state that tolerance can be avoided if the genotype possesses some of the mechanisms such as a drought avoidance mechanism, a dehydration reduction mechanism, and a dehydration tolerance mechanism. Early maturation provides an escape mechanism. Namely, genotypes of various plant species possess this mechanism where they complete their life cycle before the onset of a period of intense drought. This is achieved by accelerating metabolic processes as well as their accelerated growth (Shavrukov et al., 2017). The mechanism for reducing dehydration represents a physiological adaptation of the plant. Genotypes that possess this mechanism have a characteristic slowdown in metabolic processes, slower growth, and high-water potential and turgor in cells (Kereša et al., 2008). The mechanism of dehydration tolerance mainly represents tolerance to changes that drought causes in cells.

Conclusion

Microorganisms play a significant role in the adaptation and increase of the tolerance of numerous legumes to drought, as well as to other abiotic stresses. Numerous studies have shown that certain types of microorganisms increase the tolerance of numerous legumes to various abiotic stresses such as drought. This applies especially to nodule bacteria, which are characterized by the ability to form biofilms and the production of exopolysaccharides, as well as other numerous mechanisms that thereby increase the tolerance of legumes to various abiotic stresses.

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Stručni rad

Mehanizmi tolerantnosti kvržičnih bakterija i biljke domaćina na stres izazvan sušom

Sažetak

Poljoprivredna proizvodnja vrlo je podložna promjenama u okolišu te na nju izravno utječu povoljne i nepovoljne klimatske promjene. Globalne klimatske promjene kao drugi biotički i abiotički stresori predstavljaju veliki izazov za poljoprivrednu proizvodnju danas. Ekstremni uvjeti kao što su poplave, suše i temperaturni ekstremi koji su danas sve češći izravno utječu na biljni svijet kao i mikroorganizme tla. Neke od posljedica globalnih klimatskih promjena promjena su otežano klijanje sjemena, inhibicija procesa fotosinteze, loša raspoloživost nutrijenata, smanjenje broja mikroorganizama u tlu te promjene u ekspresiji gena biljaka i mikroorganizama. Prema tome, globalne klimatske promjene predstavljaju poseban izazov u proizvodnji visoko kvalitetne hrane te povećavaju potražnju za novim tipovima usjeva koji moraju udovoljavati kriteriju visoke produktivnosti.

Ključne riječi: *kvržične bakterije, klimatske promjene, suša, tolerantnost, adaptacijski mehanizmi*