QUANTITATIVE VARIATION OF MACROELEMENTS FROM
Claviceps purpurea PARASITED RYE PLANTS

ŞTEFANIA SURDU*, ZENOVIA OLTEANU*, VIORICA DULMAN*, MARIA-MAGDALENA ZAMFIRACHE**

Abstract: In the pathogen system Claviceps purpurea – rye, the parasite produces profound modifications. In order to study the alkaloids’ influence upon the mineral metabolism we have investigated the sodium, potassium, magnesium, calcium and silicon ions from the rye plants parasitized by Claviceps purpurea, as well as from rye plants treated with ergocryptine and ergotoxine solutions. The sodium, potassium and magnesium ions are found in different amounts, depending on the alkaloids that act upon the plant. The value of the potassium/magnesium report is higher than the one mentioned in references. There is a convergence of the manifestations for the calcium ions, depending on the predominant alkaloid that acts upon the plants. The value of the calcium/magnesium report varies between 1.12 and 0.27. In the presence of the parasite these values are reduced and they range according with the alkaloid type of the parasite (the lowest report was found at the ergocristine type).

Key words: Claviceps purpurea, rye, pathogen system, ergotic alkaloids, mineral metabolism.

Introduction

Between plants and soil there is a tight interaction, as the soil influences the growth and development of plants through the nourishing substances it provides, through the moisture, temperature, pH conditions; on the other hand, plants can produce important modifications in soil by the organic substances they produce at the surface of the roots, thus transforming some organic or mineral combinations. The carbon dioxide resulted in respiration, as well as organic acids or enzymes increase the solubility of some compounds or increase their accessibility.

The mineral elements have a special role in the growth and development of the organisms. They influence the enzymatic activity, maintain the protoplasmic membranes integrity, regulate the osmotic pressure and the acid-basic equilibrium, they contribute to the establishing the electrochemical equilibrium and the colloidal systems. They also influence the hydrating and viscosity of the living material, the pH of the vacular and citoplasmatical liquid, the reversibility of the sol-gel state, the forming of complex compounds (Caramete C., 1973, Milică C.I., 1977, Neamţu G., 1995).

The literature information illustrates that in rye plants the distribution and the content of mineral substances vary according with numerous factors (Gaşpar, I., 1978).
Material and Methods

The biological material is represented by Ergo race autumn rye plants, artificially infected with conidia suspensions of *Claviceps purpurea* obtained in submerged cultures. There have been used three strains of each alkaloid type with biochemical determined alkaloid content and spectral composition. The fungal strains have been codified after the predominant alkaloid name (P-ergocryptine, S-ergocrystine and T-ergotamine), accompanied by a number.

At the same time, there have been used two pure alkaloid solutions in equal concentrations of active substance, but with different compositions used on plants. At first, the ears were pricked and afterwards pulverized. The *3P* variant represents the lot of plants which have been treated with ergocriptine and the *SRP* variant represents the lot of plants which have been treated with ergocristine, ergocornine and ergocriptine, in equal shares. Ten days after the first treatment and then every 5 days, leaves and ears of the plants have been pulverized with the two-alkaloid solutions. The samples for the chemical and biochemical analysis have been taken 10 days after the inoculation date and than every 5 days, up to 35 days, which is the day of the sclerotia harvesting.

In order to determine the total alkaloid content we have used a spectrophotometric method (Rumpel, W., 1955). Extinctions were correlated with concentrations by using an etalon curve made with ergotamine tartrate. The determining of the alkaloid content was made on half a sclerotium, the second half being used for obtaining in vitro culture.

The alkaloids spectrum was determined by thin layer chromatography (Brevet RSR, 1977). The determining of dry substance and ash used the gravimetrical method; the determining of calcium and magnesium ions used the complexometric method, and the determining of sodium and potassium used the flamphotometric method (Halga P., 1984).

Results and Discussions

The *Claviceps purpurea* strains used for obtaining submerged conidia cultures have different biosynthetic qualities. The main selection criterion consists in their capacity of producing mostly one of the alkaloids, as well as the possibility of producing alkaloids. The selected strains have morphological, physiological and biochemical manifestations characteristics to the biochemical sclerotia type appreciated by the capacity of producing mainly ergotamine, ergocrystine or ergocryptine (Surdu St., 2001).

The ergocryptine type has as a characteristic a reduced capacity of the sclerotia to produce total alkaloids, the ergocryptine being rarely represented in a proportion of 80 – 90%. The descent of these strains is less numerous. P – 509 sclerotium, from which were obtained the conidia used for artificial infection of rye, has a reduced biosynthetic potential. The quantity of total alkaloids from the origin sclerotium is 0,28g%, out of which 80%
represent ergocryptine and the rest represent ergotamine and ergocrystine. The ergotamine sclerotium T-506 has a higher biosynthetic potential. The total alkaloid quantity is 0.68g% of which 80% ergotamine is accompanied by ergocrystine.

The highest biosynthetic potential is typical for the sclerotia that produce mainly ergocrystine. The selected sclerotium, S – 515, contains 0.79g% total alkaloids, with 70% ergocrystine, plus ergotamine and ergocrystine.

The capacity of these strains to transmit their biosynthetic potential differs by the average amount of total alkaloids content, by the intensity of alkaloid biosynthesis process and by the spectrum modification during the growth period and the sclerotia development in the rye’s ears (Fig. 1)

Fig. 1 – Accumulation of total alkaloids in Claviceps purpurea sclerotia parasitizing rye plants

It is notable that the alkaloid biosynthesis processes are fast and intense at the ergocrystine strain whereas the lowest are found at the ergocryptine strain. The small decreasing of total alkaloids quantity at ergocrystine descent can be explained by the different age of sclerotia formed on the analyzed plants, as they are more numerous at this alkaloid type than at the other two types.

The alkaloid spectrum changes during the growing period of the sclerotia (fig. 2) by progressive increasing or decreasing of the predominant alkaloid proportion, with the changing of the accompanying others.

On rye plants that have been administered only alkaloid solutions, sclerotia have developed as well; however, the infection is initiated by the conidia from honey dew produced by the parasite which inhabited the ovary of the host flowers, consequently to the artificial infection. The alkaloid spectrum of these sclerotia is different and it is influenced by the composition of the alkaloid solution. The usage of the solution, which contains ergotoxinic alkaloids in equal proportion, is found in the alkaloid spectrum of these plants’ sclerotia. On the other hand, the sclerotia of the plants treated with ergocryptine solution are characterized by predominant presence of the alkaloid in the younger sclerotia and by
diminishing of the ergocryptine proportion, in favor of ergocristine and ergotamine, along with their maturing (Fig. 2).

Fig. 2 – Modification of alkaloid spectrum in Claviceps purpurea sclerotia during growth and development on rye plants

In order to establish the influence of the alkaloids upon the host plant’s metabolic processes, especially upon the ions’ presence, we have reported the chemical analysis results of the plants parasitized by Claviceps purpurea to parasite free rye plants, treated and untreated with alkaloid solutions. The up mentioned ions have been determined at 10, 20 and 30 days from the inoculation of the alkaloid solutions and the infection of rye plants, each analysis time corresponding to a different stage of the parasite’s life cycle. The first analysis corresponds to the intense conidia producing, marked by the honeydew. The diminishing of this process is accompanied by the intensification of alkaloid biosynthesis started in the phase of maximum secretion (Taber W.A, 1985). The growth of the sclerotia, the reduction of their humidity and the intense alkaloid synthesis take place in the third decade of the parasite’s life cycle, when rye plants are almost ripping phase (Gaspar, 1978).

The role of the ions for the plants’ physiological balance as well as their implication in the functioning of numerous enzyme systems are well known facts and they constitute a possible explanation for the significant modification of some enzymes’ activity at rye plants parasitized by Claviceps purpurea, according to the predominant alkaloid type of the sclerotia present in the ear (Olteanu Z., 1998, Oprică –Antohe L., 1998, Surdu St., 1998, Tănase A., 1998).

For the witness plants (fig. 3), the sodium ions are in the highest amount during the maximum flowering of the rye, immediately after the administration of the solution. The ergocryptine solution has an action similar, which manifests later on, during the ripping period (variants SRP and 3P respectively). Significant modifications occur to treated and parasitized plants (SRP pz and 3P pz), as the sodium ions are present in smaller amounts.
The difference between parasitized and a parasite free plant is significant. The values are very low in the presence of ergocristine type sclerotia (variant S – 515) and ergocryptine type sclerotia (variant P – 509) and significantly high in the presence of ergotamine type sclerotia (variant T – 506), for which there is also an increased tendency of diminishing the values.

Potassium is found in small amounts in all vegetative tissues and organs, and it is well represented in the tissues with a high physiological activity. It circulates with high velocity in the vegetal tissues, sometimes against the concentration gradient. It is involved in the freezing resistance and drying periods, as well as in pest and diseases attacks. Potassium has an important role in the biosynthesis, transport and depositing of sugars, especially reserve sugars.

For the control plants (Fig. 3) there is a clear diminishing of the potassium amount at the flowering stage, comparing to the ripening stage. Ergocryptine has a strong influence upon the dynamics of potassium ions that are found in higher amount immediately after the solution has been administered. Higher values than the ones present at control plants or at plants treated with ergotoxine solution are maintained during the whole investigation period. The diminishing tendency of the values is an obvious characteristic of the three mentioned variants. The presence of the parasites on the treated plants modifies significantly the values only for the 3P pz variant.

The presence of the parasites on plants untreated with alkaloid solutions determines quantitative and dynamical modifications. Ergotoxine type sclerotia determine a diminishing of potassium amounts in the parasitized plants. This diminishing is more severe in the presence of ergocristine sclerotia immediately after the plants infection with
The ergocryptine type sclerotia produce a persisting diminishing of potassium amount after the ceasing of honeydew production.

Ergotamine type sclerotia maintain a high content of potassium during the two decades of the life cycle, when there are present the most significant variations, both for the control plants and for the treated and/or parasitized plants. During the intense alkaloids biosynthesis the potassium amount is similar for most of variants; only ergocrystine type sclerotia are an exception.

Magnesium, as well as calcium, has an important structural and functional role. It accumulates especially in young tissues, rich in protean substances and it has a special role in maintaining the macroergic compounds’ equilibrium.

At control plants (Fig. 5), the magnesium amount remains quite constant during investigated period. The influence of parasites or alkaloids from the administered solutions modifies significantly the dynamics and the quantity of magnesium from rye plants, by emphasized the differences from the control plants. Repeated administration of alkaloids solutions determines an increasing of the magnesium amount and maintains high values during most of the period. The presence of sclerotia determines a significant increasing of magnesium concentration at plants treated with ergocryptine solution (variant 3P pz).

Parasites determine quantitative modifications similar to the ones present at plants treated with alkaloids solutions. As far as the dynamics is concerned, only plants parasitized with ergocrystine type sclerotia behave similarly to plants treated with alkaloid solutions. Ergocryptine and ergotamine types’ sclerotia determine a progressive diminishing of the magnesium amount, which is more severe in the first case.
It acts as a calcium metabolism regulator. For the well development of plants a certain report between calcium and magnesium (2.1/1.1) and between potassium and magnesium (4/1) is necessary. (Caramete C., 19, Milica C.I., 1977).

The value of the potassium/magnesium report at rye plants is higher than the one mentioned in references and it diminishes during the considered period, from the flowering to the ripping phase. At parasitized plants the value of the report modifies according with the parasite’s age and with its alkaloid type. Thus, it is higher at plants parasitized by the ergotamine type strain and lower at plants parasitized by ergotoxine type strains. The ergotoxine solution applied on leaves and ears determines a decreasing of the report, the values being close to the normal limit in the second part of the investigated period; the ergocryptine solution maintains the report within high values in the first 20 days, whereas during the next stage values would drop to approximately 4/1. The presence of the parasite associated with alkaloids administered on leaves increases the potassium/magnesium report for both of the variants.

![Graph](image)

Fig. 5 – Modification of Mg\(^{2+}\) (g\% x 10\(^{-4}\)) at rye plants treated with ergotic alkaloids and parasitized by *Claviceps purpurea*, during growth and sclerotia forming

Similarly to the other ions, calcium has both structural and functional roles. It is an antagonist of the potassium, sodium and magnesium ions. Calcium content in plants depends on species, age, vegetation stage, soil. It accumulates in large quantities in old tissues, in leaves, stalks and less in roots. Cereals contain little calcium.

As far as rye plants are concerned (Fig. 6) the dynamics of the calcium ions is characterised by wide variations not only for parasitized plants, but also for control plants. Massive diminishing of the calcium amounts is produced after the flowering period and it increases during the beginning of the ripping period.
Plants parasitized by ergotamine type sclerotia have a similar response to the one of the control plants. Plants treated with ergotoxine solution and the ones parasitized by ergocrystine type sclerotia are similar in values and dynamics (variants SRP and S515). The response of the variant with a predominant ergocryptine presence is similar (variants 3P and P-509). Each of the alkaloids predominantly present in plants, regardless of source, act upon calcium in the same manner.

![Graph showing modification of Ca²⁺ ion](image)

**Fig. 6 – The modification of the Ca²⁺ ion (g% x 10⁻⁴) in rye plants treated with ergotic alkaloid solutions and parasitized by *Claviceps purpurea*, during the growth and sclerotia forming period**

The presence of sclerotia on plants treated with alkaloid solutions increase the variation tendency of the values for the respective variant. Besides ergocryptine applied on leaves and ears there is also the ergocryptine produced by the sclerotia (Fig. 2) and the increasing of the concentration in plant probably determines the modification of calcium quantity. The large number of sclerotia per ear, as well as the high proportion of ergocrystine is added to the one present in ergotoxine solution, thus acting upon calcium. In both cases, alkaloids modify the dynamics of ions, more obviously the one of calcium, when it reaches certain concentrations.

The value or the calcium/magnesium report varies between 1,12 – 0,27 and the values are present at control variants and SRP variant, respectively, 10 days after the artificial infecting of rye. Values are reduced and vary according with alkaloid type of the parasites, ergocrystine type increasing their diminishing. The lowest values of the calcium/magnesium report characterises the 20 days age. Parasite increases significantly the value of the report for the variants where alkaloids have been administered on leaves and ears.

Silicon is an element that contributes to the mineralization of the plants cell walls and it has structural and functional role. It is found predominantly as hydrated silica and, in
small amounts, as organic compounds. In cereal stems, as well as in some weeds the amount of hydrated silicon represents approximately 50% of the ash. Silicon has an important role in the growth of plants as well as their defence against parasites.

In control plants and plants treated with alkaloid solutions, the silicon amount is maintained at a high level, with relatively similar periodic variations. Parasites determine the diminishing of the silicon content, more significant at ergocryptine addition (Fig. 7).

![Fig. 7 - Modification of the Si^{2+} ion (g% x 10^{-4}) in rye plants treated with ergotic alkaloid solutions and parasitized by *Claviceps purpurea*, during the growth and sclerotia forming period](image)

At artificially infected plants the silicon amount is lower in the presence of ergocryptine and ergocrystine type strains. There is a significant maximum at ergocrystine type at the age of 20 days, which otherwise is present at all plants, with a lower amplitude. The ergotamine type does not modify the silicon dynamics or content in parasitized plants.

We can conclude that ergotic alkaloids, regardless of the path they use to influence rye plants’ metabolism, by their concentration and alkaloid type produce modifications of some ions concentration. During the entire life cycle the parasite influences the host plants’ metabolism, inducing different manifestations according with the bioproductive abilities of the *Claviceps purpurea* strains.
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