




Article

Influence of 24-Epibrassinolide on the Energetic Parameters and Early Stages of Growth and Development in Seedlings of Two Maize (*Zea mays* L.) Genotypes

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Abstract: Brassinosteroids (BRs) are a class of plant hormones that play important roles in regulating various physiological and developmental processes in plants. One of the most effective BRs involved in modulating crop growth is 24-epibrassinolide (24-EBL). The effects of different concentrations of 24-EBL on various biochemical and biophysical parameters critical to early growth stages and seedling development were investigated using two maize hybrids, ‘ZP 434’ (a new-generation hybrid) and ‘ZP 704’ (an older-generation hybrid). The evaluation of results is based on measurements of germination percentage, morphometric parameters, redox status, comparative analysis of thermodynamic parameters (such as Gibbs free energy, enthalpy, entropy), and the concentration of specific sugars in different parts of maize seedlings. The results indicate that the germination and initial growth of maize seedlings are influenced by the flow of crucial sugars from the remaining seed (as a source of nutrients) towards the plumule and radicle (as sink organs). Furthermore, alterations in Gibbs free energy play a significant role in these sugar transfers within the maize seedlings. The seed germination was most affected by the highest concentrations of 24-EBL, showing inhibitory effects, whereas lower and moderate concentrations of exogenously added 24-EBL exhibited a beneficial influence on the initial phases of seedling growth. The mentioned approach gives new insights into source–sink relationships and can be used as a quantitative measure of the germination energy, which until now has been a qualitative criterion in seed science.

Keywords: brassinosteroids; plant seedling growth; sugars; thermodynamic changes; total redox status



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1. Introduction

Brassinosteroids (BRs) are one of the last identified classes of phytohormones [1], with a coordinating effect on the various developmental processes of plants, thereby decisively affecting the processes of growth and development in normal and stressful environmental conditions [2–8]. The coordination action of BRs is reflected in complex signaling pathways, triggered by the binding of BR molecules to a specific plasmalemma receptor [5,9]. The BR signaling pathways interfere with other signaling pathways in the plant cell: other

phytohormones and light reception systems (e.g. phytochrome system, etc.), as well as other signaling pathways (Ca signals, signals from lipid molecules, etc.) [5,9–12].

Seed germination and seedling establishment are developmental stages of plants that are highly sensitive to variations in environmental conditions, where the impact of BRs is crucial (late frost, early drought, parasite attacks, etc.) [13]. 24-Epibrassinolide (24-EBL) is a phytohormone belonging to the BR group. Studies have shown that 24-EBL has a significant effect on seed germination, seedling growth, and plant development. It acts by interacting with specific receptors in plants, activating signaling pathways that regulate growth and development processes. In agricultural production, the application of 24-EBL can be useful for improving yield and improving plant quality and resistance to stressful conditions. However, it is important to note that the optimal concentrations and application time of 24-EBL may vary depending on the plant species, the stage of plant development, and growing conditions [14,15].

The energy required for developmental activities in young plants is derived from the breakdown of stored starch. The regulation of seed germination and growth is influenced by BRs affecting α -Amylase expression and activity and the degradation of starch in the endosperm in rice (*Oryza sativa*) [16]. This process involves the conversion of starch into intermediate saccharides and eventually into monosaccharides, primarily glucose. These monosaccharides then act as a substrate for respiration and other synthetic processes during the plant's growth and development [17,18]. Glucose and its derivatives play a significant role in signaling processes as indicators of carbon status in plant cells via hexokinase enzymes as "sugar sensors" [19], thereby directing carbon metabolism toward catabolism or anabolism by modulating different sets of genes. Sucrose, as a disaccharide, is the major transport sugar in the phloem of the vast majority of higher plants [20] in addition to having a signaling function similar to glucose, reflecting the carbon status of plant cells [21]. Some other mono-, di- and tri-saccharides (e.g., fructose, arabinose, raffinose) have different functions in plant metabolism, such as acting as monomers or products of degradation of different more complex saccharides with different functions, e.g., in cell walls and in seeds [22,23]. BRs may influence the status of this saccharides, possibly to affect homeostasis of the cell wall of plants by acting on the ester linkages of pectin and polyphenolic compounds [24]. Observing the various effects of 24-EBL on different sugars, the influence of 24-EBL on disaccharide trehalose, which contributes to the protection of plant proteins under stress [25–27], common when forming or germinating seeds, is of great importance for germination and the early stages of seedling development.

During the initial stages of germination, seeds present a metastable and unstable system with a high risk of disruption of structures and functions, mainly due to the production of reactive oxygen and nitrogen species and oxidative stress [17,28,29], so it is important to determine what make it possible to overcome unstable stages (crisis stage) in the development of each plant, called seedling establishment. Recent results suggest that there is a certain tendency to maintain the stability of these metastable structures of living matter through controlled modulation of system entropy [30,31] and other thermodynamic parameters, such as enthalpy and Gibbs free energy, which may be of interest in considering seed germination and the first stages of seedling growth. The Gibbs free energy in seedlings refers to the amount of free energy available or required during growth and development [32]. During the growth of maize seedlings under the influence of 24-EBL, there are changes in the chemical composition of the plant, such as the breakdown of stored starch in the seed and conversion to saccharides, which provides energy for the initial growth of the seedling and which result in changes in the Gibbs free energy [33].

Among various thermodynamic approaches which found applications in plant systems [34,35], the cybernetic approach based on the Aristide Lindenmayer's L-Systems [36,37] has proven fruitful in modeling the various situations that occur during plant growth and development [38,39] and even in the case of the very complex, so far mostly qualitatively described source–sink relationships, which are very important in determining the yield of cultivated plants [40]. The problem of quantitative modeling of plant systems, which

would take into account the interconnections of biochemical, energetics, and information parameters, has not yet been satisfactorily resolved. This approach is very demanding, but it can be applied to plant systems such as germinating seeds, seedlings, or small model plants, such as *Arabidopsis* sp.

One of the areas where BRs have been shown to have an influence is in the regulation of source–sink relations in plants, where source tissues such as leaves are responsible for photosynthesis and the production of carbohydrates, while sink tissues such as roots, flowers, and developing seeds are responsible for using these carbohydrates for growth and development [41–44]. Studies have shown that BRs can influence source–sink relations by regulating the expression of genes involved in carbohydrate metabolism, transport, and partitioning [45,46]. A thorough explanation of the biochemical and biophysical stages that govern these processes still needs to be provided. It is crucial to comprehend the impact of BRs on the mechanisms underlying plant responses in order to anticipate an optimal source–sink relationship.

The intention of this work was to test the effect of BRs on processes in plants by testing physicochemical processes in maize seedlings, which are also a model system for both source–sink relationships and plant seed germination processes. The actual innovative approach provides a more comprehensive understanding of plant seedling growth and the effects of 24-EBL on maize seedlings from a thermodynamics standpoint. Instead of the existing qualitative indicators of plant seed germination (“germination energy”), the same process can be described quantitatively, in real thermodynamic terms, by using parameters, such as Gibbs free energy, enthalpy, entropy, so that the germination of seeds of various genotypes can be compared quantitatively. In addition, it can help determine the optimal dose of 24-EBL that can enhance the growth and development of the seedlings without compromising their energetics or thermodynamic stability.

2. Materials and Methods

2.1. Plant Material, Treatments, and Growth Conditions

Two maize hybrids, ZP434 (drought tolerant) and ZP 704 (older-generation hybrid, which is a standard hybrid, more susceptible to stressful conditions), were used in the experiments. The seeds were produced in the “Maize Research Institute ZEMUN POLJE”, Republic of Serbia. Concentrations of 24-EBL used for treatments and growth conditions were previously described and confirmed in several papers [2,47–51], and based on findings (inhibitory and promotion effect), a range of concentrations from 5.2×10^{-9} to 5.2×10^{-15} M were chosen [52].

2.2. Seed Germination and Determination of Morphometric Parameters of Seedlings

Seed germination and determination of morphometric parameters of seedlings was performed using International Seed Testing Association (ISTA) rules for seed testing [49,51]. The 800 previously measured seeds were divided into 4 equal portions, a control group and groups intended to be treated with different targeted concentrations of 24-EBL (5.2×10^{-9} , 5.2×10^{-12} , and 5.2×10^{-15} M). Seeds were surface sterilized with 0.5% (*v/v*) sodium hypochlorite solution and washed thoroughly with several changes of sterile distilled water. Each group of 200 seeds was germinated in 4 replicates (50 seeds per one box) in 2 L plastic boxes, on filter paper sheets. At the beginning of the experiment, each replicate was topped with 60 mL of different concentrations of 24-EBL solution, and the control was topped with distilled water [50], under the phytotron (Loške tovarne hladilnikov Škofja Loka, d.d., Slovenia) conditions at 24 °C (over day) and 21 °C (overnight), with a 12 h of light ($110\text{--}160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$)/12 h of dark regime [51,52]. After 7 days, 25 uniformly grown seedlings from each box (in total, 100 seedlings per treatment) were divided with a scalpel into plumule, radicle, and RoS. Seedling parts chosen for the further experiments were measured using an analytical balance (Ohaus Pioneer, model PA413) and stored in a deep freezer at -70 °C.

2.3. Electron Paramagnetic Resonance Spectroscopy

Electron paramagnetic resonance spectroscopy (EPR) was used to assess the total redox status associated with the production of the reactive oxygen species (ROS) and reactive nitrogen species (RNS) in seedlings of the maize hybrids. All EPR recordings were performed on a Elexsys II E-540 X/L EPR spectrometer (Bruker, Rheinstetten, Germany) in the X-band. During the EPR measurements, experimental parameters were as follows: Center field 3506.3 G, field width 75 G, microwave frequency 9.85 GHz, microwave power 6.325 mW, modulation amplitude 2 G, and modulation frequency 100 kHz. Quantification of the EPR results consisted of measuring the remaining signal in the system after the reduction of the pyrrolidine membrane-permeable spin probe 3CP by maize seedlings. A 30 μ L 0.075 mM 3CP solution dissolved in deionized water was used as the control (Sigma–Aldrich, Steinheim, Germany). Whole seedlings of measured mass were immersed in 10 mL of 0.075 μ M 3CP solution. After 60 min, 30 μ L of the 3CP solution was sampled and transferred into the EPR spectrometer for signal reading. Sampling was performed by drawing the solution into 5 cm long gas-permeable Teflon tubes (Zeus industries, Raritan, NJ, USA) with a wall thickness of 0.025 mm and an inside diameter of 0.6 mm. The obtained EPR signals were compared with the control by assessing the double integral values of the EPR spectra. All recordings were performed at room temperature and analyzed using Xepr-2.6b.170 software (Bruker GmbH, Billerica, MA, USA).

2.4. Thermodynamic Calculations

Isothermal measurements of thermodynamic parameters were performed at a high temperature in an oven (Carbolite Gero GmbH & Co. KG, Neuhausen, Germany) operating at a heating rate of 30 $^{\circ}$ C min^{-1} . All measurements were carried out in a static oxygen atmosphere (without continuous gas flow). The previously separated portions of the 25 seedlings of both maize hybrids (ZP 434 and ZP 704) treated with appropriate concentrations of exogenously added 24-EBL (5.20×10^{-9} M, 5.20×10^{-12} M, and 5.20×10^{-15} M and control) were individually thermally treated at $T = 60, 105,$ and 130 $^{\circ}$ C. Samples were placed in an oven and subjected to temperature treatment in the time range $t = 8$ min to $t = 30$ min. Subsequently, the samples were placed in a desiccator and then measured. The masses recorded represent the average mass losses of the sample. Each experiment at a given operating temperature was repeated three times. Water activity in the system can be described as the “effective” water content. The temperature dependence of the change in water activity (isothermal displacement) can be described by the Clausius–Clapeyron equation:

$$\ln\left(\frac{a_{w2}}{a_{w1}}\right) = \frac{q + \lambda_w}{R} = \left(\frac{1}{T_2} - \frac{1}{T_1}\right), \quad (1)$$

where q is the heat of sorption, λ_w is the latent heat of evaporation for water (44.0 kJ kg^{-1} at 25 $^{\circ}$ C), and R is the universal gas constant, while a_{w1} and a_{w2} are water activities at different temperatures (T_1 and T_2) at a given equilibrium water content. Changes in external (temperature and/or humidity) and internal factors (“glassy state” stability) affect the energy status of seedlings with respect to the respiratory processes of living cells, which can be estimated (calculated) using the sorption isotherm to which the following equations for different thermodynamic parameters apply [28]:

$$dH^{\circ} = \frac{R \cdot T_1 \cdot T_2}{(T_2 - T_1)} \ln\left(\frac{a_{w1}}{a_{w2}}\right), \quad (2)$$

$$dG^{\circ} = R(T_2 - T_1) \ln\left(\frac{a_{w1}}{a_{w2}}\right), \quad (3)$$

and

$$dS^{\circ} = \frac{(dH^{\circ} - dG^{\circ})}{dT}, \quad (4)$$

where at a given water content, a_{w1} and a_{w2} represent relative humidity at lower and higher temperatures T_1 and T_2 , respectively. dH^o , dG^o , and dS^o represent differential enthalpy, differential Gibbs free energy, and differential entropy dehydration, respectively. The total (cumulative) change in Gibbs free energy can be obtained from equation [28]:

$$\Delta G^o = \Delta H^o - T_i \Delta S^o, \quad (5)$$

where T_i represents the selected temperature. Other definitions of thermodynamic parameters are given in [49,53]. Obtained results are not shown and were used for statistical evaluation.

2.5. Statistical Evaluation

The results represent the mean values of the measurements on the three experimental samples. Multivariate linear regression analysis (Partial Least Square Regression—PLSR method) was used to evaluate the relationship between the content of selected sugars (trehalose, arabinose, glucose, fructose, sucrose, raffinose (results of sugars used for statistical evaluations can be founded in refs. [49,53])) and differential Gibbs free energy (ΔG^o ; kJ mol^{-1}) as a measure of synthetic processes in the maize hybrids. PLSR can analyze strongly collinear data, reducing the high-dimensional data matrix to a much smaller and interpretable set of latent variables (LVs). LVs were calculated for both independent and dependent variable matrices, plus a relationship between them. The quality of the models was monitored with the following parameters: (i) R2cal, the cumulative sum of squares of the Ys explained by all extracted components, and R2CV, the cumulative fraction of the total variation of the Ys that can be predicted by all extracted components, and (ii) root mean square errors of calibration (RMSEC) and root mean square errors of cross-validation (RMSECV). Validation of the models was performed using venetian blinds cross-validation. The data were mean-centered and scaled to unit variance before statistical analyses. PLSR was carried out using PLS Toolbox, v. 6.2.1 (http://www.eigenvector.com/software/pls_toolbox.htm, accessed on 15 February 2023, Eigenvector Research, Inc., Wenatchee, WA 98801, USA) for MATLAB (7.12.0 (R2011a)).

The results of seed germination, morphometric parameters, and total redox status were obtained from the average of four biological replicates. Data significant difference was analyzed using one-way ANOVA with Fisher's Least Significant Difference (LSD) post hoc test. Analyses were performed using Microsoft Excel 2016 (Data Analysis Package).

3. Results and Discussion

3.1. Influence of 24-EBL on Seed Germination

Based on the obtained results (Figure 1), it can be seen that different concentrations of 24-EBL have a different effect on the germination of hybrid seeds ZP 704 and ZP 434. The greatest effect is observed at the highest concentrations, where germination is 93% for ZP 434 and 92% for ZP 704, unlike the control samples, whose germination rate is almost 96%. Observing the differences in the influence of different concentrations on germination, small variations are observed at all tested concentrations except for the highest concentration. In addition, taking into consideration differences between the hybrids, it can be seen that the hybrid ZP 704 shows a higher sensitivity than the hybrid ZP 434. Taking into consideration the results shown in Figures 1 and 2, as well as our previous study, there is an indication of a better germination and a higher Vigor index (as an important quality parameter combining seedling growth rate and dry weight) [53] of the maize hybrid ZP 434 in comparison to the hybrid ZP 704 at a higher gradient of concentration of the 24-EBL solution in which the seeds of these maize genotypes germinated. This finding is in accordance with the declared higher resistance of the plants of the ZP 434 hybrid to stressful environmental conditions confirmed by Waisi [49].

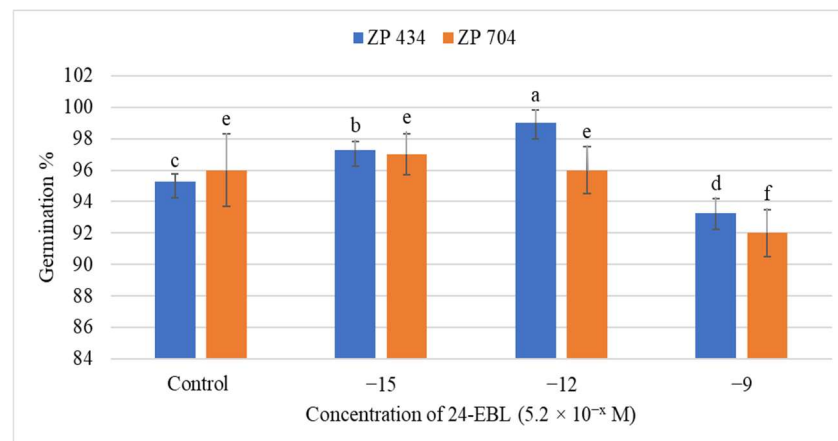


Figure 1. Effect of different concentrations of 24-EBL on germination of 7-day-old seedlings of ZP 434 and ZP 704 maize hybrids. Values indicated by the same letter were not statistically different ($p < 0.05$).

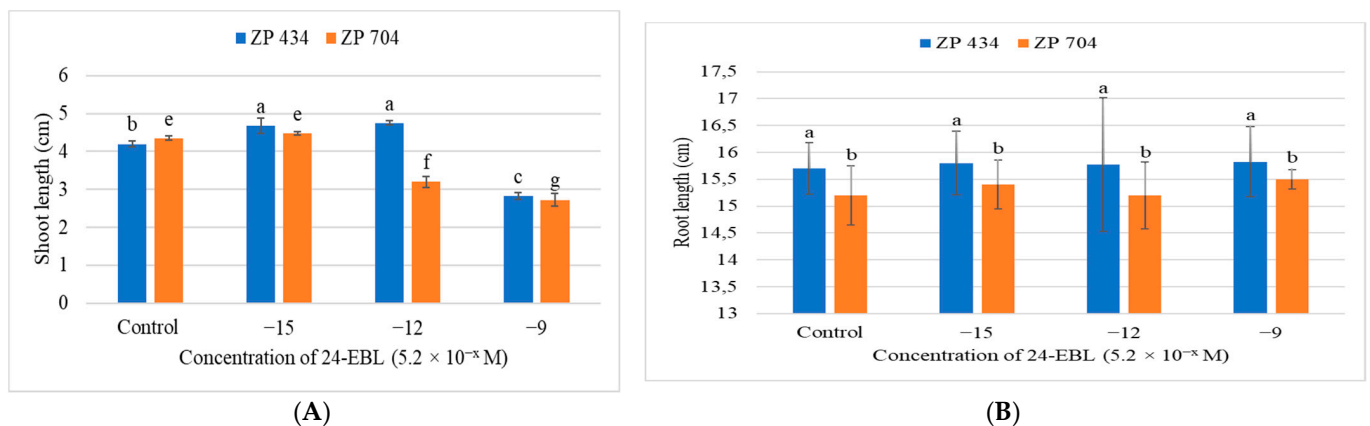


Figure 2. Effect of different concentrations of 24-EBL on morphometric parameters of 7-day-old seedlings of ZP 434 and ZP 704 maize hybrids. (A) Shoot length; (B) root length. Values indicated by the same letter were not statistically different ($p < 0.05$).

3.2. Determination of Morphometric Parameters of Treated Seedlings

The hybrid ZP 434 showed a positive impact on plumule elongation for all 24-EBL concentrations, with the exception of the highest concentration (5.2×10^{-9} M), as compared to the control. Previous results suggest that higher concentrations (5.2×10^{-8} , 5.2×10^{-7} M) have an inhibitory effect, while concentrations of 5.2×10^{-6} M have a total inhibitory effect on the germination of seeds and growth [53]. The results from Figure 2A suggest that the hybrid ZP 704 exhibited plumule length values similar to control samples only at the lowest concentration of 24-EBL (5.2×10^{-15} M), while higher concentrations inhibited plumule length. It is important to note that the germination of seeds and the elongation of seedlings are highly influenced by the ABA and the combined effect of BRs and GAs [54–56]. In germinating cereal grains, GAs trigger the expression of a number of genes encoding enzymes such as α -Amylase, crucial for the breakdown of starch reserves [57,58].

The mechanism of action of BRs on root growth is complex and includes the interaction of hormones with receptors on the cell membrane. This interaction leads to the activation of signaling pathways that influence the growth and differentiation of root cells. One of the main effects of BRs on root growth is the stimulation of cell elongation, leading to root elongation [59]. A series of studies performed on different plant species showed that the application of BRs usually leads to an increase in root length. In a study conducted on barley (*Hordeum vulgare* L.), the application of 24-EBL led to a 30% increase in root

length compared to the control group [60]. Similar effects of BRs have been reported in other crops, such as maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), and soybean (*Glycine max* L.) [59]. However, the results of the present study show that there are slight differences, but they are not statistically significant (Figure 2B). Taking into account the differences in the literary data (some point to an increase and others to a decrease in root length), it can be assumed that the influence of 24-EBL is different for different species, even hybrids [59,61]. It is necessary to carry out analyses on a large number of species and varieties in order to be able to draw conclusions about the precise application of 24-EBL in agriculture.

3.3. Impact of 24-EBL on The total Redox Status

It can be observed from Figure 3 that the EPR signal magnitude of the 24-EBL-treated maize seedlings for the concentration range from 5.2×10^{-12} to 5.2×10^{-9} M is greater for the ZP 704 seedlings than for the ZP 434 seedlings, indicating a greater spin probe reduction potential of ZP 434 in comparison to ZP 704 hybrids and/or greater spin probe reoxidation potential of ZP 704 in comparison to ZP 434 hybrids. These results could derive from either lower antioxidant content in ZP 704 in comparison to in ZP 434 hybrids, higher production of ROS and RNS in ZP 704 in comparison to in ZP 434 hybrids, or both.

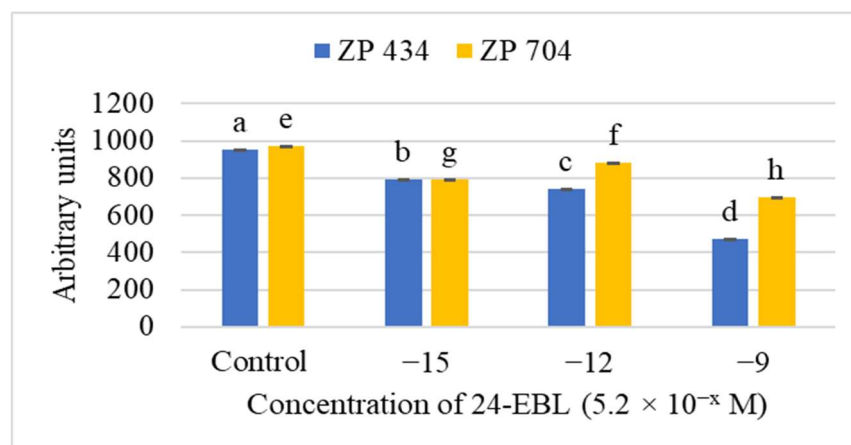


Figure 3. Effect of different concentrations of 24-EBL on total redox status of whole seedlings of ZP 704 and ZP 434 maize hybrids, determined using the EPR spectroscopy method. The results are given in arbitrary units of the EPR signal, double integral values per sample mass. The axis values are displayed in ten thousands. Values indicated by the same letter were not statistically different ($p < 0.05$).

The increased presence of ROS and RNS in both hybrids could be attributed to the regular auxin-mediated relaxation of the cell wall, which is a necessary part of the cell elongation process for seedlings [62]. The level of response to stress is noticeably higher in hybrid ZP 434 compared to hybrid ZP 704, and this can be attributed to increased concentrations of polyphenolic compounds during the germination of hybrid ZP 434, which has more polar phenolics in both the root and shoot compared to ZP 704 [63]. Auxins and BRs are known to act independently but in synergy [2,3], especially since it was found that one of the first genes found to be induced by the action of BRs was xyloglucan endotransglucosylase (XET; [3]), one of the key enzymes in cell wall remodeling during plant cell elongation. The aforementioned enzyme is also known to be controlled by auxin-mediated acidification of the cell wall [23]. Furthermore, the exposure of treated ZP 704 seedlings to 24-EBL could lead to oxidative stress, which is a common occurrence during seed imbibition and rehydration in the first growth stages of maize seedling. Oxidative stress is a common event in the rehydration of dry seed tissue [64]. It is also known that the production of ROS occurs in the cell wall synthesis process [23], which is relatively common in the early stages of growth and development of maize seedlings, a plant of subtropical origin [29], especially in stressful conditions.

3.4. Influence of 24-EBL on Thermodynamic Changes in Maize Seedlings

The model parameters of the regression relationship between the content of selected sugars (trehalose, arabinose, glucose, fructose, sucrose, raffinose) and differential thermodynamic parameters (entropy, enthalpy, and Gibbs free energy) assessed in 7-day-old maize seedlings (hybrids ZP 434 and ZP 704) are presented in Tables 1 and 2.

Table 1. Statistical parameters of the models of regression relationship between the content of selected sugars (Tre, Ara, Glu, Fru, Sah, Raf) and Gibbs free energy assessed in 7-day-old maize seedlings (hybrid ZP 434).

Thermodynamic Parameter	Seedling Parts	Model Parameters	Regression Coefficients in Model *
Entropy	Plumule (P)	Low statistical significance model	
	Radicle (R)	RMSEC: 0.0020 RMSECV: 0.0046 R^2_{Cal} : 0.7561 R^2_{CV} : 0.2554	Tre (+), Sah (+), Ara (+) Glu (−), Fru (−) Raf (0)
	Rest of seed (RoS/S)	Low statistical significance model	
	Whole seedling	Low statistical significance model	
Enthalpy	Plumule (P)	Low statistical significance model	
	Radicle (R)	RMSEC: 0.3711 RMSECV: 0.8215 R^2_{Cal} : 0.7563 R^2_{CV} : 0.2823	Fru (+), Glu (+) Ara (−), Sah (−), Tre (−) Raf (0)
	Rest of seed (RoS/S)	Low statistical significance model	
	Whole seedling	Low statistical significance model	
Gibbs Free Energy	Plumule (P)	RMSEC: 0.0152 RMSECV: 0.0539 R^2_{Cal} : 0.9714 R^2_{CV} : 0.8479	Raf (+), Tre (+), Fru (+), Glu (+) Ara (−), Sah (−)
	Radicle (R)	RMSEC: 0.0357 RMSECV: 0.0454 R^2_{Cal} : 0.8517 R^2_{CV} : 0.7779	Glu (+), Fru (+), Tre (+), Sah (+), Raf (+), Ara (+)
	Rest of seed (RoS/S)	RMSEC: 0.1866 RMSECV: 0.2947 R^2_{Cal} : 0.6471 R^2_{CV} : 0.3319	Sah (+), Raf (+) Ara (−), Glu (−), Fru (−), Tre (−)
	Whole seedling	RMSEC: 0.5123 RMSECV: 0.5931 R^2_{Cal} : 0.7188 R^2_{CV} : 0.6328	Glu (+), Ara (+), Tre (+) Raf (−), Sah (−), Fru (−)

* + (positive influence on the dependent variable, in descending order); − (negative influence on the dependent variable, in descending order).

Table 2. Model parameters of regression relationship between the content of selected sugars (Tre, Ara, Glu, Fru, Sah, Raf) and differential thermodynamic parameters (entropy, enthalpy, and Gibbs free energy) assessed in 7-day-old maize seedlings (hybrid ZP 704).

Thermodynamic Parameter	Seedling Parts	Model Parameters	Regression Coefficients in Model *
Entropy	Plumule (P)	RMSEC: 0.0072 RMSECV: 0.0098 R^2_{Cal} : 0.7339 R^2_{CV} : 0.5425	Raf (+) Glu (−), Sah (−), Ara (−), Tre (−) Fru (0)
	Radicle (R)	RMSEC: 0.0087 RMSECV: 0.0097 R^2_{Cal} : 0.6509 R^2_{CV} : 0.6180	Raf (+), Fru (+), Glu (+), Sah (+) Ara (−), Tre (−)
	Rest of seed (RoS/S)	RMSEC: 0.0077 RMSECV: 0.0102 R^2_{Cal} : 0.8687 R^2_{CV} : 0.8117	Glu (+), Ara (+), Sah (+), Raf (+), Fru (+) Tre (−)
	Whole seedling	Low statistical significance model	
Enthalpy	Plumule (P)	RMSEC: 1.3083 RMSECV: 1.7839 R^2_{Cal} : 0.7340 R^2_{CV} : 0.5433	Tre (+), Ara (+), Sah (+), Glu (+), Raf (−) Fru (0)
	Radicle (R)	RMSEC: 1.5849 RMSECV: 1.7754 R^2_{Cal} : 0.6508 R^2_{CV} : 0.5632	Tre (+), Ara (+) Sah (−), Glu (−), Fru (−), Raf (−)
	Rest of seed (RoS/S)	RMSEC: 1.4122 RMSECV: 2.0551 R^2_{Cal} : 0.8687 R^2_{CV} : 0.7782	Tre (+) Fru (−), Sah (−), Raf (−), Glu (−), Ara (−)
	Whole seedling	Low statistical significance model	
Gibbs Free Energy	Plumule (P)	RMSEC: 0.0709 RMSECV: 0.1692 R^2_{Cal} : 0.3456 R^2_{CV} : 0.4042	Tre (+), Raf (+), Fru (+), Ara (+), Glu (+) Sah (−)
	Radicle (R)	Low statistical significance model	
	Rest of seed (RoS/S)	RMSEC: 0.0904 RMSECV: 0.2579 R^2_{Cal} : 0.8282 R^2_{CV} : 0.4600	Sah (+), Ara (+), Fru (+), Tre (+) Glu (−), Raf (−)
	Whole seedling	RMSEC: 0.5291 RMSECV: 0.6804 R^2_{Cal} : 0.8075 R^2_{CV} : 0.7112	Glu (+), Ara (+), Tre (−), Raf (−), Sah (−), Fru (−)

* + (positive influence on the dependent variable, in descending order); − (negative influence on the dependent variable, in descending order).

The Gibbs free energy can be used to estimate the thermodynamic feasibility of a particular reaction or process in maize. A negative Gibbs free energy value indicates that a reaction is spontaneous and thermodynamically favorable, while a positive Gibbs free energy value suggests that the reaction is non-spontaneous and requires energy input to occur. By measuring the Gibbs free energy changes of various biochemical reactions in maize, a better understanding of the metabolic pathways involved in processes such as

photosynthesis, respiration, and carbohydrate metabolism could be achieved. As can be seen from Table 1 and particularly from Table 2, the regression between the content of sugars against differential entropy vs. enthalpy is inversely proportional, especially with respect to the monitored parameters in plumulas and radicles of the seedlings of both maize genotypes. This relationship is very reminiscent of the enthalpy–entropy compensatory dependence, already observed in related biological material in the works of [49,53], suggesting that the enthalpy–entropy relationship may influence the redistribution of assimilates in the maize seedlings examined and thus the changes in Gibbs free energy and the growth of seedlings.

The presented results are significant (Tables 1 and 2), since there is a possibility of maintaining the stability of these metastable structures of living matter through the controlled modulation of system entropy (by the inverse relationship of the entropy and enthalpy changes) [30]. These results indicate that the changes in Gibbs free energy, in particular seedling organs (plumule (P), radicle (R), rest of seed (RoS/S)), in both corn hybrids and its linear dependence on monitored sugars are not statistically significant, contrary to the significant relationship obtained for whole young plants (Tables 1 and 2). The observed correlations suggest that the regulation of these processes occurs at the plant seedling level. This indicates that different plant growth regulation processes (mainly regulated by different phytohormones) and/or source–sink relationships are important in the redistribution of assimilates and therefore in the regulation of plant growth and development in maize seedlings. This can be explained in more detail by examining the regression relationship between Gibbs free energy and the content of monitored sugars in whole young plants and individual organs (P, R, RoS/S).

The parameters of the PLS model for whole seedlings of ZP 434 maize hybrid (conducted using 30 samples of parts of a young plant: rest of seed + plumule + radicle) were statistically significant, with relatively high values of R^2_{cal} and R^2_{CV} and a low difference between the RMSEC and RMSECV values (Table 1, Figure 4).

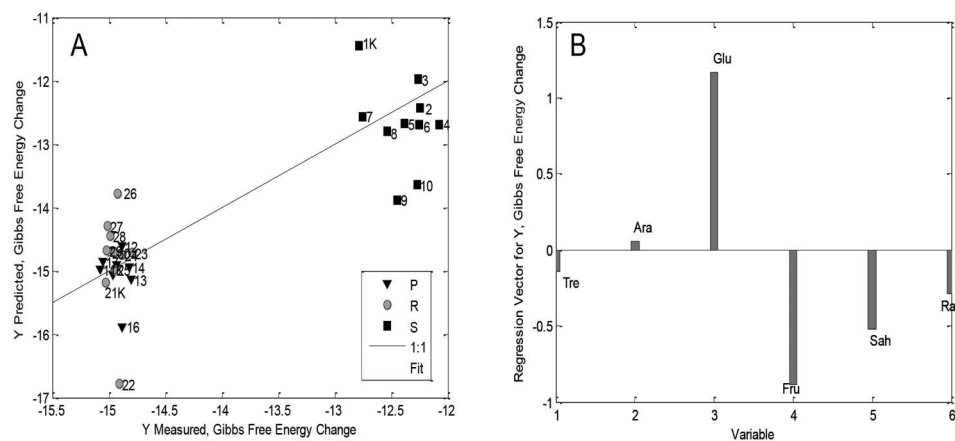


Figure 4. (A) The plot of the measured versus the predicted ΔG^0 values obtained from PLS model for whole 7-day-old corn seedlings of the ZP 434 hybrid. “P” represents plumule, “R” represents radicle, and “S” represents rest of seed. K refers to control samples. Numbers from 1K to 30 represent concentrations of exogenously added 24-EBL to seedlings, from higher to lower concentrations, respectively; (B) plot of the coefficients of descriptors (sugars: trehalose, arabinose, glucose, fructose, sucrose, raffinose) in PLS model of the whole 7-day-old maize seedlings of the ZP434 hybrid.

3.5. The multilinear Regression Model for Maize Seedlings

The plot of the measured versus the predicted ΔG^0 values indicates grouping of samples of P and R on one side of the regression line and samples of the RoS/S on the opposite end (Figure 4A). Additionally, in a group of RoS/S samples, the control samples are distinguished from the treated samples. Figure 4A is proof of source–sink relationships, such as between maize plumules and radicles, as young developing organs

and net importers of assimilates (sink organs) and RoS/S, as a net exporter of assimilates (source organ) [64]. For the ZP 434 hybrid model, it is observed that the medium values of the exogenously added 24-EBL had the greatest influence on the source–sink ratios, which coincides with the results of the shoot length in seedlings (Figure 2A). It can be observed that the values of the ΔG^0 parameter of thermodynamics for the plumule and radicle samples have lower values compared to the RoS/S samples, which indicates that the transfer of energy (and matter) takes place from RoS/S to the plumule and the radicle. This observation is understandable considering the fact that the RoS/S in maize seedlings is the source organ, i.e., the origin of the net production of assimilates (mainly mono- and oligosaccharides) produced by the degradation of the reserve starch, while the plumule and radicle are sink organs, i.e., they consume or are net “importers” of assimilates from the RoS/S [18,64]. Furthermore, the onset of grass seed germination is characterized by the activation of α -Amylase enzyme synthesis (crucial in the process of starch degradation during grass seed germination, which is again controlled by the production of GA in the aleurone layer and scutellum of grass seedlings) [64]. In addition, a positive effect of the examined medium concentrations of 24-EBL on the source-sink relationship for the ZP434 maize hybrid can be seen in Figures 1 and 4A, and based on the results, it can be assumed that the source–sink relations could be driven by BRs.

Glucose has the highest positive impact on ΔG^0 changes in the whole ZP434 hybrid seedling, followed by arabinose and trehalose (Figure 4B). Glucose is a major substrate of mitochondrial respiration, which, in addition to ATP production, produces the organic acids necessary for transamination during nitrogen fixation [65]. Furthermore, glucose is the major monomer of the cell wall polysaccharide [23] but also one of the constituents of sucrose, the major transport sugar in the vast majority of plants [22]. Bear in mind that BR phytohormones affect some respiration patterns [6] but also the dynamics of cell wall construction [24], which is not surprising because the enzyme XET is induced among other factors by the action of BRs, which explains the observed relationship of the Glu and ΔG^0 thermodynamic parameter. A positive effect of arabinose on the change in the ΔG^0 parameter of thermodynamics would be similarly interpreted, since arabinose is one of the important monomers in the arabinogalactan polymers of hemicellulose, an important building block of the cell wall [24].

Furthermore, a positive effect of trehalose on the changes in the ΔG^0 parameter of whole ZP 434 hybrid seedlings is also observed (Figure 4B). Trehalose has recently been found to be of great importance in plant metabolism, although present in a much smaller amount than other sugars [26]. The significance of this sugar so far stems from its exceptional properties (maybe due to its participation in the formation of glassy state, possibly through the water replacement mechanism [27]), which contribute to the protection of plant proteins under stress [46]. Thus, trehalose complements the effect of other sugars and LEA proteins that are considered as crucial in the formation of glassy state, which strongly contributes to the stability of macromolecular structures in dry seeds [27], but this disaccharide also appears to contribute to their stability during the rehydration and germination of seeds [29]. It is probable that the primary processes affecting the changes in the ΔG^0 parameter of thermodynamics in whole seedlings of ZP 434 maize hybrid are related to cell wall remodeling, increased respiration of the young plant, and protective processes related to trehalose. The redistribution of assimilates (associated with the sugars fructose and sucrose) does not appear to have the positive effect of changing the ΔG^0 parameter of thermodynamics in the seedlings. In addition, it is notable that the PLS model of the radicle of seedlings for the ZP 434 maize hybrid is good because of the high values of R^2_{cal} (=0.851748) and R^2_{CV} (=0.777918) and small differences between the parameters RMSEC (=0.0357414) and RMSECV (=0.0454265), which may indicate different metabolism in these two organs of the ZP 434 hybrids but also of different “sink strength” and different affinity of these organs to assimilates [66,67].

As stated, glucose plays a variety of roles in the seedlings of different plants [23,65]. Since there is a positive effect of arabinose on ΔG^0 , it is possible that cell wall remodeling

processes are of great importance during seed germination and seedling establishment in the ZP 704 maize hybrid.

The statistical parameters of the multilinear regression model for the whole seedlings of the ZP 704 maize hybrid are not the best due to the relatively low values of R^2_{cal} (=0.807494) and R^2_{CV} (=0.711153), and the difference between the parameters RMSEC (=0.529099) and RMSECV (=0.5680484) is not insignificant. Although the results for the entire seedlings of ZP704 maize hybrid (Figure 5A) are more scattered relative to equivalent data for whole seedlings of the ZP434 maize hybrid (Figure 4A), it is observed (Figure 5A) that the values of the ΔG^0 parameter of thermodynamics of the ZP 704 hybrid plumule and radicle are lower or the same for the RoS/S of the ZP704 maize hybrid, a trend that is observed for the whole seedlings of the ZP 434 maize hybrids (Figure 4A). This could be associated with the source–sink relationship between plumules and radicles as a sink organ (both young developing organs and net importers of assimilates) and RoS/S as a source organ (net exporter of assimilates) [64].

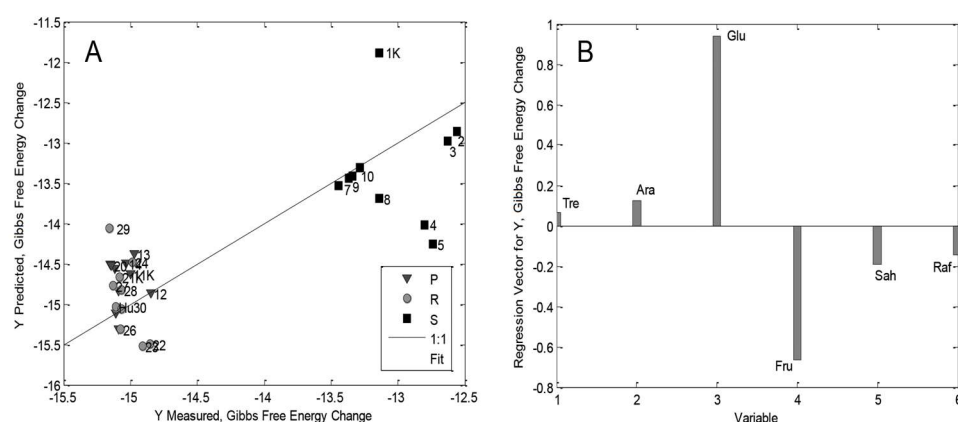


Figure 5. (A) The plot of the measured versus the predicted ΔG^0 values obtained from PLS model for whole 7-day-old corn seedlings of the ZP704 maize hybrid. “P” represents plumule, “R” represents radicle, and “S” represents rest of seed. K refers to control samples. Numbers from 1K to 30 represent concentrations of exogenously added 24-EBL to seedlings, from higher to lower concentrations, respectively; (B) plot of the coefficients of descriptors (sugars: trehalose, arabinose, glucose, fructose, sucrose, raffinose) in PLS model of whole 7-day-old maize seedlings of the ZP 704 hybrid.

It is obvious that the changes in the thermodynamic parameter ΔG^0 (as a measure of the biosynthetic capacity of the system) depend on the source–sink relationships, whereby ΔG^0 flows from the RoS/S to the plumule and radicle (Figures 4A and 5A). For the ZP 704 model, it is observed that the lower values of the exogenously added 24-EBL have the greatest influence on the source–sink ratios, which is in accordance with the results of the shoot length in seedlings (Figure 2A). Although the vast majority of scientific papers about source–sink relationships are related to fully formed plants, it is clear that due to the simple hydrostatic rules on the transport of matter (and energy) through phloem [68], similar rules can apply to seedlings. Moreover, it has been observed that source–sink transitions of young tissues are determined by the presence of sucrose synthase and invertase enzymes on target cells [67], which leads to the degradation of sucrose as the major transport sugar to its hexose components and thus to the formation of a concentration gradient, which directs the phloem flow to the sink organs. Without going into the question of whether a phloem system has been developed in 7-day-old maize seedlings or whether sugars from the RoS/S are transported to the plumule and the radicle in a simple manner, these processes, and thus the overall source–sink relationships, are highly influenced by plant hormones [69], affecting sucrose-degrading enzymes.

The results (Tables 1 and 2) indicate the importance of sugar redistribution in different organs (P, R, RoS/S) of maize seedlings and their effect on ΔG^0 changes within a metabolic network whose activation depends on the genotype, the concentration of exogenously

added 24-EBL, and the type of seedling organ. It was mentioned that this diversity depends on the phenomenon of sink strength [66,67]. In addition, the effects of exogenously added 24-EBL on these processes in maize seedlings depend on the presence of BR plasmalemma receptors, the BRI proteins, which can vary in different tissues (root) [70]. Although these phytohormones are thought to be produced in the plant meristem tissue [71], since it is a young plant with very pronounced meristem tissue additionally exposed to the exogenous treatment of 24-EBL, the described experiment corresponds to the concentration of BR-type phytohormones in a wide variety of plant tissues and species [2].

The canalization transport of auxins through the plant, which greatly influences the various processes of plant growth and development [72] and related phenomena of apical dominance and phyllotaxis [73], is the reasons why appropriate cybernetic solutions have been proposed [74]. However, other phytohormones have been reported to be transported through the plants and partially modulate the aforementioned effects of auxin [73]. BRs are one of them [74], which agrees with the intention of Hartwig and Wang [75] to present a “molecular circuit of steroid signaling in plants”. Our contribution to this problem consisted of taking into consideration both the energetics and thermodynamics of the plant, with a presented solution to the problem of source–sink relationships in plant seedlings. The presented study convincingly demonstrates that it is also possible to describe the process of plant seed germination quantitatively by using thermodynamic parameters.

4. Conclusions

The results show that the different concentrations of 24-EBL exhibit varying effects on seed germination. It was found that the highest concentrations of 24-EBL had the greatest impact on seed germination (inhibitory), while lower and medium concentrations have a positive effect on the early stages of seedling development. The enthalpy and entropy correlation affects the redistribution of assimilates in the examined maize seedlings. In addition, the changes in Gibbs free energy influence the growth of seedlings. The changes in the thermodynamic parameter ΔG^0 (as a measure of the biosynthetic capacity of the system) depend on the source–sink relationships, whereby ΔG^0 flows from the RoS/S to the plumule and radicle. This study confirms that the flow of important sugars from the RoS/S to the plumule and radicle, which are the sink organs, as well as the changes in Gibbs free energy between these parts are responsible for controlling and regulating the germination and early growth of the maize seedlings. This study suggests that this approach could be used to measure the “germination energy” quantitatively (as ΔG^0 presents a measure of the chemical potential of living systems), which was previously evaluated qualitatively (as a percent of germinated seeds) in seed science.

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