

RESEARCH ARTICLE

Effect of gibberellin and tebuconazole on the use of seed reserve oil by *Zea mays* L. seedlings under photomorphogenesis and scotomorphogenesis conditions

Kuryata V.G., Kuts B.O.* , Poprotska I.V., Golunova L.A., Baiurko N.V., Nikitchenko L.O., Frytsiuk V.A.

Vinnytsia Mykhailo Kotsiubynskyi State Pedagogical University, Ostrozhskogo Str. 32, Vinnytsia, 21000, Ukraine;
* danotchka@gmail.com

Received: 06.10.2021 | Accepted: 22.10.2021 | Published: 01.11.2021

Abstract

Elucidation of the particular use of seed reserve substances under different germination conditions, the role of phytohormones in the regulation of processes, allows developing new effective means of regulating the vigour and intensity of seed germination. The peculiarities of the utilization of reserve oil of corn seeds by seedlings emerging in the light and the dark have been established under Gibberellic Acid (GA₃) action and gibberellin antagonist-retardant tebuconazole. The process of germination under the influence of gibberellic acid was accompanied by the increase in reserve substances utilization for organogenesis, intensive use of reserve oil, rising under scotomorphogenesis condition; light inhibited phytohormone activity. Under the action of GA₃, the processes of saturation of Fatty Acids (FA) were significantly enhanced, the unsaturated/saturated fatty acid ratio was reduced, and the linolenic acid outflow from the seed for the needs of morphogenesis was enhanced. The inhibitor of gibberellin synthesis had the opposite effect compared to GA₃. The obtained research results can be used in the development of new technologies to increase the germination energy of seeds.

Keywords: *Zea mays* L., scotomorphogenesis, photomorphogenesis, gibberellins, retardants, lipids

Introduction

Being one of the most important external factors that significantly affect morphogenesis, the light changes the rapidity and duration of growth for both the total plant and its parts (root, epicotyl/hypocotyl, leaf). Plants, germinating in darkness, develop according to the scotomorphogenesis program: the epicotyl/hypocotyl is elongated, a hypocotyl loop is formed, the cotyledons turn yellow and the first leaves are formed corrugated. The photomorphogenesis program is activated in the light: hypocotyls/epicotyls are shortened, hypocotyl loops are straightened completely, cotyledons and primary leaves turn green, the leaf blades are straightened and expanded (Golovatskaya & Karnachuk 2007). In monocotyledonous seedlings, the length of both axial organs and leaves are stretched during etiolation. At the same time, in dicotyledonous plants, only stem internodes are stretched,

and primary leaves and cotyledons change a bit (Polevoy 2001). The ability to switch from scotomorphogenetic to photomorphogenetic development is essential for seedlings' survival. Its central mechanism is a system of photoreceptors that regulate the activation of photomorphogenesis (Hornitschek, et al. 2012; Wu 2014; Franklin 2016). Nowadays it is known that light modifies growth and morphogenesis through restructuring of plant hormonal complex, being a mechanism of influence on the source-sink system (Poprotska 2017). The initial stages of photomorphogenesis are accompanied by active metabolic shifts, phytochrome modification of the hormonal status of seedlings, transport and formation of phytohormone gradients, in particular, gibberellins, as well as changes in the intensity of biosynthesis of natural inhibitors (Kutschera & Briggs 2003; de Wit & Pierik 2016).

On the soil surface, light acts as the main exogenous agent that inhibits the activity of the main protein-suppressor COP1, synthesized in nuclear space. Light determines the activity of other transcriptional regulators providing realization of gibberellin (DELLA) and brassinosteroids (BZR1/BES1) signaling as well as activating trans-factors such as HY5, which initiate the transition to autotrophic nutrition (Folta, et al. 2003).

Due to the involvement of phytohormone in the light-signal system of transduction, several light-regulated development factors equally respond to treatment with hormones (Josse & Halliday 2008). Phytohormone modifiers also have a significant morphophysiological effect on the plant (Khodanitska, et al. 2019; Kuryata, et al. 2019). It should be noted that only a few works study scotomorphogenesis and photomorphogenesis under the action of retardants (Poprotska & Kuryata 2017; Poprotska, et al. 2019). Blocking the synthesis of gibberellins under the influence of retardants leads to significant changes in morphogenesis and rearrangement of source-sink relations in the plant (Kuryata & Golunova 2018; Kuryata, et al. 2019). Consequently, the work aim was to study the use and redistribution of reserves during the transition from scotomorphogenesis to photomorphogenesis under changing conditions of source-sink relations under the action of exogenous growth regulators.

Materials and Methods

The work was carried out on *Zea mays* L. seedlings of the Aromatna variety. During the heterotrophic phase (seed germination), pre-treatment of seeds with growth regulators was used to create different tensions of the source-sink system. The seeds were soaked for 24 h in a 0.025% aqueous solution of Gibberellic Acid (GA₃) and tebuconazole at a concentration of 0.05%. The seeds of the control variant were soaked in distilled water. After soaking, the seeds were sown in ditches with wet sand. Biological repeatability was fivefold. The experiment was performed under the action of light (about 500 lx) and in dark to study the implementation of scotomorphogenesis and photomorphogenesis programs. The utilization rates of reserve substances were determined on the day of 18 of germination as the ratio of dry matter mass of the organ to dry matter mass of the whole seedling, expressed as a percentage. Total oil content in dry seeds and cotyledons was determined by extraction in the Soxhlet apparatus. Petroleum ether with a boiling point of 40°C–65°C was used as an organic solvent for oil separation. The content of higher Fatty Acids (FA) in oil was determined by gas chromatography on chromatograph Chrom-5 (Czech Republic) (AOAC 2010). The gas flow rate was 50 ml/min, the gas carrier was nitrogen. Chromatography was

performed under isothermal conditions (200°C), and evaporation temperature was about 230°C. The flame-ionization detector temperature was 240°C. Chromosorb W AW100-120 mesh with a mixture of stationary phases SP-2300 2% and SP-2310 3% was used as a solid carrier for column packing (length 3.5 m, internal diameter 3 mm). Statistical data processing was performed using the software program Statistica 6.0. The reliability of the difference between control and experiment was determined by Student's t-test. The tables and figures show arithmetic mean values and their standard errors.

Results

In our previous work, it was established that gibberellin stimulated more intensive use of the main reserve substance of corn seeds—starch both in light and in the dark and the use of reserve nitrogen-containing compounds was better in the dark and started after intensive hydrolysis of starch. It was found that the use of tebuconazole led to the opposite effect—the intensity of the use of reserve carbohydrates and nitrogen-containing compounds in both scotomorphic and photomorphic seedlings decreased compared to control (Kuryata, et al. 2021). However, there is no data on the peculiarities of the use of reserve lipids by seedlings under scotomorphogenesis and photomorphogenesis conditions, the influence of gibberellins and retardants on these processes is not clarified.

Analysis of germination of corn seeds showed that under the influence of GA₃ germination was stimulated, and under tebuconazole action, this process was inhibited in comparison to control. There was also no significant difference in the presence or absence of light in experimental variants. Thus, under GA₃ action, germination energy in light was 93.75% ± 4.68% (in dark 97.30% ± 4.80%); under the action of tebuconazole 76.75% ± 3.83% (77.45 ± 3.87); in control 82.60 ± 4.13 (83.50% ± 4.17%). As shown in Fig. 1, coefficients of use of seed reserve compounds for the formation of above-ground and underground parts were higher under GA₃ action and lower under the action of tebuconazole. The utilization in scotomorphic seedlings was higher in all experimental variants. It was found GA₃ stimulates the use of reserve starch and nitrogen-containing compounds (Kuryata, et al. 2021) along with the use of lipids with the light signal participation in heterotrophic period: under darkness and light action barely differ from control. Thus, light blocks gibberellin activity during germination. Using tebuconazole, the lipid residue was maximal in seeds, which is consistent well with the lowest germination and growth rates in comparison to control and GA₃ variants.

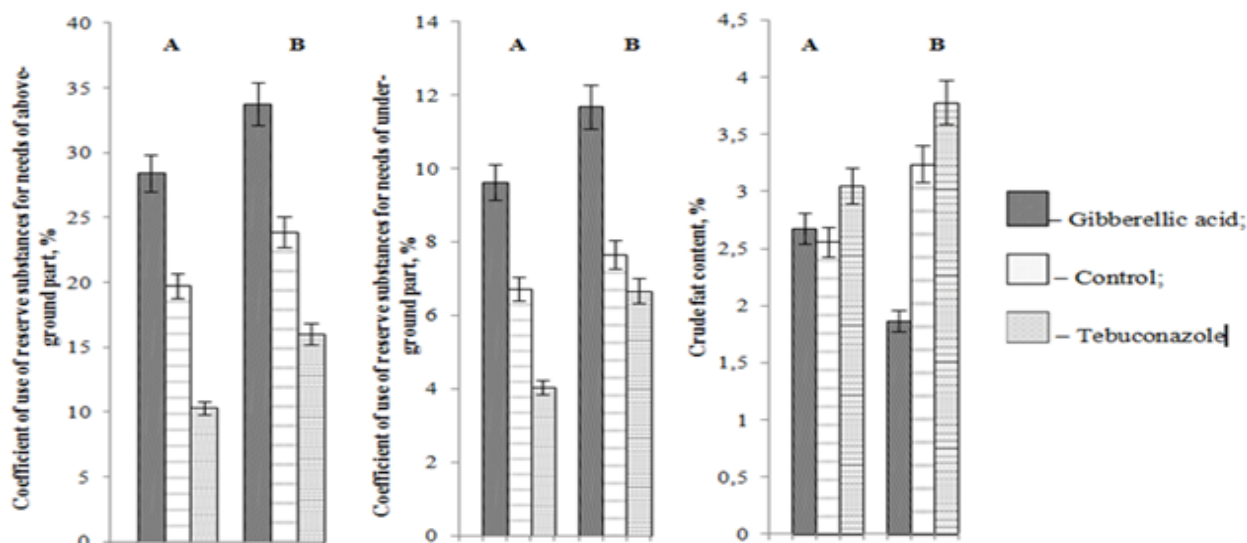


Figure 1. The influence of gibberellic acid and tebuconazole on the coefficients of the use of reserve substances and fat (oil) content in cotyledons of scoto- and photomorphous plants (the day of 18).

Chromatographic analysis of corn oil revealed the presence of ten higher FA—myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidonic, behenic, and gondoic (which content was distinct) (Tab. 1). There is a clear pattern of changes in saturated and unsaturated acids content during germination in comparison to dry seed oil, as well as the impact of the applied growth regulators on this process. In some studies, it was noted that germination is accompanied by a change in the unsaturated/saturated FA ratio in favor of the latter due to saturation processes (Poprotska 2017). Our research confirms this pattern.

There was a total increase in the content of saturated and a decrease in the content of unsaturated FA in seeds on the day of 18 in comparison to dry seed oil, decreasing the unsaturated/saturated FA ratio. GA₃ accelerated seed germination and the formation of seedling organs. It was characterized by the lowest ratio. It was found that light did not affect the saturation intensity, unsaturated and

saturated FA content was almost equal under the action of gibberellin. The smallest changes of this indicator were due to the action of tebuconazole.

In our opinion, there is interesting data on Linolenic Acid (LA) content in cotyledon oil as compared to control—in all variants it was higher than in ungerminated seeds. Similar data was found during pumpkin seed germination (Poprotska 2017). It was the lowest in the GA₃ variant in comparison to control. It was minimal under light action. Likewise, it has been already established that the formation of green leaves in light is accompanied by the accumulation of glycolipids in chloroplastic membranes, which include linolenic acid (Tremolieres & Mazliak 1967). Reduced LA content in the GA₃ variant may be explained by a more intensive outflow of this FA for the needs of plastid apparatus formation due to the more intensive growth as well as chloroplastogenesis occurs more rapidly in photomorphous plants. The increased content of LA under the tebuconazole action indicates

Table 1. FA content in corn oil during germination under gibberellin and tebuconazole action, %.

Indicator	Dry seed	Gibberellin		Control		Tebuconazole	
		A	B	A	B	A	B
Myristic	0.03 ± 0.01	0.1 ± 0.01 [*]	0.07 ± 0.01 [*]	0.02 ± 0.01	0.03 ± 0.001	0.06 ± 0.01 [*]	0.04 ± 0.01 [*]
Palmitic	7.98 ± 0.39	9.1 ± 0.46 [*]	8.38 ± 0.42	7.71 ± 0.39	8.01 ± 0.40	5.53 ± 0.28 [*]	6.85 ± 0.34 [*]
Palmitoleic	0.10 ± 0.01	0.08 ± 0.01	0.06 ± 0.01 [*]	0.08 ± 0.01	0.08 ± 0.01	0.11 ± 0.02 [*]	0.14 ± 0.01 [*]
Stearic	2.52 ± 0.12	4.72 ± 0.24	5.12 ± 0.26	4.79 ± 0.24	4.9 ± 0.25	4.21 ± 0.21 [*]	4.49 ± 0.22 [*]
Oleic	30.97 ± 1.55	29.98 ± 1.50 [*]	29.65 ± 1.48 [*]	25.76 ± 1.29	25.89 ± 1.30	30.44 ± 1.52 [*]	27.94 ± 1.40 [*]
Linoleic	57.48 ± 2.87	53.67 ± 2.68 [*]	53.84 ± 2.70	57.68 ± 2.88	56.95 ± 2.85	55.32 ± 2.77	54.32 ± 2.72
α-Linolenic	0.38 ± 0.02	1.64 ± 0.08 [*]	2.12 ± 0.11 [*]	2.95 ± 0.15	3.33 ± 0.17	3.35 ± 0.17 [*]	5.26 ± 0.26 [*]
Arachidic	0.24 ± 0.01	0.31 ± 0.02 [*]	0.35 ± 0.02 [*]	0.55 ± 0.03	0.41 ± 0.02	0.52 ± 0.03	0.51 ± 0.03 [*]
Gondoic	0.07 ± 0.01	0.09 ± 0.01 [*]	0.07 ± 0.01 [*]	0.2 ± 0.01	0.15 ± 0.01	0.12 ± 0.01 [*]	0.17 ± 0.01 [*]
Behenic	0.22 ± 0.01	0.31 ± 0.01 [*]	0.34 ± 0.02 [*]	0.26 ± 0.01	0.25 ± 0.01	0.34 ± 0.02 [*]	0.28 ± 0.01 [*]
Saturated FA	11.00 ± 0.54	14.54 ± 0.73 [*]	14.26 ± 0.71	13.33 ± 0.67	13.6 ± 0.68	10.66 ± 0.53 [*]	12.17 ± 0.61 [*]
Unsaturated FA	89.00 ± 4.46	85.46 ± 4.27	85.74 ± 4.20	86.67 ± 4.33	86.4 ± 4.32	89.34 ± 4.47	87.83 ± 4.39
Unsaturated/saturated FA ratio	8.09	5.88	6.01	6.50	6.35	8.38	7.22

Note: ^{*} significant difference at p<0.05; A: photomorphogenesis; B: scotomorphogenesis

the least intensive outflow of this FA for the needs of chloroplastogenesis.

Conclusion

Thus, corn seed germination is accompanied by the use of reserve oil and is enhanced under scotomorphogenesis. GA₃ enhances the use of lipids for morphogenesis. Moreover, light inhibits the activity of phytohormone. Under the action of GA₃, the saturation of FA is significantly enhanced. The unsaturated/saturated FA ratio decreased and the outflow of linolenic acid from the seed for the needs of morphogenesis increased. Tebuconazole has the opposite effect compared to GA₃. The results can be used in the development of new accelerating technologies seed germination.

References

- AOAC (2010).** Official methods of analysis of association of analytical chemist international 18th ed. Rev. 3. 2010. *Ass of Analytical Chemist Gaithersburg, Maryland, USA*. <https://www.worldcat.org/title/official-methods-of-analysis-of-aoac-international/oclc/62751475>
- de Wit M., Pierik R. (2016).** Photomorphogenesis and photoreceptors. *In Canopy Photosynthesis: From Basics to Applications* 171-186. https://doi.org/10.1007/978-94-017-7291-4_6
- Folta K.M., Pontin M.A., Karlin-Neumann G., Bottini R., Spalding E.P. (2003).** Genomic and physiological studies of early cryptochrome 1 action demonstrate roles for auxin and gibberellin in the control of hypocotyl growth by blue light. *The Plant J* **36**: 203-214. <https://doi.org/10.1046/j.1365-313X.2003.01870.x>
- Franklin K.A. (2016).** Photomorphogenesis: plants feel blue in the shade. *Curr Biol* **26**: R1275-R1276. <https://doi.org/10.1016/j.cub.2016.10.039>
- Golovatskaya I.F., Karnachuk R.A. (2007).** Dynamics of growth and the content of endogenous phytohormones during kidney bean scoto- and photomorphogenesis. *Fiziologija Rastenij* **54**: 461-468.
- Hornitschek P., Kohnen M.V., Lorrain S., Rougemont J., Ljung K., López-Vidriero I., Fankhauser C. (2012).** Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *The Plant J* **71**: 699-711. <https://doi.org/10.1111/j.1365-313X.2012.05033.x>
- Josse E.M., Halliday K.J. (2008).** Skotomorphogenesis: the dark side of light signalling. *Curr Biol* **18**: R1144-R1146. <https://doi.org/10.1016/j.cub.2008.10.034>
- Khodanitska O.O., Kuryata V.G., Shevchuk O.A., Tkachuk O.O., Poprotska I.V. (2019).** Effect of treptolem on morphogenesis and productivity of linseed plants. *Ukr J Ecol* **9**: 119-126.
- Kuryata V.G., Golunova L.A. (2018).** Peculiarities of the formation and functioning of soybean-rhizobial complexes and the productivity of soybean culture under the influence of retardant of paclobutrazol. *Ukr J Ecol* **8**: 98-105.
- Kuryata V., Kuts B., Prysedsky Y. (2021).** Utilization of spare substances of *Zea mays* L. seeds during germination under the combined effect of growth regulators and presence or absence of light. *Biologija* **67**: 23-34. <https://doi.org/10.6001/biologija.v67i1.4401>
- Kuryata V.G., Polyvanyi S.V., Shevchuk O.A., Tkachuk O.O. (2019).** Morphogenesis and the effectiveness of the production process of oil poppy under the complex action of retardant chlormequat chloride and growth stimulant treptolem. *Ukr J Ecol* **9**: 127-134.
- Kutschera U., Briggs W.R. (2013).** Seedling development in buckwheat and the discovery of the photomorphogenic shade-avoidance response. *Plant Biol* **15**: 931-940. <https://doi.org/10.1111/plb.12077>
- Polevoy V.V. (2001).** Fiziologiya tselostnosti rastitelnogo organizma. *Fiziol rast* **48**: 631-643. [https://doi.org/10.1016/S0944-7113\(96\)80059-6](https://doi.org/10.1016/S0944-7113(96)80059-6)
- Poprotska I., Kuryata V., Khodanitska O., Polyvanyi S., Golunova L., Prysedsky Y. (2019).** Effect of gibberellin and retardants on the germination of seeds with different types of reserve substances under the conditions of skoto- and photomorphogenesis. *Biologija* **65**: 296-307. <https://doi.org/10.6001/biologija.v65i4.4123>
- Tremolieres A., Mazliak P. (1967).** Biosynthese de d'acide α -linolenique au cours du verdissement des cotyledons etioles de trefle. *Compt Rend Acad Sci* **265**: 1936-1945.
- Wu S.H. (2014).** The expression regulation in photomorphogenesis from the perspective of the central dogma. *Annual RevPlant Biol* **6**: 311333. <https://doi.org/10.1016/j.crbiot.2021.02.004>