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Biochemical Characteristics of Allelopathic Activity of Germinating Seeds*

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Abstract. Biochemical characteristics (enzyme activities, contents of vitamins, phytohormones, nucleic acids, proteins, saccharides, *etc.*) in germinating seeds and developing seedlings are summarized, and the inhibitory and stimulatory actions of these compounds on plant growth and development are discussed.

At present much attention is paid by plant breeders to the development of biotechnology as a basis for creating biologically active substances to be used in agriculture and medicine. The solving of this problem includes both artificial synthetic substances of high biological activity and physiologically active composite substances of natural origin. As is known, the discovery of the physiologically active natural compounds has resulted in putting them to use in agriculture, medicine and biology. However, the extent of their usage is limited.

As far as the agricultural practical purposes are concerned, one of the most creative approaches to expand the usage of biologically active substances is that of the allelopathically active composite substances (the allelochemicals) of plant origin. Much attention has been paid to the physiological and biochemical properties of germinating seeds. At the same time the techniques of their practical usage in agricultural production became an object of scientific research.

Such research of the biological significance and functions of the extracts obtained from germinating seeds helped in discovering both their complicated biological structure and their multifactor effect. Besides, it allowed us to work out the theoretical foundation for the use of seed allelopathic properties (NAUMOV *et al.* 1984).

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* This paper is based on and employs a broader definition of allelopathy than most other contributions to this collection of colloquium reports, in that it includes all interactions of plants, not merely inhibitory ones

It should be mentioned that there is an essential difference between dry and germinating seeds as far as their composition and biological structure are concerned (NOVOTELNOV and ESZHOV 1957).

As was pointed out by GRODZINSKY (1973) the process of extraction of growth retarders in hard seeds is passive – the substances are washed out, leached out or pass into the soil solution. With the beginning of swelling and germination of seeds the character of their extracts is noticeably changed, the quality of the extracted compositions being raised. The latter may be defined as a heleolike mucus containing different organic compound where epiphytic microorganisms actively breed.

Physiologically active substances that are given off into the outer medium as a result of swelling and following germination of seeds are characterized by diverse biochemical structure and a wide range of the biological functions they fulfil. It is natural that the biochemical structure and the functions of the exudates depend upon the species origin and the agrotechnical features of plant growing and seed formation process as well as upon the conditions of seed storage, the age of seeds and their sowing quality.

As far as the biological activity of exometabolites is concerned different components are of much importance. They are the enzymes: proteolytic, hydrolytic,

TABLE 1

Levels of the allelochemicals of growing wheat seeds

	Allelochemicals	Unit	Index
Vitamins of B group	Thiamine (B_1)	$\mu g\ cm^{-3}$ (extract)*	0.01
	Pantothenic acid (B_3)	$\mu g\ cm^{-3}$ (extract)*	0.21
	Pyridoxine (B_6)	$\mu g\ cm^{-3}$ (extract)*	0.096
	Nicotinic acid	$\mu g\ cm^{-3}$ (extract)*	0.05
	Inositol	$\mu g\ cm^{-3}$ (extract)*	1.19
Enzymes	Dehydrogenase	arbitrary units	7.2
	Catalase	mm^3 (O_2 evolved) s^{-1}	113.0
	Peroxidase	arbitrary units	3.7
	Amylase	μmol	3.08
Phytohormones	Gibberellins	$mg\ cm^{-3}$ (extract)*	10^{-3}
	Cytokinins	$mg\ cm^{-3}$ (extract)*	10^{-3}
Nucleic acids	DNA	$\mu g\ cm^{-3}$ (extract)*	4.74
	RNA	$\mu g\ cm^{-3}$ (extract)*	44.59
Protein	Raw protein	% of dry matter	14.8
Saccharides	Monosaccharides	$mg\ cm^{-3}$ (extract)*	989
	Disaccharides	$mg\ cm^{-3}$ (extract)*	159

* Extract preparation: NAUMOV and NASONOVA (1987).

respiratory, phosphatases, *etc*; the phytohormones: auxins, gibberellins, cytokinins, abscisic acid; the vitamins of B group: thiamine, pantothenic acid, pyridoxine, nicotinic acid, inositol, biotin; nucleic acids – DNA, RNA; organic acids – oxalic, malic, succinic; different amino acids, and monosaccharides and disaccharides (Table 1).

The complex structure of the allelochemicals of germinating seed extracts defines their physiological activity and diversity of their functions.

The metabolites of germinating seeds may play important endogenous and exogenous parts in both development and productivity of future plants. It is the metabolites that enable establishing phenocenotic links between the plants and the soil microflora providing vitality of seeds and their genetic stability.

The variety of the biological components of growing seed secretions influences their allelopathic activity and different biological functions that are to be taken into account in agricultural activities, *i.e.* in developing new techniques of cultivation of agricultural crops and creation of new cultivars and hybrids of agricultural plants. The allelochemicals give rise to a concrete biochemical and microbiochemical medium around the germinating seeds, which provide the establishment of phytocenotic mutual relations, and at the same time are stimulators and regulators of growth. They also play an important part in raising vitality of growing seeds, seedlings and plants, help them to struggle with diseases, unfavourable environmental conditions, chemical and physical effects, fulfilling the control and restoration functions in seeds, *etc.* Depending on the specific structure of the participants in the cenose and due to the concentration of physiologically active substances they may be inhibitors of germination. The physiologically active substances have always played an important role in creation, conservation and variability of species.

It is worthy of saying that the above-mentioned functions have some peculiarities. The medium-forming function is of high importance. The formation of an adequate biochemical medium around the swelled seed is an important condition for seed germination and seedling formation. The distortion of this function may result in slowing germination. The increase of the secretory activity of seeds and raising their allelopathic functions provide vigorous and normal germination of all the seeds.

Due to the presence of the physiologically active substances in the extracts of the growing seeds, allelopathic mutual relations are established between them. The seeds exchange different substances raising their own allelopathic activities and density of the biological medium. Secretions of the normal seeds provide vigorous germination of all the seeds of the given species and facilitate the establishment of phytocenosis. The allelopatic role of the secretions depends upon the species, origin, and variety of the seed structure and may be stimulating or inhibiting. Since the secretions contain phytohormones, enzymes, vitamins and some other compounds, they are stimulators relative to seed acceptors. This is a very important feature from the agricultural point of view because it allows us to work out the techniques of obtaining the biologically active stimulators from the germinated seed-donors

possessing a wide range of biological functions. The functions in question must be taken into account in practical work of plant breeders; otherwise it is possible to develop new crop cultivars the seed secretions of which will stimulate the development of weeds (and we are sorry to state that this sometimes occurs in practice). Besides, the allelopathic factor should be taken into account in developing the norms and methods of sowing.

The availability of substances of inhibitory effect (those of phenolic nature, abscisic acid, *etc.*) and the biologically active substances of certain concentration in seed secretions provide the seeds with their own inhibition properties.

For example, seeds having lower germinative characteristics such as old seeds possess lower allelopathic activity. The treatment of normal seeds with extracts of such seeds influences negatively both seed germination and microbiological processes around the seeds (NAUMOV and VORONKOV 1983). The seeds having poorer sowing characteristics if planted under field conditions have poorer index of germination and lower plant productivity. To sum it up, sowing of seeds treated improperly is a result of disregarding the allelopathic properties of seeds. The above-mentioned inhibition functions are to be considered in practical work of plant breeders to create new crop cultivars possessing seed secretions which could suppress weed development, as was outlined by GRODZINSKY (1969, 1982).

The protective role of the secretions of germinating seeds is also of much importance. As early as 1905 Mechnikov (MECHNIKOV 1947) paid much attention to the problem saying "Plants protect themselves with their own membranes and secretions". Phytocides are of great significance in this process. The complex protective function mechanism of the biologically active substances of seeds is composed of fungicidal and bactericidal effects on the pathogen and saprophyte microflora of both seeds and plants. It also envisages therapeutic activity and plant immunity as well as the influence on forming an ecologically favourable environment (NAUMOV and SEVRYUKOVA 1984). The increase in exchanging reaction index within the seeds and plants causes physiological immunity, at the same time raising the fungicidal effect of groups of substances which are responsible for biological protection of seeds and are suppressing pathogens. Such substances also inactivate toxins and exoferments given off by fungi into the neighbouring medium. Such ability of growing seeds to suppress pathogenic microflora and on the seeds may be defined as an allelopathic effect on factors causing diseases.

The defining of various biochemical structures of growing seed extracts and their biological functions allowed the working out of techniques to get the allelochemicals from seed donors of grain crops and to use them for presowing enrichment of seed acceptors of field crops. The extracts are obtained by germination of seed donors during 72 h and subsequent extraction of physiologically active substances from them (in the form of water extract). According to the methods developed, 1 kg of initial seeds give 2 litres of the extract (NAUMOV and NASONOVA 1987).

The extract obtained by this method is a multicomponent and balanced system of

physiologically active substances of metabolic origin with a high level of allelopathic activity. Research on some physiological and biochemical characteristics of the extract showed that it represented an acid medium with pH ranging from 4.5 to 6.0; the contents of dry matter were 0.6 %, and those of nitrogen 2.6 %, phosphorus 1.4 % and potassium 2.1 % per dry matter. The highly active substances of germinating seeds mentioned above are present in full in the extracts and provide its allelopathic activity. Many of them (enzymes, vitamins, phytohormones, *etc.*) take an active part in the exchange of germinating seeds or are used as energy material, the quality of the extract being greatly dependent upon them (Table 2).

TABLE 2
Dependence of enzyme activity in seeds of winter wheat Mironovskaya 808 on seed treatment

Presowing treatment	Catalase [mm^3 (O_2 evolved)s^{-1}]	Peroxidase [arbitrary units]	Amylase [μg (maltose) g^{-1} (dry m.)]
Dry seeds (control)	8.0	35.0	270.5
Humidified seeds	14.1	37.0	291.2
Seeds enriched with extract	14.4	50.0	313.2

The presowing treatment of field crop seeds with the physiologically active extracts not only enables enriching seed acceptors with the complex of the allelochemicals and other compounds but also strengthens the biological functions of their exometabolites during the germination period, raising the allelopathic activities of the seeds.

It is known that individual presowing treatments of seeds have an essential influence on the exchange processes in vegetating plants. This in fact is manifested in directing physico-biochemical processes and therefore in changing biological activity of the ferments which take part in the exchange reactions. The seed treatment activates such processes in seeds. It is hardly possible to give an account of all of them within the scope of the present paper, so examples will be given here.

The study of several enzymes showed that the peroxidase activity is most subject to change (Tables 2,3).

The phosphatase activity is closely connected with the exchange of carbohydrates, and with that of nucleotides and phospholipids. The change was also observed in protein exchange processes. Substances of hormonal nature, such as gibberellins, auxins, and cytokinins have a high influence on the processes taking place in the

TABLE 3
Influence of seed treatment on dehydrogenase activity [arbitrary units] in seeds of winter wheat Mironovskaya 808 during seed germination

Presowing treatment	Seeds after treatment	Phases of germination			
		Swelling up (1 d)	Primary rootlets (3 d)	Seedling development (7 d)	Seedling establishment (14 d)
Dry seeds (control)	0.085	0.233	0.426	0.856	1.109
Humidified seeds	0.096	0.318	0.467	0.879	1.265
Seeds enriched with extract	0.054	0.445	0.518	0.668	1.271

enriched seeds. The defining of their contents allows the different biological functions of the given extract, *i.e.* its allelopathic activity, to be accounted for.

Along with the increased level of the exchange processes in the enriched seeds their secretory activity is also raised. It facilitates medium formation, raising phytocenotic and protective functions of the secretions, *i.e.* seed treatment with the allelochemicals raises the level of the allelopathic activity of seed acceptors. For example, by the treatment of winter wheat seeds the area of mucous shells on the root ends was increased by 37 %, that of treated peas by 33.18 % as compared with the roots of untreated seeds, the root hairs being developed better. This, in turn, enabled the formation of a more developed adsorbing surface of roots.

How do we finally evaluate the presowing enrichment of field crop seeds with allelopathically active extracts? No doubt we observe a significant improvement in sowing qualities of seeds and their yield properties (NAUMOV *et al.* 1984). The average increase of field germination index in different crops is raised by approximately 9–12 %, the seedlings appear from 1 to 4 days earlier as compared with untreated seeds. The mass of the treated seeds is 1.2–1.5 times as high as that of control samples. The further development of the experimental plants is more active; they form a better developed leaf area and roots.The structure and characteristics of the useful microflora within the rhizosphere is more diverse due to raised activity of the roots, including the microflora participating in nutrition and in nitrogen and phosphorus transformations in soil, which in turn improves plant nutrition.

The better plant growth and development provide higher productivity (NAUMOV and NASONOVA 1987). For example, the yields of grain crops and sunflower on average raised by 0.25–0.53 t ha^{-1}, those of sugar beet by 3–5 t ha^{-1}, of fodder beet by 5–15 t ha^{-1}, *etc.*

Thus we may say that the experiments carried out allowed us to define a high

allelopathic activity of the exometabolites of growing seeds and to develop techniques for their highly effective use in agricultural production.

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