Use of biochemical methods of research for studying stability of soya to cultivation conditions

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Abstract. The influence of cultivation conditions of glycine and cultural soya of various eco-geographical origin on economically valuable attributes and biochemical structure of it seeds is investigated. It has been determined that the variation of environmental conditions causes change of specific activity and multiple forms of enzymes influencing soya adaptive potential. Stable parameters of specific activity and number of forms of soya enzymes, and also their increase, under the influence of varied of environmental conditions testify to high adaptive soya potential.
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Introduction

The efficiency of selection work in many respects depends on as far as the genotype is fully estimated and the selection lines structure is srudied [1; 2]. The increase of applied research quality is possible due to application of fast and reliable biochemical methods corresponding to modern requirements.

The ecological balance disruption results in changes in biosphere, rearrangement of genofonds and organisms abilities to adapt to adverse environment al factors [3]. The problem of biochemical adaptation of living organisms to constantly varying environmental conditions is one of central in modern biological research and demands knowledge of genetic bases of adaptive reactions. The generals trategiclaws of biochemical adaptation of animals are widely enough investigated [4]. However, for plants especially cultural such processes are understudied [5].

Enzymes as universal catalysts and regulators of exchange processes in wildlife refer to biochemical test-systems, suitable for the estimation of an environment. The detection of enzymes is important for fast and accessible studying biochemical adaptation processes and for solving practical selection tasks [6]. Till now there have been insufficiently investigated changes in a set of the enzymes, occurring during adaptation to varied fancying conditions of new soya varieties.

The important role in studying deep regulation processes of living belongs to multiple forms of enzymes and, in particular, genetically determined izoenzymes [6; 7; 8]. The practical importance of studying enzyme systems of polymorphism consists that izoenzims are effective genetic markers [9; 10; 11].

Recently there has been much research devoted to studying certain soya genom [12; 13; 14; 15], protein polymorphism including enzymes [16; 17; 18; 19; 20; 21; 22; 23]. Extension and generalization multiple forms enzymes of soya can become one of the tools of adaptation studying.

There are 70% of soya cultivated in the Russian Far East. Soya varieties adapted to soil and climatic conditions are required. The development of soya varieties resistant to unfavorable environmental is one of the tasks for the selectors [24].

Currently, selectors widely use as base line *Glycine soya* for introduction prepotent genes in culture, determining ecological plasticity, high homeostasis, illness and wrecker resistance and also the genes controlling protein load, precocity, high productivity [25].

The research in this direction will be more successful if the knowledge of biochemical adaptation mechanisms is used. To solve the problem then enzyme spectral electroforetic catalogue of glycine and cultural soya is needed.

The analysis of works on studying polymorphism izoenzymes spectra of various enzymes systems among sort Glycine has revealed insufficient level of the scientific development in this direction. The urgency of the use of izoenzymes analysis for studying soya is connected also with the low scientific level of development of genetic domestic varieties, cultivated in the Far East. The essential gap in studying multiple forms soya enzymes consists in the data shortage about their functional role during its adaptation.

Materials and methods

The research object was seeds of lines of glycine soya (*Glycine Soya Sieb. Zucc.*); seeds of grades of soya (*Glycine max (L.) Merrill*), mainly selections of State National University All Russian Scientific research soya Institute cultivated in 1999-2002 on three state experimental areas in the Amur region (various agroclimatic conditions); a type collection of seeds of various eco-geographical origin(from the Amur, Bryansk, Voronezh, Leningrad, Moscow, Novosibirsk, Omsk, Ryazan, Saratov and Ulyanovsk areas, Poland, Germany, Sweden, France, Japan and USA),cultivated in 2000-2002 on an experimental farm plot of the Far Eastern State Agrarian University. The soya collection obtained from Saint Petersburg All-Russian institute of plant growing we raised in 2003 on Blagoveshchensk State Pedagogical University agrobiological station and in 2003 and 2006 on the demonstration site of All-Russian Scientific Research Institute of Selection and Vegetable-seed Industry (s. Odintsovo, Moscow region). Since 2004 at Blagoveshchensk State Pedagogical University molecular biological laboratory a research program has been initiated with the aim to better study the influence of biologically active substances and heavy metals on enzymes activity of soya to reveal its adaptive potential to environmental conditions.

Electrophoretic enzyme spectra were studied by electrophoresis method according to Davis. Gel staining of zones with enzyme activity was carried out with the use of histochemical methods [26]. Peroxidase activity, amilase, esterase and acid phosphatase were determined by photo colorimeter, catalase was analyzed by gasometrical method, ribonuclease by spectrophotometrical method, protein content by Loury method or bybiuret methods. Biochemical analysis of soya seeds structure (protein, oils, amino acids and the high fatty acids) was carried out at All-Russian Scientific Research Institute of soya by chromatographica method on the IR-scanner.

The weather conditions during the research period (1999-2006) differed from long-time annual average in temperature mode and amount of precipitation.

Results

Earlier we investigated physical and chemical properties of enzymes, its activity in seeds and soya sprouts were examined, methods of analytical definition of electrophotetic spectra were tested, cultivation conditions and its influence on soya enzyme activity were determined [20; 27]. Each enzyme form has been assigned its shortened designation according to their electrophoretic mobility values (Rf) [28].

While examining specific activity of researched enzymes during 1999-2002 in seeds of various phylogenetic origins its higher activity of *Glicine Soya* is determined [29].

The study of influence of meteorological conditions of the vegetative period and agroclimatic zones of cultivation on soya enzymes activity (*Glycine max L.*) and plant efficiency demonstrated that an increase of precipitation and air temperatures causes the change in its activity. It has been determined that in zoned soya seeds specific activity catalase, peroxydase, sourphosphatase, esterase and amylase is higher in northern agroclimatic zone. Moreover, it has been revealed that occurrence of various forms of researched enzymes depends more on meteorological conditions of the year than on agroclimatic zone of cultivation. It has been demonstrated by us that high specific activity and increase of number of variety forms of enzymes improves quality of soya seeds: the contentof oleic and linolenic acids increases as well as the quantity or seeds weight [30].

There has been revealed wide biochemical variability of soya parameters in collection of seeds from various eco-geographical origin cultivated. The analysis of a collection of seeds from various eco-geographical origin cultivated in different agroclimatic regions (the Amur and Moscow region). It has been determined that soybean varieties cultivated in the Amur region have higher heterogeneity of studied hydrolyzing enzymes.

The enzymes variability to temperature changes presents significant interest. As a result our previous temperature stress research stated the decrease of enzymes specific activity under such conditions which is compensated by increase of its multiple forms number [31].

The elimination of negative consequences of stressful factors on an organism is provided with system antioxidant protection to which peroxidase and catalase refer, regulating metabolism in case of stress. Ribonuclease belongs to protective enzymes. It has been determined that catalase, peroxidase and ribonuclease can serve of stressful condition indicators of soya.

Scientific works studying Dihydroquercetin influence on the activity of soya seeds peroxidase present significant interest. Dihydroquercetin refers to flavonoids taking part in oxidation-reduction processes. It has been determined that Dihydroquercetin application of low concentration causes a substantial increase of activity and change of peroxidase multiple forms due to biochemical processes of activation of soya seeds.

As a result of research we suggested the application of sensitive test - enzyme peroxidase as a

marker of soya adaptation to environmental conditions.

Using ribonuclease and catalase as a markeres we have revealed that filling in $ZnSO_4$ solution of different concentration into a nutrient medium has resulted in a decrease of specific activity of soya sprouts enzymes. The minimal concentration solution of $CuSO_4$ affected positively ribonuclease and catalase specific activity of soya. It has been stated that filling in heavy metals salts into a nutrient medium leads to an increase of new fast-moving soya catalase forms. Heavy metals salts reduced the heterogeneity ribonuclease in the row Cu>Zn>Pb.

Conclusions

We demonstrated that adaptive opportunities of wild-growing soya are caused by high specific enzyme activity and in cultural soya - by an increase of number of variety forms of enzymes.

For the first time as a result of long-term research of electrophoretic spectra of soy seeds enzymes obtained in various agroclimatic conditions we attained eight catalase forms (C1-C8), eighteen peroxidase forms (P1-P18), ten amylase (A1-A10), thirteen acid phosphatase forms (AP1-AP13), fourteen esterase forms (E1-E14) and twelve ribonuclease forms (R1-R12).

On the basis of the obtained data, cultural soya grades and hybrids and also a *Glycine soya* line perspective for adaptive selection and introduction in various regions of the country have been selected [5].

Thus, the use of biochemical research methods allow better assess soybean genome and solve major agricultural problem.

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