

INFLUENCE OF HUMIC NATURE SUBSTANCES (LG) ON THE POLYPEPTIDE POLYMORPHISMS OF SOME GLYCINE MAX L. SORTS WITH DIFFERENT RESISTANCE TO DROUGHT

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Abstract

The content and quality of proteins are genetically determined characters. Individual complex of soybean proteins determines the economic values of the species. In order to identify the particularities of plant response to humic compounds, the polypeptide spectrum of soybean proteins extracted from seeds of two varieties with different drought resistant was analysed. The comparative analysis of protein spectra of extracted proteins from beans of the control plants highlighted the presence of common polypeptides to both studied sorts, and the presence of specific polypeptides for each genotype with a varying amount share appreciated through colorant intensity and band dimension. Protein spectra included polypeptide bands with molecular mass comprised between 106 and 20 kDa. Notable qualitative differences between polypeptide pattern of genotype with medium resistance to drought (Horboveanca) and those resistant (S4-04) were detected at the polypeptide level with Mr 104, 60, 43 kDa, present in resistant genotype and absent in medium resistant. Under our previous research was established that treatment of seeds with humic compound (LG) significantly increase productivity of soybean plants growing under field conditions, which determined substantial changes in the protein content and enhance grain biomass. From the reported data we conclude that the chemical compounds such as humic compounds may influence production potential of soybean and resulted substantial changes in the protein complex of the plant.

Key words: biologically active substances, drought resistance. soybean, soybean proteins

INTRODUCTION

Soybean (*Glycine max* L.) is an important source of protein for human and animal nutrition. The quantity of soy protein in the environment can vary from 38-42 % [14]. Soybean proteins can be distinguished by the structure and function, amino acids composition, the content of nitrogen, sulfur, phosphorus and molecular weight of the components [2,20,21], being composed especially in globulin and insignificant quantity of albumin. The content and quality of proteins are genetically determined characters. Individual complex of soybean proteins determines the economic values of the species.

Representing one variable strongly influenced by environmental conditions, genetic potential of plants could be improved with the help of some physiologically active substances [6,17,18,22,23]. The data from the literature

relates about the possibility of using such humic compounds in the process of plant growing for the purpose of resistance modulation and to increase their productivity. Mechanism of action and role of these compounds in plants is diverse and incompletely elucidated [14].

MATERIALS AND METHODS

In order to emphasize the response reaction and particularities detection of soybean protein spectrum, it was analyzed the polypeptide pattern of extracted protein from beans of two species with different resistances to drought and treated with humate solution (LG) in a concentration of 0.5 and 0.1 % and grown under field conditions. In the study was used genotypes Horboveanca – with medium resistance and S4-04 – resistant to drought. Extraction of total soluble protein was carried out in Tris-HCl buffer solution, 6.25 mM

pH=6.8. Protein precipitates were dissolved in Tris-HCl buffer solution (pH=6.8), contained SDS – 4.25 %, sucrose about 20 %, β – mercaptoetanol – 6% and bromophenol blue – 0.004 %. Protein electrophoresis was performed in the system of Laemmli tampons, in the polyacrylamide vertical plates with 1mm thickness, under denaturing conditions and post electrophoresis operations were carried out according to the standard method [8].

For the determination of relative molecular mass of separated polypeptide fractions were used protein markers with known molecular weight: carbonic anhydrase – 29 kDa, ovalbumin – 45 kDa, bovine serum albumin – 67 kDa and phosphorylase B – 97.4 kDa.

RESULTS AND DISCUSSIONS

The main protein reserve in soybeans are represented by glycine (globulin 11 S) and β -conglycine (globulin 7 S) that representing approximately 70 % of total protein deposited in beans [7]. Glycine is composed by acid subunits with molecular weight approximately 45 and 38 kDa and base subunits with the Mr 22 kDa, and β -conglycine is composed by major subunits α , α and β , subunits with 76, 72 și 48 kDa [19].

The comparative analysis of protein spectra of extracted proteins from beans of the control plants highlighted the presence of common polypeptides to both studied sorts, and the presence of specific polypeptides for each genotype with a varying amount share appreciated through colorant intensity and band dimension. Protein spectra included polypeptide bands with molecular mass comprised between 106 and 20 kDa (Fig.1).

SDS-electrophoresis revealed the presence of some polypeptides with high content in electrophoresis gel (Mr 88, 79, 73, 53, 41, 35, 32, 29 kDa) common to analyzed genotypes, the maximum intensity which is attested in the three bands corresponding to the polypeptide with Mr 79, 73, 35 kDa. Major quantitative differences between polypeptides spectra of the witnesses were confirmed, especially, at the level bands which corresponding to

polypeptide with molecular mass \approx 88, 32–29 și 20 kDa, Mr 88 and 29 kDa polypeptide with a more pronounced expression in the resistant sorts and polypeptide with the Mr 45, 35, 32 and 20 kDa – genotype which has an medium resistance.

Notable qualitative differences between polypeptide pattern of genotype with medium resistance to drought (Horboveanca) and those resistant (S4-04) were detected at the polypeptide level with the Mr 104, 60, 43 kDa, present in resistant genotype and absent in medium resistant.

The relevant differences between control variants and studied genotype were found in the bands with the Mr 88 kDa, 79 kDa, 73 kDa, 53 kDa, 32 kDa, 29 kDa, 20 kDa, from that polypeptides with the Mr 88, 79, 73, 53 and 29 kDa have more intense expression of resistant genotype S4-04 compared to the medium resistant - Horboveanca. Biosynthesis intensification of proteins with the Mr 29 and 70 kD was attested to the action of different types of stress – heat, salt and oxidative, changes in protein metabolism constitutes an adaptation type at the molecular level [24,25,26,27]. It is know that polypeptide with the Mr 29 kDa plays a key role in plant reaction to stress and the Mr 70 -73 kDa polypeptide represent heat shock proteins being codified by the gene HSP70 [11,28].

A significant number of data shows that stress proteins have an important role in the cellular protection and in rapid reestablishment of an initial cellular metabolism after removing of stress action [1,3,4,9,10,11,16]. From those observed we mention the presence of larger quantities of polypeptides with the Mr 104, 88, 60, 43, 29, 26 kDa at resistant sort and the absence or presence in small quantities of them with the medium resistant sort to drought which demonstrates that drought may be caused by synthesis capacity of stress proteins by genotype under normal conditions of cultivation, resistant sort to drought being characterized by more emphasized protein polymorphysm. The result obtained are in compliance with the some authors data which mention that resistant sorts to hyperthermia synthesize a greater amount of PST in

chloroplasts and mitochondria [5] or demonstrate a greater variety of polypeptide composition under heat stress [26]. In previous research conducted by us on the some parameters with the determinant role in the biological potential manifestation of plants was established that treatment of seeds with 0.5 % solutions LG significantly increase

productivity of soybean plants growing under field conditions, determining substantial changes in the protein content and enhance grain biomass about 13-14 %. For these reasons, presents interest changes produced by substance administration in this concentration.

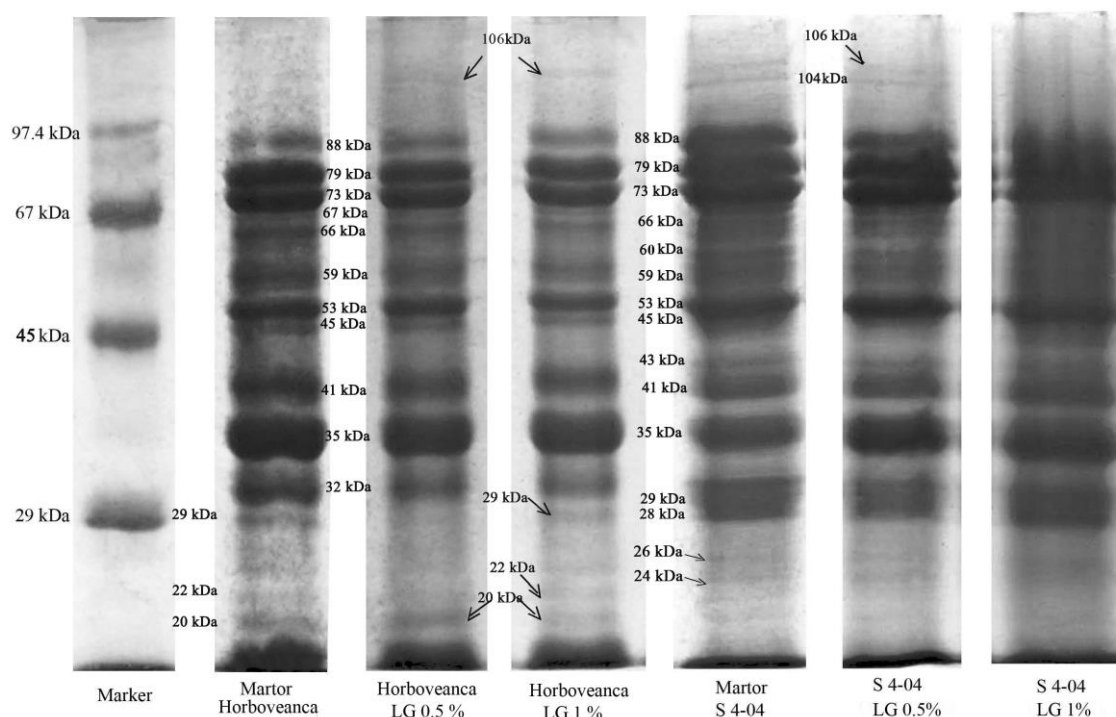


Fig. 1. Electroforeogram of extracted soluble proteins from soybeans subjected to treatment with humic substances (LG)

The polypeptide patterns of treated sorts with humic nature substances (LG) demonstrated a general spectrum of protein bands with a similar molecular weights diapason of witness variants.

However, treatment of seeds before seeding with the humate determined diverse quantitative and qualitative changes in the protein extracted content from harvested beans.

The treatment with LG resulted in band expression with the Mr 106 kDa, polypeptide color intensification with the Mr 20 kDa and also reduction of band content with the Mr 29 kDa in the case of Horboveanca sorts. S4-04 sorts responded to treatment by intensifying bands with the Mr 79, 73, 53 and 35 kDa and through reduction content of the band with the

Mr 22 kDa. The 41 kDa, 22 kDa and 32 kDa polypeptides were expressed as medium resistant sorts while for better resistant sorts were expressed polypeptide with the Mr 28 kDa and polypeptide with the Mr 26 kDa.

According to the data from the literature, polypeptide with the Mr 26 kDa correspond to the osmotine and represents a responsible protein for adaptation of plants to osmotic stress, and the 22 kDa bands corresponds to class IV for proteins responsible for adaption to heat shock, codified by HSP22 gene [10,11].

This protein results from the precursor with the Mr 26 kDa, from which as a result of post-translational modifications is removed polypeptide with the Mr 4 kDa, and resulting PST with the Mr 22 kDa [12].

CONCLUSIONS

From the reported data we conclude that the chemical compounds such as humic nature may influence production potential of soybean, determining substantial changes in the protein complex of the plant. Response to chemical treatment is determined by genetic character of the sorts. Under optimal conditions of cultivation for the resistant sorts is characteristic the presence of a varied number of stress proteins in harvested seeds.

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