Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 4 (2023)

Soybean Seed Peroxidase

Sh. Yunuskhanov[1], M. Sh. Jaynakov[2], Z. L. Abdurazakova[3]

[1,3]Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan.

[2]Andijan State University.

Abstract: Research has been carried out to study the peroxidase activity of seed coats and the surface layer of seeds of various soybean varieties. It has been shown that the peroxidase activity of soybean seeds is located mainly on the surface layer behind the thin soybean shell, which plays a protective role against various agro-ecological stress factors. The content of proteins with peroxidase activity in soybean shells of the Vilana variety is 3.29-3.36% and the surface layer of seeds after separation of the shells is 0.95-1.1% of proteins containing peroxidase activity. The total protein content with peroxidase activity on whole seeds averages 1.7%.

Keywords: *Soybean, vegetable oil, nutritious feed, livestock, peroxidase, o-diphenoloxidase, electropherograms, Khosildor, Vilana, Tumaris.*

1. Introduction

Soybean grain production in the world increases every year, and at the beginning of the 21st century. In terms of gross yield, this crop has taken fourth place among field crops after wheat, rice and corn [1]. Soybean is a unique and valuable agricultural crop due to its rich content in the grain of complete amino acid composition and digestibility of protein and high-quality oil, which determines its wide distribution for multifaceted use in feed, food and technical purposes.Currently, in the Republic of Uzbekistan, a gradual increase in the volume of sowing and cultivation of soybeans is being carried out to better meet the population's needs for vegetable oil, nutritious feed for livestock and poultry farms, as well as the efficient use of production capacities of processing enterprises. In this regard, comprehensive research in the field of biochemistry, physiology and genetics of this crop and the creation on their basis of new high-yielding and promising soybean varieties are relevant. Such studies on the scale of the Republic of Uzbekistan have not yet been carried out sufficiently. Previously, the protein content and oil content of soybean collection samples of the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan were studied [2] and it was shown that the total protein content in soybean seeds ranges from 17.4% to 28.6%, and the oil content from 18% up to 27%[3].Among the physiological and biochemical factors of plant protection from stress, peroxidase is considered one of the most important catalytic systems actively involved in the autoregulation of metabolism under stress [4-5]. Literature data indicate that peroxidase is associated with a number of metabolic transformations occurring in cells within the pH range from 3 to 14 [6], and also that the isozyme spectrum of peroxidase in soybean seeds strongly depends on the variety and agro-ecological conditions and also the year of cultivation [7 -9]. It has been established that the enzyme has not only peroxidase, but also oxidase properties, catalyzing the oxidation of a number of compounds. Peroxidase, which simultaneously possesses o-diphenoloxidase activity, was also noted in works [10,11].Data were obtained on the absence of peroxidase in electropherograms of 11 samples among 40 studied collection samples of the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan. It has been proposed to use peroxidases as a diagnostic trait to assess the degree of plant resistance to stress factors [12].Soybean peroxidase is of interest due to some unique characteristics: high thermal stability (inactivation temperature above 80°C), high reactivity and stability at low pH values and in a number of organic solvents [13]. It is noted that soybean peroxidase is contained in large quantities in the soybean seed coat. Isolation of peroxidase from soybean seed coats was carried out by A.L. Fedulov et al. [15]. For this purpose, an assessment of soybean

varieties released in Belarus by activity was carried out. Among the varieties used “Severnaya Zvezda”, “Yaselda” and “BOS37-15”, the variety “Severnaya Zvezda” turned out to be the best in terms of peroxidase activity. As a result of the research, the enzyme peroxidase was isolated from soybeans with high physicochemical parameters (activity - 276 U/mg, RZ - 0.9). Studies have been conducted to study the peroxidase activity of extracts of seed coats and seed flour from samples of a genetic collection of soybean seeds and some varieties cultivated in the Republic of Uzbekistan, as well as studies to study the effect of seed defatting on peroxidase activity [10,11,14].It has been established that some soybean varieties cultivated in the Republic differ from each other in the peroxidase activity of seed coat extracts and seed flour. It has also been shown that defatting the seed coats and seed flour on the Sok-Slet apparatus with acetone and ethyl ether leads to a sharp decrease in the peroxidase activity of extracts of both seed coats and seed flour. Similar data were obtained when samples were treated with the indicated solvents at room temperature, although the literature notes some unique characteristics of soybean peroxidase: high thermal stability, high reactivity and stability at low pH values and in a number of organic solvents [13].The loss of peroxidase activity in cotyledons during defatting of the studied varieties is observed differently. No change was detected in the Ustoz MM-60 variety, while in other varieties, from 25% to 86.4% of activity was retained. In the case of seed coats, from 8.8% to 89.9% of peroxidase activity is retained. In further studies, results were obtained indicating a difference in the peroxidase activity of the shells and kernels of soybean seeds in different batches of experiments, the results of which will be described in this article.

2. Materials and research methods

Seeds of soybean varieties Nafis, Selecta-302, Orzu, Ustoz MM-60, Slavia, Gavkhar, Selecta-201, Khosildor, Vilana, Tumaris were presented by the Scientific Research Institute of Grain and Leguminous Crops of Andijan Region. Soybean seeds were separated from the shell using a scalpel, as well as according to the method [16]. The resulting samples of seeds and shells were ground in a porcelain mortar to fine flour.A weighed portion of flour from the obtained samples was extracted with distilled water in a flour water ratio of 1:10 for 1 hour. Flour extracts were centrifuged at 6000 rpm for 30 min. and the supernatant of the samples was applied to paper filters to detect peroxidase activity in them according to the method [17].To determine the protein content in soybean shells and shelled seeds, extraction was carried out in a 10% sodium chloride solution and the protein content was determined using the Lowry method [18].

3. Results and its discussion

In Fig. Figure 1 shows the peroxidase activity of seed extracts, and Fig. 2 extracts of shells and peeled seeds of various soybean varieties. The studied soybean varieties differ in the peroxidase activity of both shells and seeds. The Vilana variety differs sharply from other varieties with high peroxidase activity of the shells.

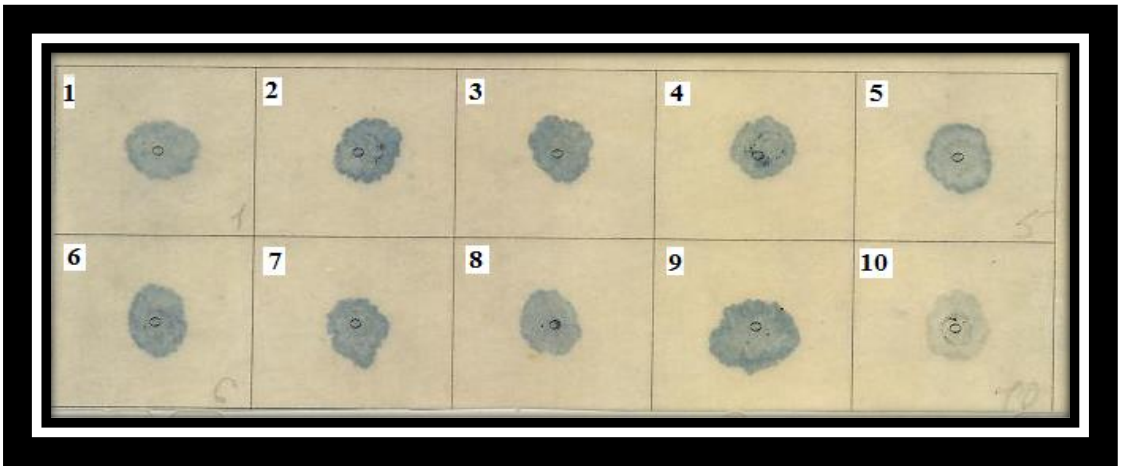

Fig. 1.Peroxidase activity of soybean seed aqueous extract,the extract is prepared in a flour: water ratio of 1:10. 10 µl applied to paper.

1– Nafis; 2 – Selecta – 302; 3 – Orzu; 4 – Ustoz MM-60; 5 – Slavia;

6 – Gavkhar; 7 – Selecta – 201: 8 – Hosildor; 9 – Vilana; 10 – Tumaris.

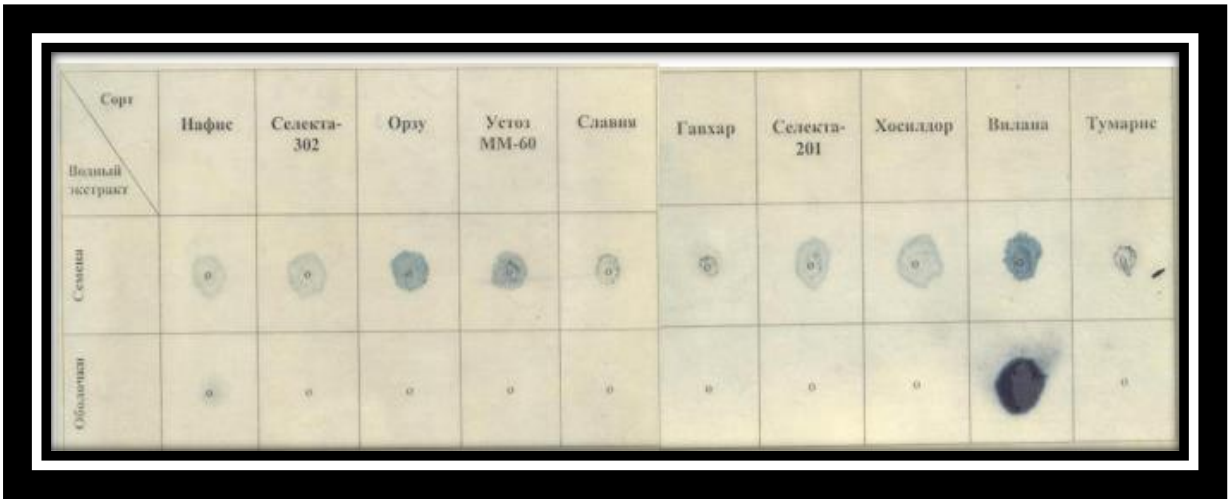

Fig. 2. Peroxidase activity of aqueous extracts of peeled seeds and shells of various soybean varieties.

In [19], it was noted that peroxidase activity in soybean seed coats (Clycine max LI Merr.) is controlled by the Ep locus. In soybean seed coats of the EpEp genotype, peroxidase activity is 100 times higher than the activity in varieties of the Epep genotype. Based on these data, it was assumed that the soybean samples studied by the authors differ from each other in the state of the loci controlling peroxidase activity. Based on these data, it can be assumed that the soybean samples we studied also differ in the locus controlling peroxidase activity.

Among the seeds, almost all varieties have easily separated and difficult to separate shells; therefore, different batches of obtained samples may differ in peroxidase activity. Next, from the seeds peeled from the shells, the outer part of the seeds was isolated by raking with a scalpel.In Fig. 3 shows peroxidase activity

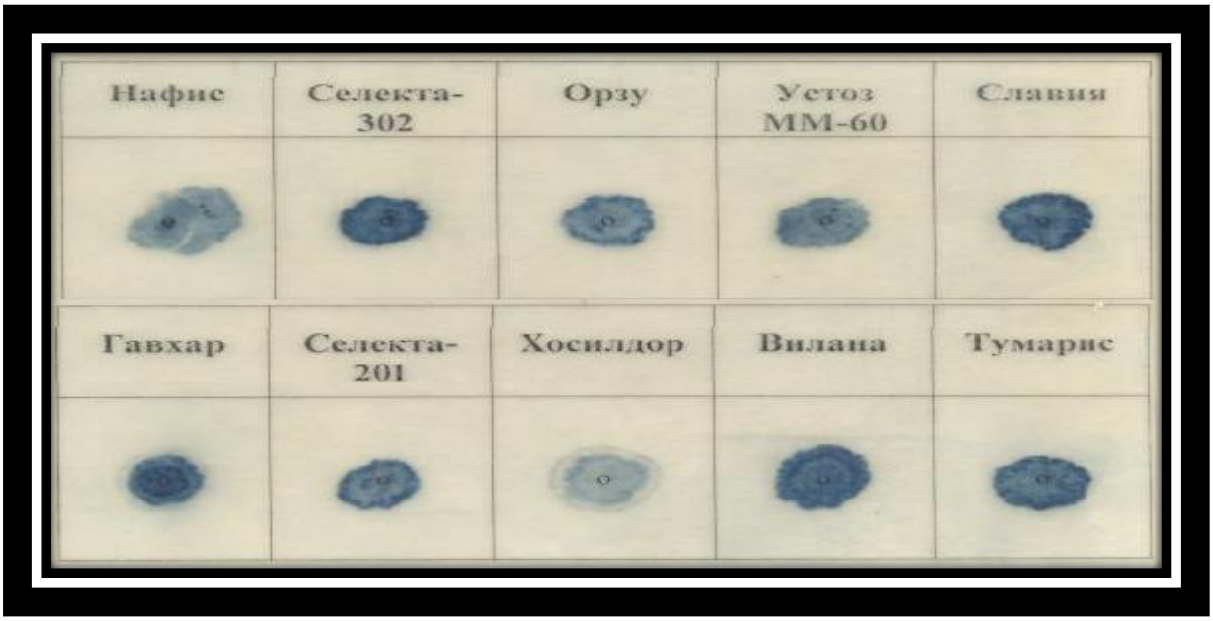

Fig.3. Peroxidase activity of aqueous extracts of the rake layer of various soybean varieties.

extracts of the rake layer of various soybean varieties. The Nafis and Khosildor varieties are inferior to other varieties in terms of intensity of peroxidase activity, although such differences are not observed when studying extracts of soybean seeds that have not been peeled from the shells (Fig. 1). When studying extracts of soybean

seed flour after raking the surface, peroxidase activity almost does not appear (Fig. 4). Next, individual seeds of the soybean varieties Ustoz MM-60 and Vilana were cleared from the easily detachable shell, some had the entire surface raked, and some had the middle part raked and tested for peroxidase activity. The results obtained are presented in Fig. 5. Weak peroxidase activity appears on the seed coats of the Vilana variety. Soybean seeds, after separating the shells, are divided into two segments. No peroxidase activity is detected on the inner part of the seeds, and its outer surface contains a layer containing peroxidase (Fig. 6).

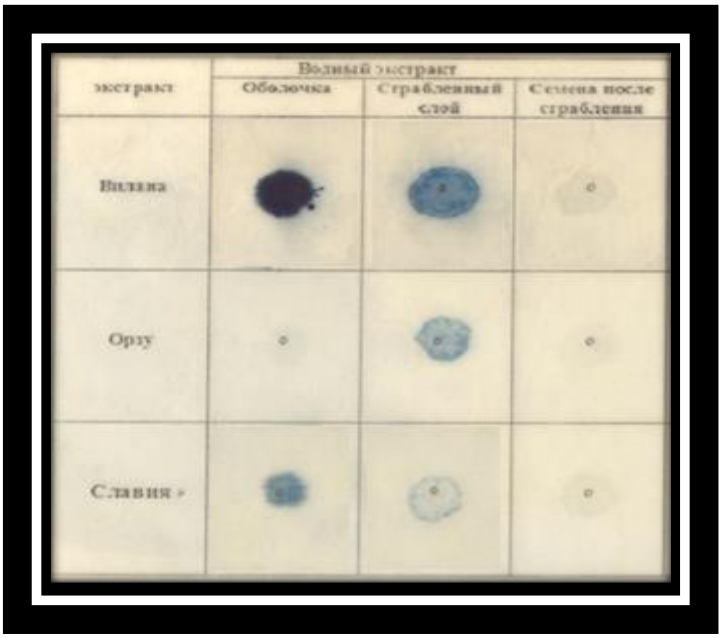

Fig. 4. Peroxidase activity of aqueous extracts of shells, rake layers and seed flour of various soybean varieties.

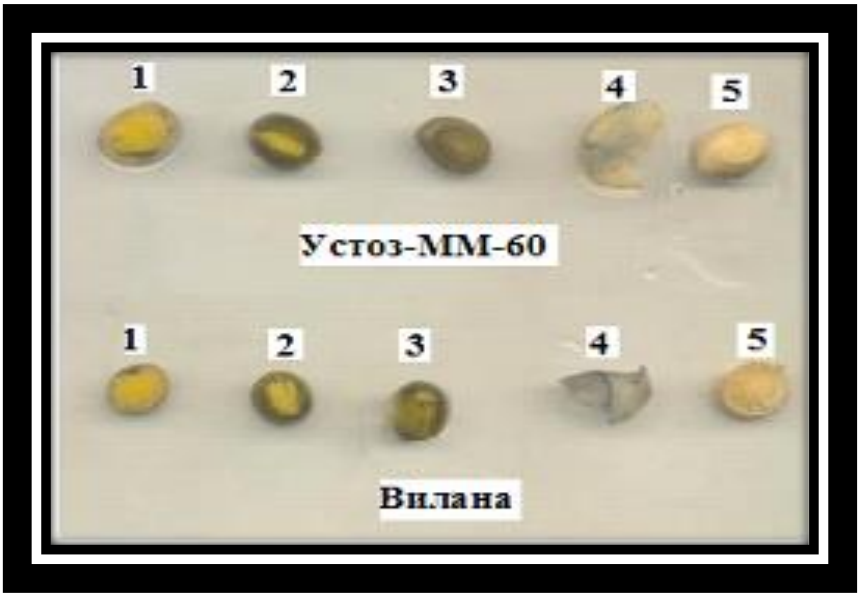

Fig. 5. Seeds tested for peroxidase activity: 1 – seed in which the layer after the shell has been cleaned by raking; 2 – a seed in which the middle part of the layer after the shell is cleaned by raking; 3 – seed in which the layer after the shell has not been cleaned by raking; 4 easily detachable shell; 5 – seed not peeled from the shell.

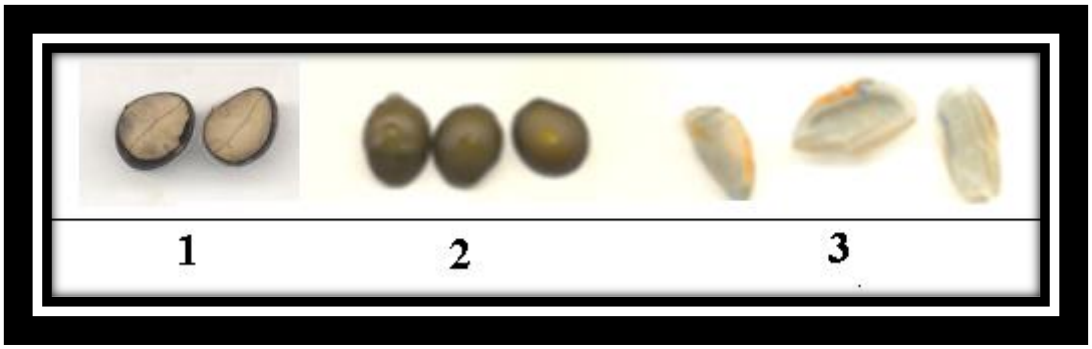

Fig.6. Seeds and shells tested for peroxidase activity: 1 – inner part of the seeds, 2 – outer part, 3 – easily detachable shell

The seed coats of the various soybean varieties we studied ranged from 4% to 11% by weight of the crude soybean seeds. In [20], data was obtained that the soybean seed coat is 2.5%, the fruit shell (husk) is 27% and the kernel is 70.5%.

4. Conclusion

Next, we studied the protein content in the seed coats and in the surface layer of the kernels, containing peroxidase activity. To do this, the seed coats and kernels were placed in a 10% sodium chloride solution and the kernels were kept in the solution until the peroxidase activity on the surface was removed. According to the results obtained, the soybean shell of the Vilana variety contains 3.29-3.36% protein and the surface layer of the seeds after separation of the shells contains 0.95-1.1% proteins containing peroxidase activity. The total protein content with peroxidase activity on whole seeds averages 1.7%. Based on the data obtained, we can conclude that peroxidase in soybean seeds is located mainly on the surface layer of the seeds after the thin shell and plays a protective role against various agro-ecological stress factors.

5. References

[1] Promising resource-saving technology for soybean production: Methodological recommendations. - M.: FGNU “Rosinformagrotekh”, 2008. - 56 p.

[2] Abzalov M.F., Kilicheva O.B. Genetic collection of the soybean plant of the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan.//Doc. AN RUZ.-2008-No. 2.-pp.77-80.

[3] Yunuskhanov Sh., Abzalov M.F., Zhainakov M., Kurbanbaev I.D., Abdurazakova Z.L. Oil content and protein composition of the genetic collection of soybean seeds // Uzbekistonagrarfani khabarnomasi.-2018- No. 4.-S. 32-35.

[4] Kuznetsova V.A. Thermal stability of soybean seed peroxidases // Oil crops. Scientific and technical bulletin of the All-Russian Research Institute of Oilseeds. - 2012. - Issue. 2 pp.151–152.

[5] Kartashova E.R., Rudenskaya G.N., Yurina E.V. Polyfunctionality of plant peroxidases and their practical use // S.-kh. biology. Ser. Plant biology. – 2000. - No. 5. – P.63-70.

[6] Andreeva V.A.//Enzyme peroxidase: Participation in the defense mechanism of plants. M.: Nauka, 1988.-128 pp.-ISBN 5-02-003944-6.

[7] Semenova E.A. The influence of water stress on the activity and electrophoretic spectra of antioxidant enzymes in soybean seeds//Modern science-intensive technologies.-2012.-No. 7.-P. 33-35.

[8] Ivachenko L.E., Egorova I.V., Fomenko N.D. Characteristics of electrophoretic spectra of enzymes as markers of soybean varieties//International Journal of Applied and Basic Research -2010.-No. 5.-P. 65-69.

[9] Ivachenko L.E. The role of multiple forms of soybean enzymes in the process of biochemical adaptation to growing conditions // Advances in modern natural science. - 2010. - No. 9. - P. 96-98.

[10] Zhainakov M., Yunuskhanov Sh. The effect of defatting on the activity of peroxidase in seeds and seed coats of some soybean varieties // Uzbek Biological Journal-2020.-No. 5.-P. 60-62.
[11] Yunuskhanov Sh., Zhainakov M., Abzalov M.F., Kurbanbaev I.D., Abdurazakova Z.L. Electrophoretic analysis of peroxidase and o-diphenoloxidase of soybean genetic collection seeds // Dokl. ANRUz. – 2019.- No. 2. – P. 90-93.
[12] SmithH.H., HamillD.E., WeaverE.A., ThompsonK.H. Multiple molecular forms of peroxidases and esterases among Nicotiana species andamphiploids//Journal of Heredity, Volume 61, Issue 5, September 1970, Pages 203–212.
[13] HenriksenA. Structure of soybean seed coat peroxidase: A plant peroxidase with unusual stability and haem-apoprotein interactions /HenriksenA. et al. //ProteinScience. – 2001. – Vol.10. – P. 108 – 115.
[14] Yunusxanov Sh., Jaynaqov M. Sh. PeroxidasesofSeedsof the Soy Genetic Collection//American Journal of Plant Sciences.-2020.-№ 11.- P. 549-553.
[15] Fedulov A.L., Spiridovich E.V., Rakhmanko E.M. Isolation of peroxidase from soybean seed coats//Proceedings of the Belarusian State University 2006: Volume 1. Part 1., pp. 212-220.
[16] Nikitchenko N. G. Agroecological aspects of the chemical composition of soybean seeds and seed coat. 03.00.16 – ecology. Abstract of the dissertation for the scientific degree of candidate of biological sciences. Krasnoyarsk-2005.
[17] Safonov V.I., Safonova M.P. Study of plant proteins and enzymes by polyacrylamide gel electrophoresis. //Biochemical methods in plant physiology. Ed. “Science” M. 1971. pp.113-121.
[18] Yunuskhanov Sh. Isolation and study of the chemical composition of cotton proteins / In: Methods of physiological and biochemical studies of cotton. Tashkent.-1973.-P.28-54.
[19] Cijzen Robert van Huystee, and Richard 1.Buzzell. Soybean seed coat peroxidase A comparison of high-activity and low-activity genotypes Mark//Plant Physiol. (1993) 103: 1061-1066.
[20] Ashurov F.F. and others. Analysis and assessment of the quality of soybean oil produced in Uzbekistan //Universum: technical sciences: electronic scientific. magazine 2021. 3(84).