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Assessment of salt resistance of some potato varieties by biochemical and RFLP markers

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Abstract. Continuous changes in climate, desertification, reduction of arable land, increase in salted land, in the conditions of continuous growth of the population, the problem of providing food and food security to humanity arises, the solution of which is one of the challenges of the 21st century that requires universal efforts. At the same time, abiotic stresses, which are the cause of 50% of global yield losses, are the motivation for the creation of new stress-resistant varieties of crops using modern technologies. The salt resistance of that idea as a physiological manifestation with a polygenic component is characteristic in the modern processes of selection management.

This work presents studies of salt tolerance of three valuable varieties of potatoes cultivated in Armenia, using biochemical and DNA-markers. Classical agronomic, molecular-biological, genetic-mathematical methods are used in the researches. In order to induce salt stress and provocation background in plants, 50, 100 and 150 mmol solutions of NaCl were used.

The results have shown that 11S-globulin of all varieties are polymorphic, forming different electrophoresis spectra and protein formulas. It is also evident that plants with different spectra of the same varieties react differently to salt stress. The DNA restriction regions of the salt-resistant variants are significantly longer than those of the non-salt-resistant forms. Thanks to the biochemical and RFLP markers, it has been possible to establish salt tolerance loci, to identify and list plants with a salt-tolerant spectrum of the same variety, which can be nominated as new salt-tolerant varieties for breeding stock producers.

Key words: DNA restriction fragment, electrophoresis spectrum, protein formula, restriction enzyme, salt stress.

INTRODUCTION

Abiotic stresses such as high temperature, drought, frost and salinity stress threaten agriculture, especially affecting yield. These factors are the main cause of crop loss worldwide, reducing more than 50% of the world's harvest (Boyer, 1982; Askari & Pepoyan, 2015; Bray et al., 2000; Sassine et al., 2022).

It is known that only 10% of the world's arable land is free from stress factors, 20% is exposed to mineral stress, 26% to drought and 15% to frost. 10% of the earth's surface is occupied by saline soils, the surface of which is especially large in dry zones. Also, it is considered that not less than 25%, maybe 50%, of the 270 million ha of irrigated land is secondary to salinization (Zhuchenko, 2001; Schroeder et al., 2013; Luo et al., 2017).

Soil salinization is one of the most common abiotic stresses in terms of negative impact on area and plant productivity (Munns & Tester, 2008). The factors that cause salinity are diverse and include not only the characteristics of soils and their composition, but also climate, terrain, and human activities. Besides, climate change brings predictions of the development of an undesirable situation (Novikova et al., 2021). Moreover, even such an agricultural approach as the use of fresh water from different sources for irrigation only exacerbates the problem, the solution of which is increasingly difficult (Pankova et al., 2017).

The effect of soil salinity on plant characteristics is diverse. It is expressed not only by changes in the biomass accumulation rate, the total and economic productivity of different crops, but also by the effect of physiology and biochemistry on the quality of the crop of plants (Parvaiz, 2008; Agarwal et al., 2013; Torabi, 2014; Sajyan et al., 2018).

Very often the cause of soil salinization is also improper irrigation, immoderate use of mineral fertilizers, constant growth of soil-soluble waste (Shrivastava & Kumar, 2015).

In the process of salinization, different salts accumulate in the soil, which are various combinations of Na^+, Mg^{2+}, Ca^{2+} cations and Cl^-, SO_4^{2-}, CO_3^{2-} anions, of which NaCl is of great importance due to its widespread scale and high concentration.

Large amounts of salts in the soil have a toxic effect on plants, even destructive.

As a result of salinization, water penetration becomes difficult, metabolism is disturbed. Due to the violation of nitrogen conversion, toxic substances, such as ammonia, accumulate in plants (Fricke, 2004). The harmful effect of salts first of all negatively affects the root system. Under the influence of salts, the transparency of the cytoplasm increases, transpiration becomes excessively intense. The harmful effect of salt concentrations in the soil is due to the high osmotic pressure caused by the excess of salts in the soil solutions and the associated water absorption difficulties (osmotic effect of salinization) (Munns & Tester, 2008). In connection with this, only those groups of plant organisms can develop regularly in the saline soils, in which the appropriate adaptation properties have been developed and strengthened under the influence of salinization in the process of evolution (Levy & Veilleux, 2007; Tian et al., 2022).

Salt tolerance is a physiological feature of halophytes in terms of obtaining water from soil solutions with high osmotic pressure, the protoplasm of such plants is endowed with high transparency for electrolytes (Levy & Veilleux, 2007; Munns & Tester, 2008; Shabala, 2013; Meng et al., 2018; Zhang et al., 2018; Han et al., 2023; Mann et al., 2023).

The most important physiological manifestation of salt tolerance is the accumulation of a large number of teratogenic substances (organic acids, sugars, etc.) in the cell sap (Fricke, 2004; Polle & Chen, 2015; Assaha et al., 2017; Shahid et al., 2020).

The composition of plants to survive under high NaCl concentrations is related to their ability to transport, partition, release and mobilize Na+ ions (Assaha et al., 2017; Alharbi et al., 2022; Wang et al., 2022).

It is obvious that salt tolerance of plants is a physiological process with multigenic manifestation, the basis of which is gene expression, during which the information of genes is transformed into a material performing a certain function, RNA or protein.

These genes that are up-regulated more than 5-fold under salt and stress may be AP2-EREBP (ATERF11, CBF4 / DREB1D, CBF1 / DREB1B, ATERF4 / RAP2.5, DREB2A, CBF1 /DREB1B, DREB12A), - Helix-Loop-Helix (bHLH) family (AtbHLH17), leucine buckle (AtbZIP55 / GBF3), C2H2 family (ZAT10, ZAT12 / RHL41, ZAT6 and ZAT102 / RHL41), heat stress (Home-Box7E) family.) and and, to the NAC family (ANAC036, ANAC029 / ATNAP, ANAC055 / ATNAC3, ANAC047, ANAC072 / RD26, ANAC002 / ATAF1, ANAC019 and ANAC032). These transcription factors are induced in response to salt stress signals transmitted through sensing and signalling molecules; then the complex gene regulatory systems consisting of transcription factors and other proteins that control the expression of many genes (Huang et al., 2006; James et al., 2006; Byrt et al., 2007; Møller et al., 2009; Shokri-Gharelo & Noparvar, 2018).

Armenia occupies not much of the north-eastern part of the Armenian volcanic highlands. In the territory of Armenia, the saline soils were formed in those parts of the Ararat plain, where the groundwater is mineralized and is on the surface of the soil (1–2 m) hectare (Manukyan & Karapetyan, 2011).

Their total area is about 29 thousand hectares. In the Ararat Valley, there are favorable soil and climate conditions for farming, particularly for the cultivation of vegetable crops, orchards and vineyards, and for obtaining a high and quality yield from them.

As a crop of global importance, potato occupies a key place in the fields of agriculture and culture of Armenia, earning the name second 'bread', as it is second only to cereals in terms of its gross output.

The commercial varieties of potatoes cultivated in Armenia are mainly imported from abroad and do not have much resistance to salt stress, which is often the reason for low yields.

It is obvious that the multigenic character of salt tolerance and the existence of new different types create difficulties in the breeding works carried out in that direction. In addition, the main emphasis was placed on useful economic characteristics (yield) in potato breeding works.

At the same time, it should be noted that imported potato varieties, even if they had highly expressed genes for salt tolerance, may not be expressed in case of changes in climatic conditions, and this is the main reason why salt-tolerant varieties should be created for specific agro-ecosystems. Moreover, the response of plants with different genotypes within the same variety to stress factors is incompletely studied or absent, which is important in the process of breeding in a very specific direction. Very often, organisms with the same genotype have different phenotypic manifestations depending on paratypic factors (Verhoeven et al., 2008).

Among the markers (phenotypic, protein or biochemical, cytogenetic, DNA or molecular) used for the assessment of genetic diversity of plants (Kanukova et al., 2019), due to their simplicity, efficiency, speed, availability and most importantly, high efficiency and applicability, protein and DNA markers were selected by us.

The electrophoretic spectrum and protein formula of 11S-globulin, a common and rather well-studied storage protein in dicotyledonous plants, was used as a protein marker. Proteins, as a result of the coding of genomic DNA loci, are not only not inferior in their molecular capabilities, but also very often have a number of advantages over other markers. They are based on multiple allelism of genes, hence polymorphism.

The study of the electrophoretic spectrum of 11S-globulin makes it possible to clarify the frequency of alleles and genotypes in populations, origin, genetic similarity, evolution, the level of manifestation of useful economic and biological traits, the degree of heterozygosity, etc., which can be applied in the fields of starting material evaluation, breeding, variety testing, seed production and genetic engineering (Konarev, 2007; Kononenko et al., 2019).

The RFLP (Restriction Fragment Length Polymorphism) DNA marker is used in our research as a DNA marker. The basis of the RFLP method is the separation of DNA restriction segments of different lengths by electrophoresis, which are generated when the double-stranded DNA molecule is cleaved by restriction enzymes. At the same time, the placement of restriction sites of the same enzyme in the DNA segment of different species or cultivars is different, or at the same time, it is polymorphic, which is a species characteristic and has a hereditary nature. The RFLP method provides an opportunity to construct a map of the restriction segments of the DNA of various organisms. The advantage of RFLP markers compared to other DNA markers is that the information contained in them can be used by cloning the corresponding restriction fragment of DNA. Moreover, the results of the analysis of the relevant segment can be used to obtain STS from the given segment of the genome. The construction of restriction maps also does not require prior cloning of the DNA under study.

In addition, in the DNA-bank or gene library of the 'Scientific Center of Agrobiotechnology' branch of the Armenian National Agrarian University /ANAU/, restriction fragments of the genomic DNA and genetic constructions of a number of valuable crops and their wild relatives are preserved, with the aim of obtaining new high-yielding and stress-resistant varieties and improving the existing ones using agrobacterial transformation (Badalyan et al., 2023).

In fact, plants with different genotypes of the same variety can show greater or lesser tolerance to salt stress. From that point of view, our studies aimed to identify the most stable genotypes of Impala, Madeline and Arizona potato varieties cultivated in Armenia under salt stress conditions of 50, 100 and 150 mmol of NaCl. The results will be used to obtain new salt-resistant potato varieties through agrobacterial transformation (cisgenesis) and to improve existing ones.

MATERIALS AND METHODS

Experiments and studies were carried out in 2021–2023, in the 'Scientific Center of Agrobiotechnology' branch of the ANAU. Impala, Arizona and Madeline varieties of potatoes (*Solanum tuberosum* L.), which are widely cultivated in Armenia, were used as a test sample. Experiments and researches were carried out in 4 stages.

First stage: Marking was carried out within each variety and formation of experimental groups. Randomly selected 500 plants (from each variety) certified according to the electrophoresis spectrum of the 11S-globulin storage protein, as a result forming experimental groups, each with 20 plants.

A number of crops, including potato tubers, also contain albumin, whose electrophoresis mobility (RF) is close to that of globulin and is often a source of confusion.

In order to disable the globulin, it was carried out:

- Cryoprecipitation – for this purpose, 10 parts of 0.2 M NaCl solution was added to 1 part of the sample and kept at 4 °C for 2 hours. Then cold distilled water was added

to the supernatant containing albumin and globulin at a ratio of 1:10 and stored in a refrigerator at 4 °C for 2 hours to precipitate globulin. Then the precipitate is dissolved in buffer (0.4 g Tris, 3 mL 1 M HCl, 1 g sodium dodecyl sulfate, 5 g sucrose, 18 g urea, 2.5 mL mercaptoethanol, 0.25 g bromophenol blue, and 100 mL distilled water), in a ratio of 1:1.

- Preparation of the gel – 12.5% resolving gel contains 12.5 g acrylamide, 0.25 g bisacrylamide, 4 g Tris, 0.1 g SDS, 0.3 g ammonium persulfate, 0.28 mL TEMED (per 100 mL gel). The pH of the solution was adjusted to 8.8 with 1 M HCl. The stacking gel (5%) contains 5 g acrylamide, 0.13 g bisacrylamide, 0.24 g Tris, 0.1 g SDS, 0.02 g ammonium persulfate, 0.15 mL TEMED (per 100 mL gel). The pH of the solution was adjusted to 6.8 with 1M HCl (Konarev, 2007).

Electrophoresis was carried out by the Davis method on a 12.5% polyacrylamide gel with Multigel-long phoresis apparatus of the German company Biometra (Table 1) (Davis, 1964).

Table 1. Necessary conditions for electrophoresis of 11S-globulin protein

Protein	Gel (%)	Gel length (cm)	Sample titer	Buffer		Current voltage (V)	Duration of phoresis (hour)
				Gel	Electrode		
11S-Globulin	12.5	12	1:1	0.05 M Tris HCl, pH = 8.8	0.025 M Tris-glycine, pH = 8.3	230	1.5

After completion of phoresis, the gel was fixed for 60 min in ethanol, acetic acid, distilled water solution (40:10:60), then it was stained with Kummas G-250 dye for 30–60 minutes, then it was washed 3 times with washing buffer (10% solution of acetic acid).

The results of phoresis were compared with the reference spectrum of 11S-globulin. The frequency of meeting of electrophoretic spectra was calculated as follows by formula $P_i = \frac{n}{N}$, where P_i is the frequency of the spectrum and n is the frequency of the spectrum the number of plants, N is the total number of investigated plants.

Second stage: According to the electrophoresis spectrum of 11S-globulin and the decoded protein formulas, further cultivation of experimental groups was carried out by *in vitro* cultivation (Murashige, 1974). Potato tubers have been planted in a 3:1 mixture of peat. After sufficient growth of the stems, the stems with 3–4 leaf buds were harvested. For the purpose of sterilization, the samples were washed in 70% alcohol for 1–2 minutes, after which were washed with sterile distilled water. Sterilized sprouts were crushed to 0.5–0.8 mm size and placed in test tubes containing 10 mL of MS and various growth promoters. Test tubes with samples were kept in the laboratory under conditions of 27 ± 2 °C and 16 hours a day be illuminated by fluorescent lamps (2,000–3,000 lux).

Third stage: The transplantation of *in vitro* grown plants was carried out in soil (peat, soil and sand: 3:1:3). Ten-twelve-day-old acclimatized plants were exposed to NaCl 50, 100, 150 mmol salt stress within two months. For each version of the saline situation were determined wet weight, dry weight, height, number of tubers, shoot weight, root weight. The plants were watered with different concentrations of NaCl solution at least 1 time a day, in principle, soil moisture should be 50–53%. The genetic-mathematical analysis of the data was performed using the SPSS Statistics software package.

Fourth stage: Molecular-biological researches were carried out. In order to obtain restriction of DNA and use it as an RFLP marker, from plants with different electrophoretic spectra of studied cultivars the following works were performed:

- The extraction of DNA – The extraction of total DNA from studied plants was carried out by the SDS method according to of the guide (Padutov et al., 2007).
- DNA concentration test – The concentration of DNA in the samples was determined with a NanoDrop One spectrophotometer.
- Restriction and ligerization – The restriction of total DNA was performed by preparing a restriction mixture, with the following composition: 2 µl 10x appropriate restriction buffer, 5 µl nucleic acids free deionized water, 2 µl DNA sample, 1 µl (5 units µl) restriction enzyme. The restriction mixture was incubated at 37 °C for 5 hours. Of necessity the restriction process was stopped by adding 1 µl of 0.5 M toluene B (pH = 7.5) to the restriction mixture adding. In order to determine the restriction sequence of DNA and make maps it necessary to cleave complementary DNA with two restriction enzymes, using by Tango universal buffer.
- Electrophoresis – Quantitative DNA's restriction fragments' electrophoretic separation was performed on a 0.8% agarose gel, by Biometra company's Compact M electrophoresis apparatus.

RESULTS AND DISCUSSION

As a cross-pollinated crop, the potato stands out to a high degree with diverse cultivars endowed with genetic and biochemical variation which are seen as complex populations.

According to F. Ayala (1984) the genetic diversity degree can be estimated if protein formulas and populations are known of their occurrence frequency (Ayala, 1984). Diversity of 11S-globulin as a protein biological specificity is manifested as a result of electrophoresis, where amount and electrophoretic mobility of polypeptides are depends on characteristics and origin of genotypes (Barta et al., 2003).

At this stage of research, we aimed to find out the genetic structure of the studied potato varieties according to the electrophoretic spectrum of 11S-globulin (protein formula), because thousands of European varieties and samples of potato have been identified in this way at the International Germplasm Center (Dementyeva, 2006; Ammarelou & Lamei, 2007; Bernal et al., 2019).

The 11S-gobulin electrophoresis spectrum of Impala, Arizona and Madeline potato cultivars was compared with the reference spectrum of 11S-globulin (Fig. 1).

As a result, the protein formulas of the mentioned varieties and their meeting frequency were decoded (Table 2).

The intensity of the protein formula of each polypeptide was evaluated with points: 3 is very intense, 1 is weakly intense.

In the case of Impala (Im) potato's variety, were recorded 4 different electrophoresis spectra of 11S-globulin, which were conventionally designated Im1, Im2, Im3 and Im4, the frequency of them is high (0.18–0.31). The variety is also distinguished by a high total number of polypeptides (48), of which 48% is rated as low intensity, 23% as intense and 29% as very intense (Table 2).

11S-globulin protein of Arizona (Az) potato variety in the selected group is forming 3 types of electrophoresis spectrum (Az1, Az2, Az3), the frequency of them is ranging

from 0.16 to 0.44, total polypeptides quantity is 18 (for each type 6), 2% of which were assessed as low, 55.5% were high and 22.5% – very high intensity.

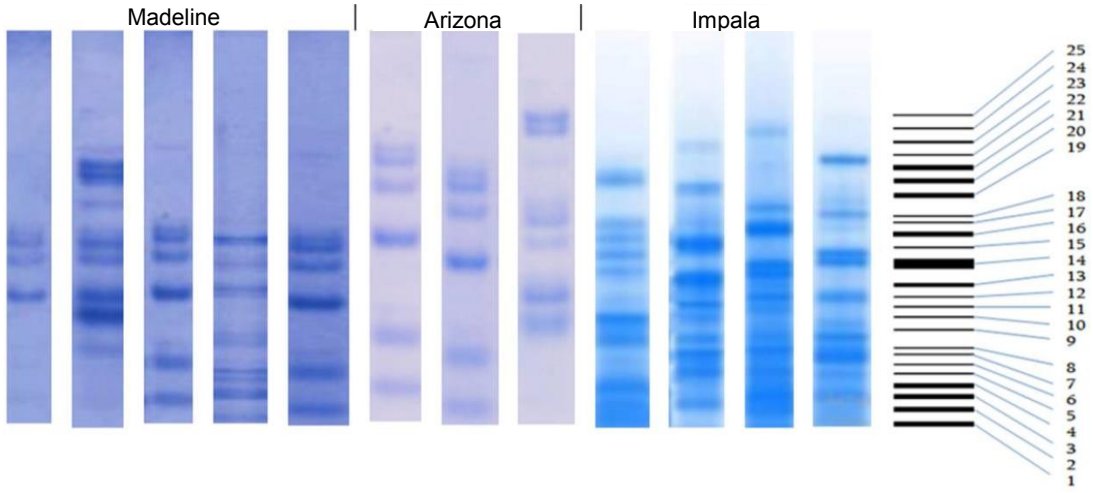

Figure 1. 11S-globulin electrophoretic spectrum of studied potato varieties according to the results of polyacrylamide gel electrophoresis.

The frequency of spectrum meeting in the selected group of Arizona potato variety was 44%, by the way, this index is the highest in the different electrophoresis types of all studied varieties (Table 2).

Table 2. Characterization of Impala, Arizona and Madeline potato cultivars by 11S-globulin electrophoresis spectrum

Varieties	Type of specrum	The frequency of meeting (n)	The number of fractions	Protein formula																							
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Impala (Im)	Im1	0.18	14	1	1	1		1	1	1		1	2		1		3	3	3	3			3					
	Im2	0.23	11		2			2			2	2	1	2		3		3		1		2			1		
	Im3	0.31	11		1	1		1			1	1		1	1	3	3				3						2	
	Im4	0.28	12	1		1		1		2		2		1	2		3	3	1		2			3				
Arizona (Az)	Az1	0.40	6				2					2								3			3		2	2	
	Az2	0.16	6		2					2							3				2	2	1					
	Az3	0.44	6									1			2					1	1						2	2
Madeline (Ma)	Ma1	0.28	6				1			1	1					3	2	2										
	Ma2	0.31	10			1						2		3	3		2	2			1		3	3			1	
	Ma3	0.11	7			2					2					3		2	2	2					1			
	Ma4	0.21	8	1		3		2	3			1				3	2			3								
	Ma5	0.09	8		2			2			1				3		3	3	2					1				

The 11S-globulin storage protein of the Madeline potato variety formed a 5-type spectrum in the electrophoresis field (Ma1, Ma2, Ma3, Ma4, Ma5) and the frequency of meetings this varies from 0.31 (Ma2) to 0.03 (Ma5). The total number of polypeptides was 39. The highest is 10 (Ma2), the lowest is 6 (Ma1), 28% of which were assessed as low, 38.5% as high, 33.5% as very high intensity (Table 2).

In recent years, studies based on protein markers at the A.G. Lorch Russian Potato Research Center prove that intervarietal differences in 11S-globulin spectra are clearly expressed, which makes it possible to easily distinguish the genetic diversity of the variety. In the same center, it was proved that most of the storage proteins present in tubers, due to differences in their molecular mass (35–78 kD), are considered to have slow and medium mobility. Moreover, plants with the same version of the electrophoretic spectrum also have phenotypic uniformity, except for those plants that have undergone somatic mutation and are distinguished not only by morphological indicators, but also by electrophoretic spectrum (Dementyeva, 2006).

In this regard, remarkable results were also recorded during the genotyping of other crops (Sayed Mohammad Reza Khoshroo, 2011; Mouzo et al., 2018; Nasrollahzadeh et al., 2023).

The highest frequency of the 11S-globulin electrophoresis spectrum of the potato varieties studied by us was recorded in the case of the Az3 version of the Arizona variety, it was 44%, and the lowest - in the case of the Ma5 version of the Madeline variety (9%) (Fig. 2).

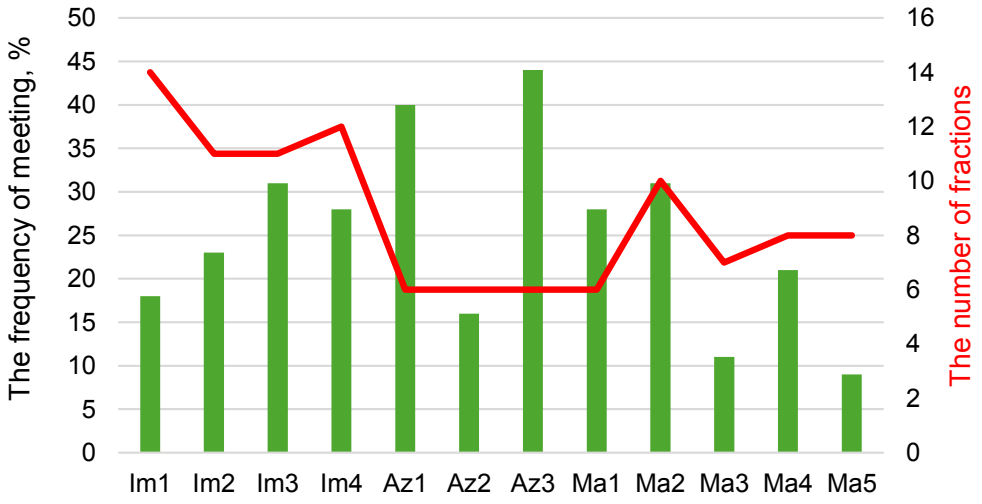

Figure 2. The frequency of meeting and number of fractions of different spectrums of potato varieties: Im 1–4 – Impala; Az 1–3 – Arizona; Ma 1–5 – Madeline.

It is obvious that in the process of evolution, plants have developed protective mechanisms against salt stress, the physiological manifestations of which have a multigenic arrangement (Askari et al., 2012; Askari & Pepoyan, 2015).

In higher plants and a number of microorganisms, the low level of Na^+ in the organism and the maintenance of the normal range of K^+/Na^+ ratio in the cytoplasm of the cells are necessary to increase the salt tolerance of plants (Ketehouli et al., 2019).

It has been established that stress proteins are active at the plasma membrane level (Horie et al., 2009), acting as transporters to remove Na^+ ions from leaves while increasing the movement of K^+ ions to cope with salt stress (Huang et al., 2006; James et al., 2006; Møller et al., 2009).

Several studies have shown that stress proteins are highly selective in transporting K^+ ions instead of Na^+ ions under salt stress conditions. Increasing calcium ion homeostasis helps maintain cytoplasmic properties and increase salt tolerance (Ardie et al., 2009; Bose et al., 2017).

The resistance of different genetic associations to salt stress within the same variety is due to the expression of genes responsible for this salt resistance (Zhang & Shi, 2013).

The studies at this stage aimed to clarify the response of different transplant plants of studied potato varieties to salt stress, taking into account a number of bio-economic characteristics.

From the results of our research, it becomes clear that different genetic associations of the studied potato varieties respond differently to salt stress, bringing some clarity to the circumstances in which the same variety exhibits different reaction rates to abiotic stresses.

Thus, among the plants with electrophoretic Im1, Im2, Im3, Im4 spectra of the Impala potato variety, the highest resistance was recorded in the case of the Im2 version in terms of all the characteristics studied at all stages of salt stress (Table 3).

Table 3. Effect of salt stress on plants with different 11S-globulin electrophoresis spectra of potato variety Impala. Numbers represent means ± standard errors

Impala	NaCl (mmol)	Wet weight (g)	Dry weight (g)	The height of plant (cm)	The number of tubers	The total weight of tuber (g)	The weight of shoot * (g)	The weight of root (g)
Im1	0	81.4 ± 06	15.5 ± 1.4	51.6 ± 0.44	7	63 ± 0.59	31 ± 0.08	8.44 ± 0.19
	50	50.7 ± 053	10.3 ± 0.8	24.7 ± 1.22	5	48 ± 0.67	17.5 ± 0.6	4.21 ± 0.7
	100	29.8 ± 1.48	7.23 ± 0.88	18.8 ± 1.55	2	15 ± 1.32	15.6 ± 1.16	3.91 ± 0.05
	150	10.4 ± 0.78	3.35 ± 1.14	14.2 ± 1.03	0	0	10.5 ± 0.41	1.1 ± 0.21
Im2	0	80.9 ± 0.53	15.31 ± 1.1	51.8 ± 0.29	7	63 ± 0.48	30.88 ± 1.13	8.19 ± 0.14
	50	62.8 ± 1.63	13.35 ± 1.3	42.5 ± 1.22	7	55 ± 1.31	25.1 ± 1.14	5.8 ± 2.8
	100	45.8 ± 0.65	11.55 ± 0.88	29.4 ± 1.66	4	28 ± 1.01	20.1 ± 1.33	3.9 ± 0.6
	150	34.5 ± 1.66	8.30 ± 0.94	18.8 ± 0.36	2	14 ± 1.14	16.6 ± 1.48	1.8 ± 0.1
Im3	0	81.0 ± 0.16	15.33 ± 1.6	50.9 ± 0.18	8	71 ± 0.41	31.8 ± 1.41	8.41 ± 0.19
	50	49.8 ± 0.43	9.68 ± 1.8	25.0 ± 1.13	5	43 ± 0.34	17.1 ± 0.9	4.26 ± 0.28
	100	30.1 ± 1.31	7.24 ± 1.16	19.0 ± 1.36	2	14.8 ± 1.63	15.4 ± 1.08	4.11 ± 0.13
	150	10.0 ± 0.18	3.36 ± 1.18	14.1 ± 1.14	0	0	10.1 ± 0.36	1.8 ± 0.18
Im4	0	81.3 ± 0.61	15.41 ± 1.4	51.1 ± 0.18	7	63 ± 0.43	31.4 ± 0.34	8.40 ± 0.18
	50	47.1 ± 1.36	9.11 ± 0.33	22.2 ± 1.63	4	33 ± 0.54	15.5 ± 0.48	3.91 ± 0.11
	100	26.6 ± 1.31	6.18 ± 1.41	16.2 ± 1.81	1	7.28 ± 0.18	13.4 ± 0.81	2.46 ± 0.24
	150	8.13 ± 0.43	4.36 ± 0.78	10.4 ± 0.31	0	0	7.84 ± 0.63	0.91 ± 0.14

Plants with Az1, Az2, Az3 electrophoretic spectra of Arizona variety showed the same level of trait reduction at all stages of salt stress. No special manifestations of salt resistance were observed in any of the variants (Table 4).

Table 4. The effect of salt stress on the electrophoretic difference of 11S-globulin of potato variety Arizona on plants with spectrum. Numbers represent means ± standard errors

Arizona	NaCl (mmol)	Wet weight (g)	Dry weight (g)	The height of plant (cm)	The number of tubers	The total weight of tubers (g)	The weight of shoot (g)	The weight of root (g)
Az1	0	88.4 ± 0.54	19.5 ± 1.21	48.8 ± 0.54	6	56 ± 1.34	26.16 ± 0.43	6.28 ± 0.18
	50	52.6 ± 1.24	12.34 ± 1.2	20.6 ± 1.12	4	33 ± 0.68	16.4 ± 0.72	3.66 ± 1.49
	100	85.2 ± 1.11	8.28 ± 1.48	16.8 ± 1.63	2	17 ± 0.68	13.3 ± 0.81	3.11 ± 0.36
	150	23.2 ± 0.71	4.85 ± 0.61	10.22 ± 0.43	0	0	11.0 ± 0.81	2.21 ± 0.32
Az2	0	87.8 ± 0.44	18.43 ± 2.16	48.91 ± 0.15	6	56 ± 1.41	25.8 ± 0.14	6.11 ± 0.14
	50	52.1 ± 1.14	11.93 ± 0.88	21.13 ± 1.34	4	32.95 ± 0.54	15.96 ± 0.61	3.13 ± 1.23
	100	34.64 ± 0.53	8.86 ± 1.13	17.0 ± 1.36	2	17.53 ± 0.86	12.95 ± 0.83	3.0 ± 0.66
	150	23.68 ± 0.43	4.17 ± 0.38	10.86 ± 0.94	0	0	10.96 ± 0.68	2.53 ± 0.49
Az3	0	88.83 ± 0.41	18.40 ± 1.63	48.1 ± 0.45	6	55.85 ± 1.33	25.0 ± 0.35	6.8 ± 0.71
	50	53.0 ± 1.04	12.93 ± 1.12	21.63 ± 1.12	4	33.48 ± 0.53	17.1 ± 0.57	3.15 ± 0.68
	100	34.88 ± 0.81	8.11 ± 1.63	16.54 ± 1.33	2	16.6 ± 0.35	13.53 ± 0.63	3.85 ± 0.66
	150	23.0 ± 0.63	4.43 ± 0.61	10.86 ± 0.38	0	0	11.35 ± 0.18	3.0 ± 0.22

Among the plants with electrophoretic spectra Ma1, Ma2, Ma3, Ma4, Ma5 of the Madeline variety, the highest indicators of resistance to salt stress of 50, 100, 150 mmol of NaCl were recorded in the case of the Ma5 variant (Table 5).

Table 5. Effect of salt stress on plants with different 11S-globulin electrophoresis spectrum of Madeline potato variety. Numbers represent means ± standard errors

Madeline	NaCl (mmol)	Wet weight (g)	Dry weight (g)	The height of plant (cm)	The number of tubers	The total weight of tubers (g)	The weight of shoot (g)	The weight of root (g)
Ma1	0	63.35 ± 0.67	16.21 ± 0.38	48.91 ± 0.45	4	35.54 ± 0.68	22.71 ± 0.84	5.88 ± 0.21
	50	51.61 ± 1.31	9.14 ± 0.81	23.68 ± 0.38	3	24.18 ± 0.17	21.5 ± 0.98	4.11 ± 0.38
	100	29.41 ± 0.32	5.39 ± 0.65	12.67 ± 1.18	1	7.84 ± 0.43	15.44 ± 0.64	3.45 ± 0.24
	150	17.44 ± 0.93	3.64 ± 0.38	9.18 ± 0.52	0	0	6.35 ± 0.26	1.58 ± 0.11
Ma2	0	62.88 ± 0.36	15.93 ± 0.51	48.11 ± 0.54	4	35.29 ± 0.44	21.89 ± 0.41	5.36 ± 0.38
	50	49.7 ± 1.32	9.65 ± 0.12	24.1 ± 0.76	3	24.64 ± 0.71	21.88 ± 0.28	3.91 ± 0.28
	100	30.11 ± 0.28	5.77 ± 0.54	12.91 ± 1.28	1	7.59 ± 0.16	16.18 ± 0.48	3.12 ± 0.13
	150	17.68 ± 1.33	3.51 ± 0.49	9.88 ± 0.7	0	0	6.53 ± 0.45	1.31 ± 0.14
Ma3	0	61.38 ± 0.39	15.48 ± 0.61	47.19 ± 0.14	3	26.29 ± 0.15	20.86 ± 0.71	5.11 ± 0.18
	50	48.36 ± 1.26	9.31 ± 0.13	23.18 ± 0.21	2	16.18 ± 0.13	19.64 ± 0.84	4.0 ± 0.13
	100	29.14 ± 0.21	5.11 ± 0.33	11.96 ± 1.01	0	0	15.96 ± 0.58	3.31 ± 0.26
	150	16.38 ± 0.16	2.99 ± 0.08	8.87 ± 0.21	0	0	6.14 ± 0.62	1.48 ± 0.27
Ma4	0	62.5 ± 0.66	15.21 ± 0.43	48.89 ± 0.16	4	35.51 ± 0.16	21.76 ± 0.41	5.81 ± 0.33
	50	49.6 ± 1.52	9.41 ± 0.84	22.86 ± 0.81	3	24.13 ± 0.73	20.89 ± 0.14	4.26 ± 0.36
	100	29.61 ± 0.38	5.13 ± 0.31	12.77 ± 1.13	1	7.68 ± 0.41	14.96 ± 0.63	3.16 ± 0.37
	150	17.13 ± 0.81	3.53 ± 0.44	9.18 ± 0.26	0	0	6.56 ± 0.46	1.38 ± 0.13
Ma5	0	63.84 ± 1.51	16.78 ± 1.11	48.53 ± 0.43	4	35.58 ± 0.36	23.9 ± 0.94	5.51 ± 0.76
	50	56.8 ± 1.23	14.22 ± 0.76	36.1 ± 0.84	4	34.86 ± 0.63	22.81 ± 1.31	4.13 ± 0.24
	100	33.68 ± 1.16	10.11 ± 0.34	22.18 ± 0.64	3	26.11 ± 0.48	16.87 ± 0.68	4.18 ± 0.16
	150	24.65 ± 1.05	8.18 ± 0.51	11.87 ± 0.35	2	13.16 ± 0.71	10.84 ± 1.11	2.65 ± 0.23

Similar results were also reported in the studies of A. Askari. The authors report that under salt stress conditions of 0, 50, 100, 150 mmol of NaCl, Sante, Arinda and Agria potato varieties showed significant intravarietal diversity in terms of a number of bio-economic traits (Askari et al., 2012; Askari & Pepoyan, 2015).

During the selection of breeding material resistant to salt stress, the results of the reaction norm of different fractions of the varieties studied under different concentrations of NaCl are undoubtedly important, considering the number and total weight of tubers as an important bio-economic characteristic (Fig. 3).

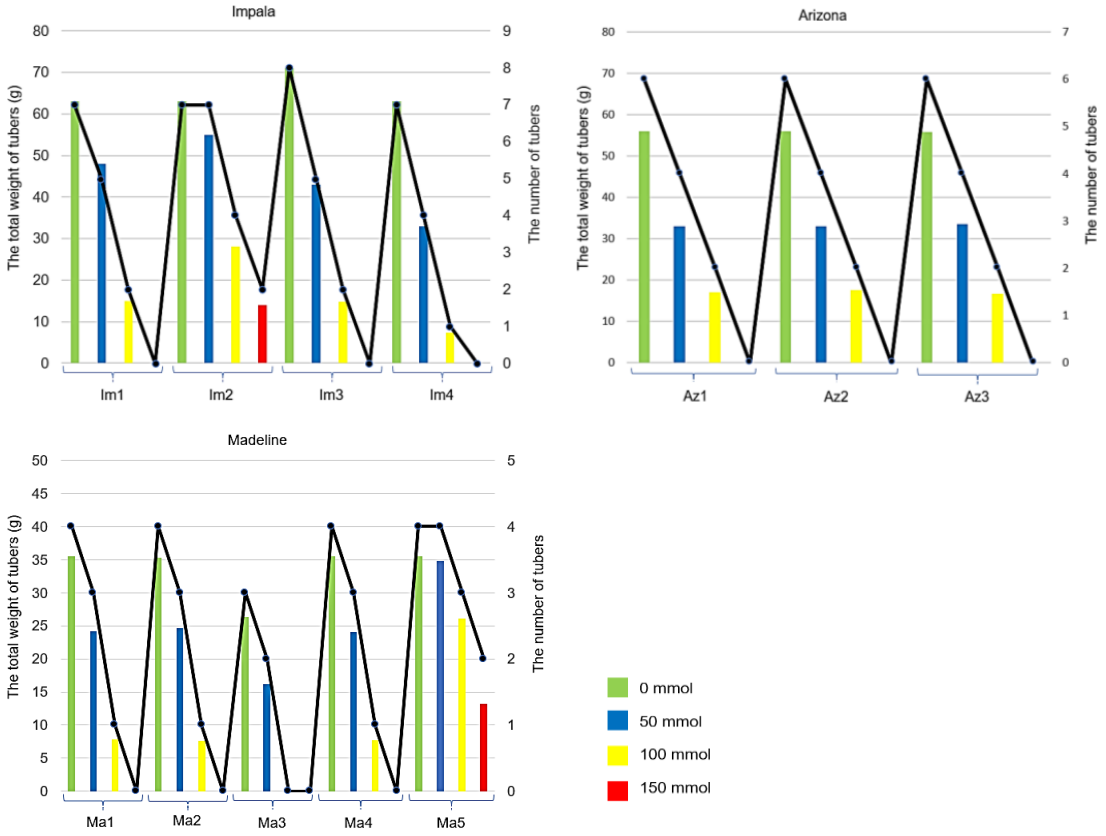

Figure 3. The effect of salt stress on the number and weight of tubers of different potato varieties according to protein spectra.

It is obvious that plant salt tolerance is a complex physiological process, which is regulated by many genes, or at the same time, it has a polygenic arrangement. By the way, as already mentioned, these genes have mutually complementary or additive effects. Therefore, in terms of a specific gene or group of genes selection is impossible, but their presence and location knowledge are very important for marker selection.

For detection the reaction of plants with different electrophoresis spectra of the storage protein 11S-globulin of potato varieties, DNA markers also have a big importance. There are many works aimed at the discovery of genes determining this or that characteristic of potatoes with the help of DNA markers (Shanina & Likhodeyevsky, 2021; Islam & Li, 2023). RFLP (restriction fragment length polymorphism) DNA markers were used in our research.

The study of the length of DNA restriction fragment (RFLP) enables the observation of genetic changes such as deletions and inversions, in which segments of DNA are shortened or lengthened due to the creation or elimination of corresponding sites (Beketova et al., 2021; Gavrilenko et al., 2021).

At this stage of the research, we aimed to construct genomic DNA restriction maps of the studied potato varieties and identify a direct relationship with salt tolerance in order to use it as a marker.

The total DNA restriction maps of plants (Im1, Im2, Im3, Im4) with different 11S-globulin electrophoresis spectra of Impala potato variety are shown in Fig. 4 (a, b, c).

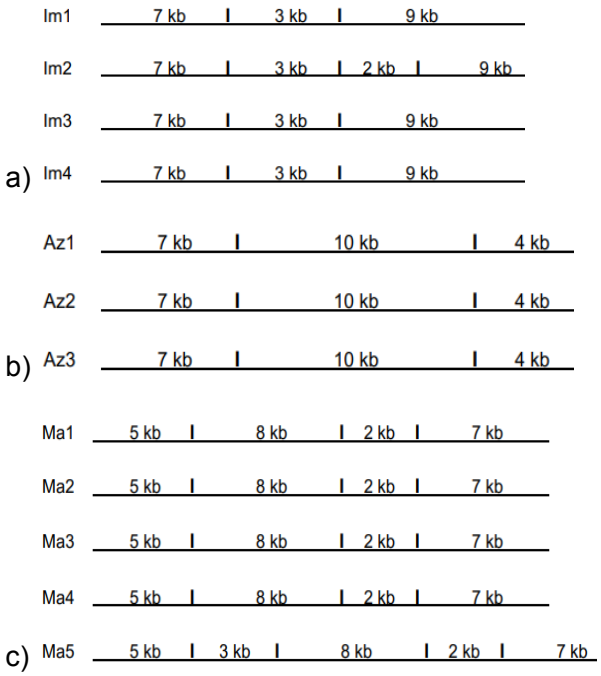

Figure 4. Quantitative DNA restriction fragment map of potato variety varieties according to RFLP analysis: a) Impala, b) Arizona, c) Madeline.

Analysis of DNA restriction maps shows that the total DNA of plants with Im1, Im2 and Im4 variants of the spectrum contains two restriction sites recognized by the *EcoR I* restriction enzyme, generating fragments of 7 and 9 kb in length. By the way, mentioned plants of variants showed similar results under salt stress conditions of 50, 100 and 150 mmol of NaCl (Table 2).

The total DNA of plants with an Im2 electrophoresis spectrum contains three restriction sites recognized by the *Eco*RI enzyme, generating fragments of 7, 3, 2, and 9 kb in length. In this variant, the genome contains a 2 kb mutational fragment, which absent in the restriction map of plants with other electrophoretic spectra. By the way, plants with the Im2 variant have all the characteristics under salt stress showed the highest stability in terms of. Most likely, the genes determining the stress resistance of the Impala potato variety is located in a 2 kb long DNA in the restriction section. Total DNA restriction fragment maps of plants (Az1, Az2, Az3) with different 11S-globulin electrophoresis spectra of Arizona potato variety are depicted in Fig. 4, b.

It is evident from the figure that the total DNA of all plants with different electrophoresis spectra contained 2 restriction sites recognizable by *Eco*RI restriction enzyme, generating restriction fragments of 7, 10 and 4 kb in length. Arizona potato variety Az1, Az2, Az3 variants had the same response to salt stress of 50, 100 and 150 mmol of NaCl, without giving an advantage to any variant. Apparently, the genes forming a certain natural resistance to salt stress are distributed in DNA sections of the specified size, not giving an advantage to any variant. The 11S-globulin electrophoresis spectrum of Madeline potato variety, maps of total DNA restriction segments of plants with variants Ma1, Ma2, Ma3, Ma4, Ma5 are shown in Fig. 4, c.

It is clear from the figure that the genomic DNA of plants with spectrum variants Ma1, Ma2, Ma3, Ma4 contains 3 restriction sites for recognition of the *Eco*RI enzyme, generating 4 restriction segments with lengths of 5, 8, 2 and 7 kb. By the way, the mentioned options plants under salt stress conditions of 50, 100 and 150 mmol of NaCl showed the same dynamics of decrease of the studied bio-economic features. The genomic DNA of plants with the Ma5 variant of the electrophoretic spectrum, in contrast to the other variants, contains 4 restriction sites recognizable by the *Eco*RI enzyme, generating fragments of 5, 3, 8, 2 and 7 kb in length.

From the restriction maps, it can be seen that there is a 3 kb long restriction fragment in the genome of plants of variant Ma5, which is absent in variants Ma1, Ma2, Ma3 and Ma4. It should be noted that the plants of this variant showed the highest resistance to salt stress and a small degree of reduction of the studied characteristics compared to plants of the same variety with other spectra. Most likely, the genes determining the salt tolerance of the Ma5 variant are located in the 3 kb DNA restriction region, which is absent in the other variants.

A group of scientists, while studying the genetic diversity of a number of potato varieties using DNA markers, came to the conclusion that there are always more regions and sites of different sizes in the DNA restriction maps of the most resistant variants to biotic and abiotic stresses. According to the authors, they are the result of aberrations and are directly related to stress resistance (Gavrilenko et al., 2021).

Professional literary sources document that the presence of deletions in the genome of the most stress-resistant forms within different varieties of both potatoes and a number of crops is an evolutionary progress and is directly related to the high expression of stress resistance genes (Bonierbale et al., 1988; Gebhardt et al., 1991, 1995; Pertuzé et al., 2002; Sharma et al., 2013; Gebhardt, 2023).

The results of our research prove that the addition of 2 and 3 kb segments in the DNA restriction maps of the most salt-resistant forms (Im2, Ma5) in different experimental groups of the studied potato varieties is directly related to stress resistance and can be used as markers in the identification of stress-resistant forms.

CONCLUSIONS

The studied potato varieties (Impala, Arizona, Madeline) are not genetically homogeneous. According to the electrophoresis spectrum and protein formula of the storage protein 11S-globulin, they are polymorphic, which is why they showed a different reaction rate to salt stress.

All described genetic associations had similar sizes of genomic DNA restriction fragments, except for the variants that showed the most pronounced salt tolerance.

The results of the research can be used as a guide in the breeding process, when obtaining new salt-resistant potato varieties and improving the existing ones.

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