Research Report on

Development of high yielding drought tolerant potato cultivars with good quality and complex resistance to important local diseases.
INN 139993

Introduction:
The potato is the third most important food crop in the world. However, its successful cultivation is threatened by sensitivity to abiotic (heat, drought, uneven water supply) and biotic stresses like infection by viruses, fungal pathogens, bacteria and pests like nematodes. Stress sensitivity may manifests in yield decrease (lower tuber number and tuber weight/plant) and quality loss (tuber malformations, internal defects, higher sugar or alkaloid content, etc.). Naturally different potato genotypes (released varieties, breeding lines) may dramatically differ in biotic and abiotic stress sensitivity. Among biotic stresses viruses, especially the Potato Virus Y (PVY) and potato late blight caused by Phytophtora infestans has the highest influence on yield. That is well-known also
that virus infected plants are even more sensitive to abiotic stresses. Under environmental conditions where abiotic stress and high virus pressure parallel appear their combined negative impact on potato production is even more dramatic. Ecological conditions of the Carpathian Basin and main potato production areas of Iran is just like that. To secure potato production in a wider range of environments and increase the yield stability in terms of quantity and quality resistance to abiotic and biotic stresses is necessary. Cultivation of resistant varieties are the most cost-effective and reliable approach to control pathogens and pests and prevent yield and quality losses. Introgression of resistance genes to main potato viruses from wild species to cultivated potato has been successfully achieved by classical breeding programs. One of the most successful resistance breeding programs in terms of combination of cultural quality traits with complex biotic and abiotic resistance is the Hungarian program operated by the Potato Research Station of MATE since 1960. Based on the success of its 60 years long resistance breeding program its released varieties and advanced parental lines can play as source of resistance genes in new research project aiming to develop new advanced breeding lines or varieties. At the Potato Research Centre DNA marker-based selection systems were developed to select potato
genotypes carrying different resistance genes. For example, tightly linked markers to Rysto PVY extreme resistance gene were identified or Rx1and Rx2 marker to detect PVX resistance genes. Adaptation of previously published linked marker to the cyst nematode resistance gene H1 was also successful. So the uniqueness of the project came from the combination and use of the decades long experience and genotypes of the resistance breeding program of Hungary with the capacity for phenotypisation under stressful Iranian conditions and expertise of both parties in the application of markers assisted selection in potato breeding.

During the duration of the research program, many problems made it difficult to achieve the set goals and to carry out the planned research tasks. Several significant changes had taking place in relation to the research project. The ownership of the Potato Research Centre was changed two times. First it was taken over by Szent Istvan University from University of Pannonia. Later Szent Istvan University was turned into Hungarian University of Agriculture and Life Sciences. This last step was also accompanied by the change of the university's operating form, which meant the transformation of a state-run university into a foundation-run university. During these processes the person of project leader was changed two times. Dr. Zsolt Polgar who started the project had to leave Pannon University soon after its start. He was replaced by Dr. Istvan Cernak for the coming 2,5 years. After the establishment of Hungarian Univ. of Agric. and Life Sciences Dr. Polgar took over the project again for the remaining 1,5 year. All these changes made it necessary to extend the term. These facts and the intervening global virus epidemy, as well as the international political/economic situation during the last year of the project significantly

hindered and slowed down the professional work. During the project 3 researchers and 2 technicians joined to the project.

Goals:
The main goal of the project was by the cooperation of Hungarian and Iranian partner to develop and characterize breeding lines and variety candidates having high yield and quality stability under environmental conditions unfavourable to potato production due to their complex resistance to biotic and abiotic stresses. To reach these goals we planned to utilise the combination of traditional and modern molecular genetic methods. After successful development and identification of such genotypes (breeding line or variety candidates) we planned to introduce them into the official variety registration process of new varieties.

Results:

The research program is basically went on two levels, on the level of the traditional breeding program and the molecular genetics program parallel in Hungary in Iran.

I. Traditional breeding programme:

We completed the research project tasks as part of the Keszthely potato breeding program. Basically, the entire breeding program that takes place at Keszthely run in an ecological environment that is not ideal for the potato. The soil of the breeding garden is medium-heavy brown forest soil, the area is not irrigated. In general, but especially during the duration of the project, anomalies characteristic of the climate of the Carpathian Basin prevailed, such as the high number of hot days above 25 C°, which are already unfavourable for potatoes, the uneven water supply, or even the continuous drought. As a result, since these conditions existed continuously made it possible to identify genotypes sensitive or resistant to biotic and abiotic stresses and with regard to the entire breeding material.
During the five-year duration of the project, we continuously carried out the research processes necessary to widen the genetic variability, identify, characterize and select the desired genotypes. Every year we carried out new parental crossings, sowing of seeds and raising of seedlings, carried out the phenotypic characterization of the single hill, clones "A, B, C and D" in the field for: skin colour, flesh colour, eye depth, skin quality, determination the length of vegetation period, carrying out pathological observations (for viruses, phytophthora, alternaria, fusarium , scab). Every year, we performed the kitchen technology and quality tests of clones A, B, C and D (variety candidates). We determined the starch content of the breeding lines, and determined the raw, cooked and fried quality and discoloration tendency by testing raw, cooking and frying discoloration. For clones C and D, these quality tests were performed twice a year, immediately after harvest and after 3 months of cold storage.
In the case of the most important variety candidates and the parental lines produced in order to pyramid the resistance genes, resistance was determined by provocation tests, after artificial infection with PVY and PVX viruses and the potato late blight pathogen *P. infestans*. The level of resistance against two quarantine pathogens, the potato cyst nematode (*G. rostochiensis*) and potato wart (*S. endobioticum*), was performed by a Polish research institute specializing in such tests. In the case of the D clones (variety candidates), we determined their productivity, yielding potential over two years in a four-repetition small-plot field experiment.

In the following, we present some examples of the results of these experiments:

Table 1. The scale of the breeding program during the project.

	Crossings	Families	F1 seedlings	Single hills	A clones	B clones	C clones	D clones variety candidates
	No.							
2018	34	75	9680	10658	436	49	13	13
2019	28	45	12680	16337	256	38	21	15
2020	24	43	10544	3698	226	42	22	19
2021	25	83	6800	7752	232	40	14	24
2022	17	74	6920	7566	232	14	7	12
	128	**320**	**46624**	**46011**	**1382**	**183**	**77**	**83**

Table 2. Phytophtora resistance test results of breeding lines.

	2020				
Breeding lines	**10.aug**	**18.aug**	**25.aug**	**02.szept**	**07.szept**
WLxMira1	0	0,025	0,05	5,125	11,375
WLxMira2	0	0	0,025	0,005	0
WLxMira3	0	0	0,025	0,2525	3,75
WLxMira4	0	0	0	0	0
VénuszxMira1	0	0,025	0,075	12,625	14
VénuszxMira2	0	0	0,025	0,0075	1,5
VénuszxMira3	0	0,025	0,025	8,34	43,75
Bintje	0,0325	0,325	1,325	37,5	85
Alpha	0	0,525	0,65	25	57,5
Mira	0,0025	0,2	0,3	0,833333	16,25
Gloria	0,0525	2,75	2,875	33,75	78,75
Escort	0,0075	2,875	2,875	17,5	35
White Lady	0	0,05	0,1	11,2525	30,125
Eersteling	0,0275	1,425	1,525	30	71,25
Robijn	0,0025	0,05	0,15	1,625	10,25
07.258	0,1275	26,25	35	80	87,5
14.21	30,125	52,5	57,5	70	86,25
14.149	0,0275	0,65	3,925	32,5	65
16.20	0,275	10,275	32,5	56,25	85
16.213	0,0275	0,525	5,75	38,75	65
16.425	0,3775	10	15	47,5	73,75
17.403	0	0,1	2,675	4	50
17.208	0,2525	0,425	0,55	7,5	23,75
17.235	0,0075	5,15	5,15	17,5	20
17.279	0,1275	0,375	3,875	28,75	45
09.688	0,0025	0,65	1,025	22,5	41,25
09.200	0,0025	0,175	0,2	4,125	35
10.437	0,0025	0,4	0,525	12,5	63,75
J101K23	0	0	0	0	0
Vénusz	0	0	0	0,05	2,5
SzD5%	***9,5***	***7,94***	***11,85***	***19,7***	***23,57***

Figure 1. Nematode resistance test results of breeding lines.

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OFFICIAL RECORD
OF ACCEPTING AN INVESTIGATION WORK

Report from realization the work was accepted 19.01.2022.

Title of the work:

Laboratory assessment of the resistance of cultivars and breeding materials of potato to *Globodera rostochiensis* (Wollenweber) and *Globodera pallida* (Stone).

Twelve lines of potato were assessed to pathotype Ro1 of *Globodera rostochiensis* resistance in biological tests according to UE protocol PM3/68.

Results are presented in table:

Number	Sample	Resistance score
1	17.91	9
2	17.140	1
3	17.151	1
4	17.208	1
5	17.235	1
6	17.279	9
7	17.306	2
8	17.318	9
9	17.348	1
10	17.380	1
11	17.384	1
12	17.389	1

Table 3. Potato warts resistance test results of breeding lines.

Laboratory assesment of breeding lines of potato to pathotype 1(D1) of Synchytrium endobioticum in 2021 U. of Pannonia, PRC, Keszthely

No	Line	Rotten aye peices	No reaction	Laboratory assessment					Total	Remarks
				Resistant			Susceptible			
				1	2	3	4	5		
1	17.91			2	1		2		5	S1
2	17.140			2	3				5	R1
3	17.151			3	2				5	R1
4	17.208			3		2			5	R2
5	17.235			2	2	1			5	R2
6	17.279					5			5	R2
7	17.318							5	5	S2
8	17.348					5			5	R2
9	17.380					1	4		5	S1
10	17.384			5					5	R1
11	17.389			5					5	R1
12	17.306						5		5	S1
		0	0	22	8	14	6	5	55	

As it can be seen from Table 1, in total we produced and examined 83 variety candidates during the project running period for the most important characters. Due to its favourable properties, we have so far introduced one variety candidate, breeding line number 09.688, for official variety registration under the name Balatoni sárga. Naturally, in the coming years, several new candidate varieties are expected to be identified among the many candidate varieties currently under investigation. Among them, the most promising lines are lines 10.437, 14.21 and 16.236. Of those, the line 10.437 was identified as potential new variety in Iran as well.

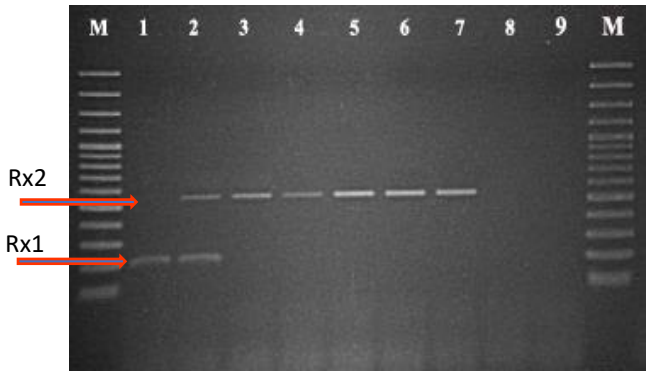
Description of the variety candidate Balaton sárga:

Maturity: Middle
Tuber: Oval, medium-sized, yellow skin and flesh, shallow eyes.
Number of tubers: 12-14 pcs.
Dry matter content: Medium.
Foliage: Medium strong with medium green leaves.
Inflorescence: White
Resistances: Immune to PVY and PVA. Highly PLRV resistant. Moderately resistant to potato blight and scab. Nematode resistant. Good heat and drought tolerance.
Quality: For fresh use and processing. "B/C" cooking type. Free from fresh and after cooking discoloration. Excellent taste.
Production and storage characteristics: Productivity 55-60 t/ha. Medium dormancy. Stores well.

II. Molecular genetic program

The effectiveness of the traditional breeding program can be significantly increased by using genetic markers linked to the genes of individual resistance traits during the selection process. In recent years, genetic markers related to resistance genes against potato Y and X viruses, potato late blight and nematode infection have been developed or adapted from the literature (Rysto, Rx1 and Rx2, R1, R2, R3a, R3b and H1) at the Potato Research Institute. During the project, we implemented the practical application of these markers in the breeding program, and optimized the methodology required for the detection of the markers, so that the probability and reliability of the detection in most cases is close to 95-98%. During the project, we determined the presence of the Rysto, Rx1, Rx2, H1 and R3a genes in more than 1,000 individuals in a total of ten segregating families.

Figure 2. Detection of Rx1 and Rx2 PVX resistance genes in breeding lines.



III. Testing of Hungarian varieties and breeding lines in Iran, by SPII.

At the beginning of the program, the following potato genotypes were sent to the Iranian partners in form of mini tubers and in vitro plantlets:
Varieties: Balaton Rose, Arany Chipke, Basa, Botond
Breeding lines: 87.3143, 98.120, 00.182, 01.536, 10.07, 02.363, 09.688 and 10.437
The research program for the Iranian partners lasted for the originally planned 3 years and ended with a final report in 2020.
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Due to the reorganization of the university system and the two times change of project manager some difficulties mentioned in the introduction as well as due to the worldwide Covid-19 epidemic, only one of the planned mutual visits could took place from each of the sides. The Iranian colleagues visited Keszthely in the first year of the project in 2018, while the Hungarian researchers were able to visit the Iranian partners in the last month of the extended term, right before the end of the professional work. During this last visit, Iranian colleagues reported that genotypes received at the beginning of the program, were propagated for the first time, then they were placed in small-plot experiments in five potato-growing regions of Iran for two years. Crosses were also made with them resulting new breeding lines between the two breeding program. Their results confirmed the high virus resistance and environmental stress tolerance of Hungarian varieties and breeding lines. In terms of productivity and environmental adaptability, varieties Balatoni rózsa and Botond, as well as the candidate variety 10.437, were found to be the most outstanding. During the visit of the Hungarian breeders, it was also

possible to see some of the F1 families that originate from the crossing with the Keszthely lines. On one of the days, we were also able to participate in the field evaluation and selection of these hybrid seedlings and "A" clones.
After this re-contact, there was hope that the joint work could continue, either in the form of a new bilateral project or on its own. The continuation of this project can be the further testing of developed new the breeding lines created on a common genetic basis to the level of candidate varieties, as well as the Iranian variety registration of Hungarian varieties that have also been found successful in Iran.

Summary

Despite the many difficulties that arose during the project, the extension of the project's duration and the re-engagement of the supervisor who initiated the research made it possible to successfully complete the research tasks. New breeding lines with complex resistance based on a common genetic basis were produced. The characterization of Hungarian varieties under Iranian conditions has begun. In the breeding program in Keszthely, the production and testing of new variety candidates was successfully carried out, including selection based on genetic markers as one of the most modern molecular genetic methods. Using the results of the program, a new variety candidate was registered in the Hungarian state registration trials, under the name Balatoni sárga.