

Crossing of potato in Tajikistan

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ABSTRACT

This article shows that in conditions of high mountains of Tajikistan (at height more than 2700 m above sea level), fertility of pollen grains of *Solanum tuberosum* L. varies from 5 to 97%. Among clones of potato studied, only 5 to 9.7% had viable pollen grains. Also, in conditions of a mountain zone, big polylythism was established for qualitative traits such as flower coloring, anther coloring and forms of stamen columns of flower of potato. As a result of carrying out crossing of varieties of potato, more than 6 kg of hybrid berries F₁ were obtained. These hybrid seeds will be used in breeding and genetic research works in the future.

Keywords: Breeding, crossing, pollen vitality, hybridization, clone, Tajikistan.

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INTRODUCTION

Potato tubers are valuable food products for most people in the world, and in many regions are considered a "second bread". The agricultural environment of mountainous areas of Tajikistan situated at an altitude of more than 1800 meters above sea level, make it possible to grow good and quality harvest of potato. Under these cool mountainous conditions, potato plants are of little affection, have intensive bloom, and form a lot of berries and botanic seeds.

During the researches made in different soil and climatic conditions a number of scientists (Perlova, 1958; Balashev, 1968; Lebedeva, 1970; Bukasov and Kameraz, 1972; Yashina et al., 1983; Mumindjanov, 2003; Shpaar, 2004; Kiru, 2007; Partoev et al., 2007; Carli et al., 2008; and Partoev et al., 2008) have ascertained the significance of breeding pair selection and locality of crossbreeding, so as to get good results of potato hybridization.

According to Mendiburu and Peloquin (1976), Frankel and Galun (1977), Kaushik et al. (1996), Gopal (1994), Kumar and Gopal (2003), Pandey et al. (2005), Gupta et al. (2004) and Luthra et al. (2006) from India, success of selective and genetic work aimed at nurture of new perspective grades, depend to a large extent on pollen grain fertility and vitality when conducting different crossing between grades and species of potato.

Thus, on basis of above researchers' work analysis and our experience on potato crossbreeding obtained in India

this year, for the first time in the highland of Tajikistan we have begun to research the rate of pollen grain fertility and to cross different grades and hybrids of potato and the results of this researchers brining in this brochure.

MATERIALS AND METHODS

To determine the vitality of potato pollen grains, the following types of work were prepared and carried out:

1. Make acetocarmine solution, dropping bottle, forceps and needle ready for potato pollen release.
2. Remove flower pistil using forceps.
3. Pour out the pollen on the slide in the acetocarmine drop by shaking the stamen filament of the flower.
4. Cover the acetocarmine and pollen mixture with a glass sheet.
5. The ready preparation is to be examined through the microscope in 3 to 5 minutes.
6. The quantity of acetocarmine coloured pollen grains (both fertile – vital pollen grains and yellow pollen grains – sterile or unviable grains) should be counted in three or five microscopic fields.
7. The counted quantity of coloured and uncoloured pollen grains is to be recorded into the log book.

Pollen vitality was analyzed in three terms: on July 20 (the beginning of blooming period), on August 1 (mass blooming period) and on August 10 (the end of blooming period) in 2009. In each term the pollen of five plants of each sample was taken, 15 plants in total. Fertile (vital) and sterile (unviable) pollen grains were counted in three microscope fields with increasing of 8x7 on the microscopes MBC-9 and MBC-10 and with increasing of 20x7 on

the microscope MBU-4A.

As a material for research, 62 clones and varieties of potato from the International Potato Center (CIP) in the network of plant gene pool investigation in the republics of Central Asia and the Caucasus were used. Also the collection of potato samples were obtained from the Institute of Plant Physiology and Phyto-genetics, Academy of Sciences, Republic of Tajikistan and Social Organization "Tukhmiparvar", from VIR in 90th years of the last century. Planting was carried out on March 13, 2009 at an altitude of 2700 meters above sea level, in the field station of Social Organization "Tukhmiparvar" situated in the Jirgital region, Sari-Kendja village, Muksu jamoat, Republic of Tajikistan.

Also straight and back crosses of different potato grades and species were carried out in order to obtain first generation hybrids. For conducting potato grades crossbreeding after 3 p.m. emas-culation of maternal plant flowers (removal of flower stamen filament with forceps) was made. After 4 p.m. we plucked paternal plant flowers, removed their pistils and kept them in a Petri dish at the room temperature from 4 p.m. to 9 a.m. of the next day.

In the morning, inside the room we shook stamen filaments with anthers using forceps and needle and picked the pollen into the glass Petri dishes of small size. The picked pollen was used for pollination of emasculated flowers by touching the pistil head with the picked pollen of paternal flowers. After pollination we attached a

paper label indicating hybrid combination (maternal form x paternal form) and the date of crossbreeding written in pencil to the stem. The number of pollinated flowers was recorded into the potato crossbreeding log book in compliance with the dates of pollination. All statistical dates (X, S, S_x , V, %, LSD_{05}) processing were done according to Dospekhov (1985).

RESULTS AND DISCUSSION

Investigations concerning identification of potato pollen grain fertility have been conducted in Jirgital region at an altitude of 2700 meters above sea level where an experimental plot of the Social Organization "Tukhmiparvar" and the Institute of Plant Physiology and Phyto-genetics of the Academy of Sciences of the Republic of Tajikistan. Fertile (vital) potato pollen grains placed in the acetocarmine drop are coloured and turned red. As to sterile pollen grains, they are not coloured and will have yellow colouring. Their number can be easily counted through the microscope (Figure 1).

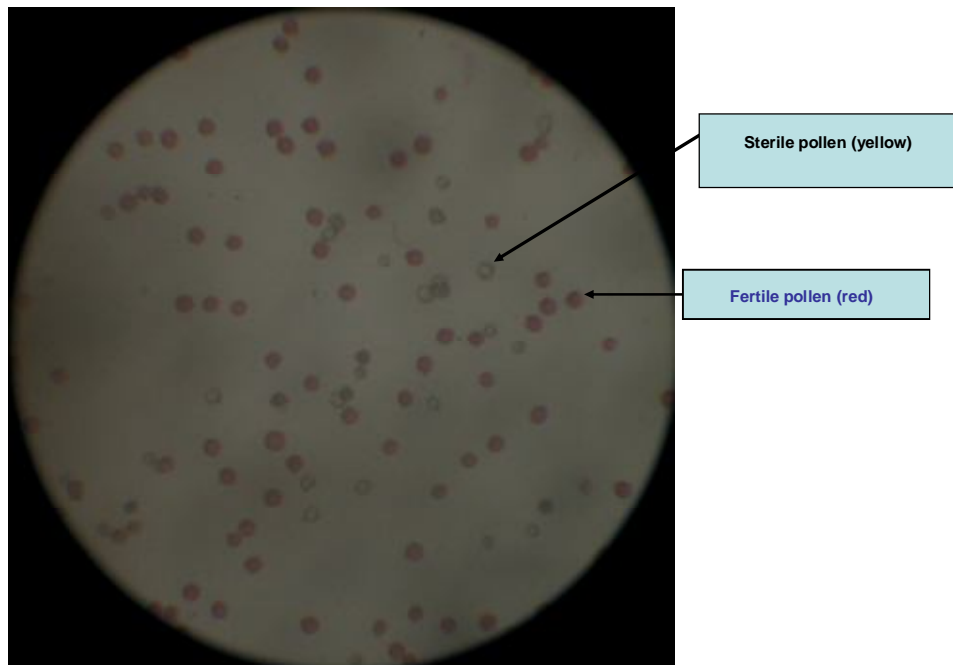


Figure 1. Sterile (yellow) and fertile (red) potato pollen grains indicate the magnification.

Pollen grain fertility of potato clones and grades in highland environment of our republic is genotypically specific. Most of the examined clones and grades have more than 80 to 97% of fertile (vital) pollen grains. At the same time it is necessary to note that pollen grain fertility of some potato grades makes only 5 to 10% (Table 1).

Among potato varieties cultivated in the mountainous area, Cardinal grade had the lowest percentage of fertile

pollen grains with an average of 26.5%, while the new perspective Dusti grade had the highest (95.2%). This grade was raised by the scientists of the Institute of Plant Physiology and Phyto-genetics, Academy of Sciences, Republic of Tajikistan; the Social Organization "Tukhmiparvar"; and the International Potato Centre (CIP). This year the grade has been given to the Tajik State Commission on quality testing and grade

Table 1. Percentage of fertile pollen grains of potato grades in highland environment (Jirgital region at an altitude of 2700 meters above sea level).

No.	Varieties of potato	Sampling dates			Mean
		Jul. 20	Aug. 1	Aug. 10	
1	Cardinal	28.0	24.4	27.1	26.5
2	Zarina	79.2	83.0	84.0	82.1
3	Dusti	95.0	96.6	94.0	95.2
4	Faizabad	85.9	86.0	84.1	85.3
5	Zhukovski	85.7	93.3	90.3	89.8
6	Condor	83.0	86.3	85.0	84.8
7	Picasso	86.4	85.2	84.1	85.2
8	Hybrid 22	84.5	87.5	90.4	87.5
9	Hybrid 23	64.7	74.9	98.2	79.3
10	Clone 27/5	64.7	63.5	63.6	63.9
11	Clone 30/9	76.7	77.0	78.9	77.5
12	Clone 36/6	66.7	68.3	70.3	68.4
13	Clone 37/2	84.1	86.2	93.8	88.0
14	Clone 40/1	79.3	81.0	87.3	82.5
15	Clone 47/4	93.4	91.5	85.9	90.3
16	Clone 47/8	83.3	85.1	89.8	86.1
17	Clone 47/11	91.7	90.2	89.7	90.5
18	Clone 50/7	85.9	85.5	86.7	86.0
19	Clone 50/9	87.4	87.6	84.1	86.4
20	Clone 52/6	75.8	73.6	74.6	74.7
21	Clone 1	94.4	88.4	97.5	93.4
22	Clone 2	5.6	7.3	6.7	6.5
23	Clone 3	5.3	6.0	8.0	6.4
24	Clone 4	62.0	63.0	60.5	61.8
25	Clone 7	5.7	5.2	6.3	5.7
26	Clone 8	95.0	97.9	98.0	97.0
27	Clone 11	11.5	12.0	11.3	11.6
28	Clone 13	95.7	97.5	90.0	94.4
29	Clone 14	10.9	11.6	8.0	10.2
30	Clone 15	5.6	7.2	7.3	6.7
31	Clone 18	81.0	80.5	75.7	79.1
32	Clone 21	87.2	94.7	82.7	88.2
33	Clone 22	85.8	84.9	91.5	87.4
34	Clone 24	47.5	38.8	42.6	43.0
35	Clone 25	94.5	98.0	90.9	94.5
36	Clone 26	75.4	73.1	75.1	74.5
37	Clone 27	90.0	88.6	92.1	90.2
38	Clone 40	32.0	35.9	33.8	33.9
39	Clone 42	73.0	70.8	72.9	72.2
40	Clone 43	83.5	84.8	75.6	81.3
41	Clone 45	96.8	88.9	86.2	90.6
42	Clone 48	5.7	4.8	5.6	5.4
43	Clone 50	9.2	10.0	9.0	9.4
44	Clone 53	95.7	97.1	84.2	92.3
45	Clone 54	89.3	87.8	94.0	90.4
46	Clone 55	90.7	89.9	90.5	90.4
47	Clone 56	95.2	97.5	96.8	96.5
48	Clone 58	10.6	9.1	9.3	9.7
49	Clone 59	88.4	86.0	93.0	89.1

Table 1. Continues.

50	Clone 60	87.6	89.4	88.1	88.4
51	Clone 63	88.8	82.8	92.1	87.9
52	Clone 64	5.0	6.6	7.0	6.2
53	Clone 65	5.2	4.7	5.4	5.1
54	Clone 66	96.6	95.0	94.0	95.2
55	Clone 67	6.0	7.1	7.1	6.7
56	Clone 68	23.6	25.0	25.0	24.5
57	Clone 71	23.9	21.0	19.8	21.6
58	Clone 73	78.0	76.0	76.5	76.8
59	Clone 75	95.4	94.9	91.9	94.1
60	Clone 76	97.9	85.6	81.1	88.2
61	Clone 79	95.3	89.0	85.4	89.9
62	Clone 80	13.0	11.8	13.0	12.6
X		64.9	64.7	64.9	64.8
S		34.3	34.2	34.3	34.2
S _x		4.3	4.2	4.2	4.2
V, %		52.9	52.8	52.6	52.5
LSD ₀₅		8.6	8.4	8.6	8.5

**a****b****c****Figure 2.** Different shapes of flowers, stamen filaments and pistils of potato clones: (a) # 43, (b) # 48 and (c) # 54.

Table 2. Pollen grain fertility (%) in potato clones with different shape of the flower stamen filament.

No.	Varieties of potato	Analysis implementation dates			Mean
		Jul. 20	Aug. 1	Aug. 10	
1	Clone 43 (plants with regular stamen filament)	83.5	84.8	75.6	81.3
2	Clone 43 (plants with modified shape of stamen filament)	45.7	50.1	47.5	47.8
3	Clone 48 (plants with modified shape of stamen filament)	5.7	4.8	5.6	5.4

Table 3. Number of pollinated flowers and set berries obtained through potato hybridization (2009).

No.	Maternal form	Paternal form	Number of pollinated flowers	Date of crossing	Number of gathered berries	Rate of berry formation
1	Clone 75	Kufri Sadabakhar	18	Jul.19	0	0
2	Clone 76	Kufri Jirgari	48	Jul.19	18	37.5
3	Clone 66	Kufri Jiota	30	Jul.19	10	33.3
4	Khahadin	Kufri Arunda	38	Jul.19	3	7.9
5	Clone 75	Kufri Anchan	15	Jul.19	0	0
6	Clone 75	Kufri Suraya	34	Jul.19	6	17.6
7	Clone 59	GTH/107	26	Jul.19	0	0
8	Clone 66	Kufri Suraya	7	Jul.19	2	28.6
9	Clone 76	Mix of pollen	29	Jul.19	5	17.2
10	Clone 68	Mix of pollen	14	Jul.19	0	0
11	Clone 63	Mix of pollen	12	Jul.19	3	13.6
12	Clone 75	Clone 76	25	Jul.19	6	24.0
13	Clone 76	Clone 75	22	Jul.19	8	36.4
14	Clone 79	Clone 66	7	Jul.19	0	0
15	Zhukovski ranniy	Dusti	72	Jul. 21	0	0
16	Dusti	Zhukovski raniy	15	Jul. 21	0	0
17	Cardinal	Dusti	70	Jul. 21	3	43.0
18	Dusti	Cardinal	20	Jul. 21	0	0
19	Dusti	Condor	191	Jul. 21	83	43.5
20	Zarina	Dusti	40	Jul. 21	0	0
21	Dusti	Picasso	44	Jul. 21	17	38.6
22	Hybrid 23	Dusti	23	Jul. 21	0	0
23	Clone 40/1	Dusti	22	Jul. 21	1	4.5
24	Clone 40/1	Cardinal	17	Jul. 22	0	0
25	Hybrid 22	Dusti	11	Jul. 23	0	0
26	Hybrid 23	Picasso	53	Jul. 23	0	0
27	Faizabad	Picasso	127	Jul. 23	41	32.3
28	Cardinal	Condor	125	Jul. 31	88	70.4
29	Cardinal	Picasso	105	Jul. 31	84	80.0
30	Clone 48	Dusti	65	Jul. 31	2	3.1
31	Clone 40/1	Dusti	22	Jul. 31	16	72.7
32	Cardinal	Dusti	50	Aug. 1	0	0
33	Hybrid 23	Dusti	85	Aug. 1	0	0
34	Clone 40/1	Dusti	30	Aug. 1	6	20.0
35	Zarina	Hybrid 23	25	Aug. 1	2	8.0
36	Dusti	Condor	85	Aug. 11	53	63.4
37	Clone 48	Condor	28	Aug. 11	14	50.0
	Total:		1650		471	28.5

protection.

It should be noted that some clones sampled individually from hybrid swarm F₁ and propagated from one plant by culture method (*in-vitro* plants), had stamen filaments of different shapes. For example, clones 43, 48 and 54 had different morphological attributes of stamen filaments (Figure 2). Clone 43 had plants with two types of stamen filament shape. One part of the plant had a regular stamen filament; the other part of the plant had a modified stamen filament when stamen filaments are not pressed to the pistil stem as it is with regular plants. They are detached from it and look friable.

Thus, according to the stamen filament shape among these new potato clones, we can observe definite variation. It should be noted that the rest of the morphological traits (plant and flower colour, leaf shape, evolution phenological stage and tuber colour of the examined clones) remained stable. In addition, the our researches indicated that the plants with modified shape of the stamen filament have weak yellow colour of the stamen filament and low fertility of pollen grains compared to regular plants (Table 2).

Plants with modified shape of stamen filament have considerably less fertile pollen grains compared to those with regular stamen (Table 2). The sign of cytoplasmic male sterility is greatly detected with clone 48 where pollen grain fertility was only 5.4%. Along with it, we have ascertained that although many potato clones have regular stamen filament shape and bright yellow colouring of the stamen filament and anther, their index of fertility is very low. They are clones 2, 3, 7, 15, 50, 58, 64, 65, and 67 that have only 5.1 to 9.7% of fertile pollen grains. These clones can serve as a good base line for potato breeding through straight crossing (without anther removal), and for obtaining botanic seeds without flower emasculation. Thus, specific potato clones with male sterility sign have been defined.

As a result of potato hybridization carried out in Jirgital region from July 19 to August 11 (1650 crosses) by early September (Table 3) regular berries F₁ (Figure 3) were obtained, which seeds will be studied in the future.

CONCLUSIONS

For the first time, study of pollen grain fertility and hybridization of potato varieties has been carried out in the mountainous area of Tajikistan.

The degree of pollen grain fertility and the variability of this genetic trait among 62 potato samples have been determined (5 to 97%).

Some potato clones have great variability based on the flower and anther color as well as on the shape of the stamen filament.

Ten potato clones were selected based on the low quantity of fertile pollen grains (5.1 to 9.7%).

Potato variety Cardinal grade showed the lowest percentage (26.5) of fertile pollen grains, while Dusti



Figure 3. Potato hybrid berries, obtained through Dusti x Picasso grades crossing.

grade showed the highest (95.2%).

Six kg of hybrid berries were obtained from crosses of potato varieties and hybrids.

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