BEHAVIOR OF CROSSES AMONG POTATO (*S. tuberosum* L.) TETRAPLOID PARENTS TO BE USED IN A CUBAN TRUE POTATO SEED PROGRAM

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ABSTRACT. Six intra-specific crosses among three commercial varieties and four clones from the International Potato Center (CIP) were studied under greenhouse conditions for two years. The aim of the work was to evaluate the behavior of the parents at the crossing time and during true potato seed stage to be used as parents in a true potato seed program. Percentages of pollen viability and germination, fruit set, total seed number, seed number per fruit, as well as berry length and diameter, were evaluated. Hybrid seeds were sown in wooden boxes containing organic matter and soil mixed at a rate of 1:1. Seed germination was determined seven and 10 days after seeding. Seedlings transplanted into nylon bags were evaluated with regard to survival percentage, stem height, uniformity, and vigor. Desirée x Kondor was the best cross to be used in a true potato seed program; however, the highest values of survival, vigor and uniformity were obtained when CIP clones were used as male parents.

Key words: potato, cross breeding, seeds, pollen, vigor; viability

RESUMEN. Se estudiaron durante dos años, en condiciones de invernadero, seis cruzamientos intraespecíficos de papa entre tres variedades comerciales y cuatro clones provenientes del Centro Internacional de la Papa (CIP). Los estudios fueron realizados con el objetivo de evaluar su comportamiento en los cruzamientos y durante la etapa de semilla botánica para su posible uso como progenitores en un programa de semilla sexual de papa. Se evaluaron los porcentajes de viabilidad y germinación del polen, el número de frutos logrados, el número de semillas totales y por fruto, y el largo y diámetro de las bayas. Las semillas híbridas obtenidas fueron sembradas en cajas de madera que contenían una mezcla de materia orgánica y tierra en una proporción de 1:1. Se les determinó el porcentaje de germinación a los siete y 10 días de sembrados. Las plántulas transplantadas a bolsas fueron evaluadas en cuanto al porcentaje de supervivencia, altura, uniformidad y vigor. El cruce Desirée x Kondor presentó el mejor comportamiento para un programa de semilla sexual; aunque, de manera general, los mayores valores de supervivencia, vigor y uniformidad fueron alcanzados por los cruces donde los clones del CIP fueron utilizados como progenitores masculinos.

Palabras clave: papa, cruzamiento, semillas, polen, vigor, viabilidad

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a highly heterozygous tetraploid plant, originating from South America. Since it was inserted in Europe in XVI, such plant has been developed, as well as extensively studied, besides being considered one of the main foods in the world. In this regard, only rice, wheat and maize are superior to potato crop (1), which is the most consumed vegetable, due to the fact that it provides human diet with calories, vitamins, proteins, and mineral salts. It also contains other substances, such as lysine and cystein amino acids, pantothenic acid, zinc and copper, which most agricultural products lack (2).

Potato production and consumption in tropical and subtropical climates are frequently very restricted, due to high costs or poor availability of high quality seed tuber. However, the demand for this product keeps increasing in developing countries. True potato seed is seen as an appropriate alternative for producing such crop in tropical climates (3). One of the main advantages of true seed, as an alternative for propagating potato crop, is the fact that it provides flexibility through its different uses, under several agroecological conditions.

The main goal of a true seed breeding program is selecting parents that, by means of their combinations, could originate uniform progenies for agricultural and reproductive characters, which are resistant to important diseases (4).

Progeny uniformity, good vigor and adequate yield are some of the main issues to be considered in the selection of hybrid families (5). Taking the formerly

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expressed aspects into account, the present work focused on selecting hybrid combinations with appropriate features, for starting a true potato seed program.

MATERIALS AND METHODS

During 1998-1999 and 1999-2000 seasons, under greenhouse conditions, six intra-specific crosses were performed among three commercial varieties and four clones from the International Potato Center (CIP) in Perú (Table I). Such experiment followed the traditional stumping method, which is used at INCA (6).

Table I. Number of hybrid combinations

No.	Hybrid combinations
1	Atlantic x Kondor
2	Desirée x 390217-03
3	Desirée x 391218-36
4	Desirée x Kondor
5	Desirée x 391218-02
6	391218-39 x Desirée

Inflorescences were collected in the morning and taken to greenhouse. After a recovering period of near two hours, open flowers as well as immature buds were removed and emasculation was performed. Afterwards, pollination was carried out, following the established cross design.

Berries were harvested between 25 and 30 days after pollination and stored at room temperature until they were fully ripe. Then, their seeds were extracted, washed, dried, as well as preserved at a temperature of 20°C, near nine months. At this stage, pollen germination and viability percentage (6), number of pollinated flowers (NFlP), number of set fruits (NFrL), total number of seeds (NTS) and seeds per fruit (NF/Fr), as well as berry length and diameter (LB, DB) were evaluated. Fruit setting percentage for each cross was also determined through fruit setting %= NFIP 100 formula.

In 1999 and 2000, hybrid seeds were sown in wooden boxes, containing organic matter and soil mixed, at a rate of 1:1. Germination percentage was evaluated seven and 10 days after sowing.

After 25 days, seedlings were transplanted into black polyethylene bags, containing organic matter and soil mixed, at a rate of 2:1. The bags were placed in a pipe and identified by cross. Flood irrigation was performed for reducing the attack of diseases and keeping steady humidity. After 25 days, each bag was supplied by 1g of the complete 9-13-18 formula. During this stage, the following characters were evaluated:

- ★ survival percentage: how many plants survived out of all transplanted seedlings.
- ★ height (cm): it was evaluated 50 days after transplantation.
- ★ uniformity: it was evaluated according to a 5-degree scale (7), where 1= very bad and 5= very good.
- ★ vigor: it was evaluated according to a 9-degree scale (8), where 0= very low and 9= very good.

Statistical análisis. Using the means from two years, single correlation analyses were performed, among fruit setting percentage, number of set fruits, number of seeds per fruit, berry diameter and length, as well as parental pollen germination and viability. Ratio comparing analysis was carried out, using values of seed germination and survival percentages, transformed to arc sen $\sqrt{}$% and compared by means of F test.

RESULTS AND DISCUSSION

After analyzing results of *in vitro* pollen germination and viability (Table II), it was noticed that CIP clones presented the highest percentages for such characters, except for clone 391 1218-39, which was low. Concerning viability, the highest value was presented by clone 391218-02 with 86,25 %, without differing considerably from clone 390217-03 (84,5 %) and showing great differences with the rest. Under Cuban conditions, CIP clones have always presented higher viability percentages than cultivated varieties (9,10).

Table II. Pollen viability and *in vitro* germination percentages of clones and varieties selected as parents

Genotipes	Viability (%)		Germination (%)	
	Transformed means	Non-transformed means	Transformed means	Non-transformed means
Desirée	44.71 d	49.5	40.53 d	42.25
Atlantic	21.3 f	13.25	14.11 f	6.00
Kondor	56.34 c	69.25	52.98 c	63.75
391218-02	68.38 a	86.25	62.21 a	78.25
391218-36	60.71 b	76.00	56.49 b	69.50
390217-03	66.83 a	84.5	55.10 bc	67.25
391218-39	24.49 f	17.25	20.66 e	12.50
X ± SE	48.96 ± 1.07 ***		43.15 ± 0.73 ***	

( ) original value
*** significant at p<0.001
Means with common letters do not differ significantly

Regarding *in vitro* pollen germination, percentages were lower, compared to the morphological staining technique. Clone 391218-02 presented the highest value with 78.25 %, differing considerably from the rest. Percentages presented by clones 391218-36, 390217-03, as well as by Kondor variety were superior to 60 %. However, even though the first clone did not differ from the second one, differences were found with respect to Kondor variety. This is the most recommended method for potato crop, as well as the most direct one, providing more reliable results (11).

Results from crosses among the selected parents are showed in Table III. A total number of 583 flowers were pollinated and 471 fruits were achieved, reporting a high fructescence percentage of 80.8 %. This last indicator was high for all crosses, presenting superior values to 70 %, in all cases. Desirée x 391218-36 achieved the highest value, with 94.4 %, whereas Atlantic x Kondor presented the lowest value, with 73.21 %.

Table III. Results of hybrid combinations performed during both seasons

Crosses	NFlP	NFrL	% F	NS/C	NS/Fr	DB	LB
Atlant. x Kondor	168	123	73.21	7494	64.58	2.04	1.58
Desirée x 390217-03	88	70	79.54	6980	99.71	2.45	1.84
Desirée x 391218-36	125	118	94.4	12900	94.4	2.6	1.89
Desirée x Kondor	48	43	89.58	5150	119.76	2.74	1.92
Desirée x 391218-02	63	47	74.6	2700	57.44	2.06	1.66
391218-39 x Desirée	91	70	76.92	2180	31.14	1.94	1.5
Total	583	471	80.8	36404	74.29	2.3	1.73

NS/C- number of seeds per cross
NS/Fr- number of seeds per fruit
% F- fruit setting percentage
LB- berry length
NFIP- number of pollinated flowers
NFrL- number of set fruits
DB- berry diameter

However, when analyzing the total number of seeds per cross and number of seeds per fruit, it was found that, even though 391218-39 x Desiree presented a high fruit setting percentage, it achieved a low number of seeds per fruit, reaching only 31.14 %, which was considerably lower than the general mean (74.9). Desiree x Kondor presented the highest number of seeds per fruit, with 119.76 %.

It can be noticed that Desirée variety was used as female parent in Desiree x 390217-03, Desirée x 391218-36 and Desirée x Kondor combinations, achieving the highest fruit setting percentages, as well as the highest number of seeds per fruit. These results coincide with others (8, 11), where the best combinations were obtained by such variety as female parent.

High fruit setting percentages were also recorded (7) when using several tetraploid parents, such as Atlantic variety. It has to be highlighted that, pollination results depend on several factors, which can influence the number of seeds obtained (12), as well as its quality (13), in a remarkable way.

Table IV shows linear correlations among the number of set fruits, number of seeds per fruit, berry diameter and length, as well as pollen viability and germination, which influence the total number of seeds obtained. Positive and highly significant correlations were found among pollen germination and the remaining characters, except for berry length. *In vitro* pollen germination provides a reliable male fertility estimate, obtaining significant correlations with the number of fruits and number of seeds per fruit (14).

Table IV. Linear correlation among the number of set fruits, number of seeds/fruit, berry diameter, berry length, pollen viability and germination

Variables	NFrL	NS/Fr	DB	LB	VP	GP
NFrL	1.000					
NS/Fr	0.015	1.000				
DB	- 0.103	0.943**	1.000			
LB	0.265	0.868**	0.788*	1.000		
VP	0.716*	0.828	0.815*	0.468	1.000	
GP	0.871**	0.888**	0.839**	0.564	0.943***	1.000

* significant at p<0.05
** significant at p<0.01
*** aignificant at p<0.001
VP- pollen viability
GP- pollen germination

Pollen viability also showed positive correlations; however, they were less significant than those presented by germination. This issue could be explained, taking into account that such technique overestimates viability, because there are stained grains that do not germinate (15).

There were positive and highly significant correlation among number of seeds per fruit and berry diameter and length. However, in this sense, big berries with few seeds can be obtained, and vice versa (16). This last issue is possible because of the incompatibility between the parents used, due to S alleles (17, 18).

Evaluation of seed germination and seedling development. Table V presents results from the behavior of hybrid families under greenhouse conditions for seed germination (after seven and 10 days), survival, as well as vigor and uniformity. Concerning germination, there were significant differences among crosses, after seven and 10 days. After seven days, Desirée x Kondor cross presented the highest values with 65.27 %, without differing significant from Desirée x 390217-03 and Desirée x 391218-3, which showed differences with regard to all the crosses, except for cross Desirée x 391218-02.

Most of the crosses surpassed 50 % germination, with the exception of Atlantic x Kondor and 391218-39 x Desirée. The same behavior was kept after 10 days, but there was an increase in values, which where higher than 70 % in Desirée x Kondor and Desirée x 390217-03. There were significant differences between such crosses and the rest, except for Desirée x 391218-36.

Hybrid seeds, preserved during three months (13), presented lower germination percentages than 50 %, which were increasing according to storage time. Results of germination could have been influenced by sowing depth, as well as by the time of evaluation, although evaluations are reported in literature from the fourth day (13); it was noticed that, under these experimental conditions, germination percentages increased gradually, even a long time after 10 days.

Table V. Behavior of germination and survival percentages, seedling vigor and uniformity

Crosses	Germination (%)		Survival (%)	Vigor*	Uniformity**
	7 days	10 days			
Atlantic x Kondor	0.46 (45.8) c	0.6 (59.8) b	0.68 (68.2) c	6.5	4.0
Desirée x 390217-03	0.64(64.3) ab	0.71 (70.87) a	0.89 (88.9) a	8.6	4.8
Desirée x 391218-36	0.58 (58.2) ab	0.69 (66.8) a	0.86 (86.30) a	8.7	4.5
Desirée x Kondor	0.65 (65.2) a	0.74 (73.6) a	0.87 (86.87) a	7.4	4.4
Desirée x 391218-02	0.56 (56.3) b	0.57 (56.7) bc	0.76 (75.6) b	8.2	3.7
391218-39 x Desirée	0.36 (36.1) d	0.51 (50.5) c	0.51 (50.5)d	2.5	2.9
ES X	0.03***	0.03***	0.02***	6.9	4.1

*** significant at p<0.001
( ) original value
Rates with common letters are not significantly different

* 0= very low y 9= very good
** 1= very bad y 5= very good

Desirée x 390217-03, Desirée x 391218-36, and Desirée x Kondor presented higher survival percentages than 85 %, which differed remarkably from the rest. The lowest value was presented by 391218-39 x Desirée, with 50.49 %. Survival percentages under 80 % were found when analyzing Desirée seeds from open pollination, as well as seeds from direct and reciprocal crosses using such variety (8). On the other hand, after studying 105 crosses using 15 parents, a high correlation was found among germination, survival percentages and vigor (19).

Table V also shows vigor and uniformity values, according to the scales used. Seedling uniformity ranged from good to very good in the first four crosses, whereas for Desirée x 391218-02 it ranged from fair to good. 391218-39 x Desirée presented the lowest value, according to the corresponding scale, showing a bad to fair uniformity. Scale values under 4 were found in crosses between commercial varieties and established clones, as well as when Desirée was used as a female parent, reaching the highest scale values (7). This coincides with the results, because in most of the crosses where such variety was used as female parent, the highest values of foliage uniformity were reached.

Plant vigor and foliage as well as tuber uniformity are two very important aspects to be taken into account, when a true potato seed program is established (19).

In general terms, it can be concluded that parents from CIP presented higher percentages of pollen viability and germination. Superior fruit setting percentages to 70 % were found, as well as positive and highly significant correlations among pollen germination and the remaining characters, except for berry length. In all crosses, seed germination and survival percentages were higher than 50 %, where combinations of Desirée x Kondor, Desirée x 390217-03 and Desirée x 391218-39 stood out, showing the highest values. A good behavior was presented for vigor and uniformity. Desirée x Kondor was the least variable and the most uniform cross for each character evaluated.

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