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Pollen viability and meiotic analysis of *Solanum commersonii commersonii* Dun., *Solanum commersonii malmeanum* Bitt. and *Solanum tuberosum* L.

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ABSTRACT - *Meiotic abnormalities in potato hamper sexual recombination, due to their influence on pollen production and viability rate. In this study we evaluated pollen viability and meiosis of three clones of* Solanum commersonii commersonii *Dun.* (SCC), *two of* Solanum commersonii malmeanum *Bitt.* (SCM) *and seven clones and four cultivars of* Solanum tuberosum *L., with the purpose of indicating promising genotypes for genetic breeding of potato. Early chromosome migration at metaphases I and II and chromosome pairing anomalies were the main causes of pollen inviability in the evaluated genotypes. Clones SCC 07 and SCM 60 are the most suitable for sexual recombination, owing to the high percentage of viable pollen grains and low frequencies of meiotic abnormalities.*

Key words: potato, meiosis, pollen, plant breeding.

INTRODUCTION

The cultivated potato *Solanum tuberosum* L. is an autotetraploid species with a narrow genetic base due to the isolation of the wild species after the introduction and domestication in Europe. Besides, the epidemics of *Phytophythora infestans* in the 19th century affected the species by genetic drift (Simmonds 1979).

One of the possibilities of broadening the genetic base of the crop is to cross the cultivated with wild diploid species (2n=2x=24). Such crosses also allow for the introgression of genes of interest to increase diversity and allele interactions (Peloquin and Ortiz 1992).

Among the wild and diploid species, *Solanum commersonii commersonii* Dun. and *Solanum commersonii malmeanum* Bitt. are particularly interesting for improvement owing to their alleles of tolerance to low temperatures, resistance to bacterial wilt caused by *Ralstonia solanacearum* and resistance to soft rot and blackleg, caused by *Erwinia carotovora*, besides high contents of tuber dry matter (Rocha et al. 2000, Carputo 2003).

However, meiotic abnormalities, which usually affect pollen viability and pollen production rate, have hampered sexual recombination in potato. Wild *Solanum* species generally produce abundant and highly viable pollen, whereas the cultivated species produce little

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pollen of low viability (Stout and Clark 1924). Therefore, variations in pollen production and viability rate between the clones of wild and cultivated species can impair the choice of male parents in breeding studies. In this paper the pollen viability and production of *Solanum commersonii commersonii* Dun., *Solanum commersonii malmeanum* Bitt. and *Solanum tuberosum* L. clones were evaluated, to identify the most appropriate genotypes for breeding programs. Besides, variations in the production and viability of pollen grain of some clones were explained based on meiotic analysis.

MATERIAL AND METHODS

The wild genotypes were represented by three clones of *Solanum commersonii commersonii Dun.* (SCC 07, SCC 176, SCC 100) and two clones of *Solanum commersonii malmeanum Bitt.* (SCM 60 SCM 57). Tubers of these clones were provided by the potato genebank of the Centro de Pesquisa Agropecuária de Clima Temperado (CPACT), of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), in Pelotas, Rio Grande do Sul.

The pollen characteristics of seven *S. tuberosum* L. clones (ESL 7-6; ESL 9-4; ESL 22-08; CBM 8-26; CBM 24-06, LT9 and BRK CQT) and the cultivars Garant, Niska, Jenseng and Chiquita were studied as well. The *S. tuberosum* L. clones denominated ESL and CBM were obtained according to Menezes (1999) and clone LT9 was provided by the Centro Internacional de La Papa (CIP).

Pollen viability was evaluated by *in vitro* germination and by staining. From each clone five flowers, which represented one replication each, were collected. The pollen grains were inoculated in culture medium containing 165 gL^{-1} sucrose, 12 gL^{-1} agar, 20 mgL^{-1} $Ca(NO_3)_2.4H_2O$, and 10 mgL^{-1} H_3BO_3 dissolved in distilled water. After germination at 20°C to 28°C, five glass slides per genotype were evaluated with 200 pollen grains per glass slide. Grains with a pollen tube size equal to or greater than the pollen diameter were considered viable.

To evaluate pollen viability by staining, anthers were fixed in Carnoy's fluid (3 parts ethyl alcohol:1 part propionic acid) and the pollen grains were stained in 2% acetocarmine. Five glass slides of each genotype were evaluated for viability, with 200 pollen grains per slide. Stained pollen grains were considered viable and the colorless unviable. The number of viable pollen grains was counted in five fields per slide. The data of the pollen viability and quantity evaluation were analyzed by the Scott & Knott (1974) test.

For the meiotic analysis, flower buds of each of the five clones of *S. commersonii commersonii Dun.* and *S. commersonii malmeanum Bitt.* and of cultivar Chiquita were collected. The flower buds were Carnoy-fixed and the meiocytes stained in 2% propionic carmin solution. One hundred meiocytes per genotype were analyzed, on six glass slides.

Chromosome pairing of approximately 100 anthers of each clone of *S. commersonii commersonii Dun.* and *S. commersonii malmeanum Bitt.* was evaluated by the technique of enzymatic maceration and air-drying described by Carvalho (1993)

RESULTS AND DISCUSSION

For all clones evaluated the viability of pollen grains was twice as high by the staining method as by *in vitro* germination. Although the correlation between the pollen viability evaluated by staining and by *in vitro* germination was moderate (r=0.69), the two methods were largely discrepant. For example, the percentage of viable pollen in cultivar Jenseng and the clones ESL9-4 and ESL22-08 were high (81.50, 78.60 and 83.80%, respectively) by staining and low by germination (8.40, 3.50 and 49.50%) (Table 1). This suggests that the selection of clones with high male fertility based on the staining method alone is not adequate and can impede selection. Techio (2006), studying *Pennisetum glaucum*, also stated that staining, although a simple and cheap procedure, is not fully reliable, but may overestimate the viability values. For Parfitt and Ganeshan (1989), acetocarmin staining does not deliver reliable results, since the test with this dye detected a high level of apparent pollen viability in pollen grain inviabilized at high temperatures.

With exception of clone SCC 100, the means of viable pollen grain were higher for *S. commersonii* spp than for *S. tuberosum* L. clones, by both evaluation methods. SCM 57 and SCM 60 stood out among the clones of *S. commersonii malmeanum Bitt.*, (Table 1) with higher pollen viability in both tests. These results are consistent with those of Pandolfi (1998) who studied pollen viability in commercial *S. tuberosum* L. cultivars

Table1. Percentage means of viable pollen grain evaluated by 2% acetocarmin staining and by in vitro germination the quantity of pollen grain per field on the glass slides

Genotypes	Staining (%)	*In vitro* (%)	Pollen quantity
LT9	6.40 e	1.00 e	127.12 c
Garant	7.00 e	1.00 e	307.40 b
Niska	36.20 d	1.10 e	147.36 c
Jenseng	81.50 b	8.40 e	176.80 c
Chiquita	63.30 c	60.00 b	198.84 c
BRK CQT	56.00 c	26.80 d	362.36 a
CBM8-26	48.80 c	4.70 e	279.76 b
CBM24-06	10.10 e	1.00 e	85.24 c
ESL7-6	60.80 c	4.50 e	306.20 b
ESL9-4	78.60 b	3.50 e	269.88 b
ESL22-08	83.80 b	49.50 c	200.00 c
SCC07	73.00 b	58.00 b	438.80 a
SCC100	52.80 c	5.60 e	514.00 a
SCC176	97.80 a	44.60 c	434.40 a
SCM57	94.40 a	78.50 a	454.20 a
SCM60	96.50 a	75.00 a	476.20 a
Overall mean	59.18	26.46	298.84
CV (%)	17.26	34.51	29.41
Correlation (staining /*in vitro*)	0.69		

Means followed by the same letter in each column did not differ from each other by the test of Scott & Knott at 5% probability

and in wild *S. commersonii commersonii Dun.* and *S. commersonii malmeanum* Bitt. species, by the techniques of staining with 2% acetocarmin and *in vitro* germination. This author verified, by both techniques, that the pollen viability is significantly higher in wild species than commercial cultivars. Pandolfi (1998) evaluated approximately 200 pollen grains per flower bud by 2% acetocarmin staining and found 78.93 and 60.33% viable pollen grains in the species *S. commersonii commersonii Dun.* and *S. commersonii malmeanum Bitt.*, respectively.

The mean pollen grain production of *S. commersonii* spp. clones exceeded the means of *S. tuberosum* L. clones, with exception of BRK CQT, with a remarkably high mean (362.36) in this test. For cultivar Chiquita, this value was lower (198.84). This could be considered a reference value for the establishment of a minimal limit for good pollen grain production, since this cultivar is considered a good pollinator in crosses (Menezes 1999).

The meiosis of cultivar Chiquita and the *S. commersonii* clones was accompanied to indicate possible clones for sexual recombination. Of the 920 cells of cultivar Chiquita, 9% were abnormal (Table 2), similarly to results obtained for the wild potato clones with the lowest rates of irregularities. It is noteworthy that among the *S. tuberosum* L. cultivars, the best results of pollen germination were observed for Chiquita (Table 1).

The meiotic configuration of most cells analyzed was normal. The clones exhibited between 7 and 20% irregular cells (Table 2). Among the frequent abnormalities the early migration of chromosomes at metaphase I and the asynchronous division at metaphase II (Table 2 and Figure 1-A and B) were salient. However, the asynchronous division in meiosis II does not seem to be the main cause of pollen inviability, since of the clones SCM 57 and SCM 60, with high percentages of this abnormality, 78.5 and 75% of the pollen germinated *in vitro* and 94.4 and 96.5% by staining proved viable, respectively. On the other hand, the early chromosome migration at anaphase I was frequent in clones SCC 100 and SCC 176 with rates of pollen grain viability of 6 and 44%, respectively, by *in vitro* germination. Problems in chromosome pairing or early chiasma terminalization are the most likely causes of these abnormalities. Pagliarini (2000) comments that the most common meiotic abnormality observed in diverse species is irregular chromosome segregation, characterized by early migration or delayed chromosomes at metaphase I and anaphase I.

Table 2. Percentage of meiotic abnormalities found in *S. commersonii* spp. clones and in cultivar Chiquita

Abnormalities	SCC 07	SCC 100	SCC 176	SCM 57	SCM 60	Chiquita
Micronucleus (PI)	7	1	12	4	3	4
Early migr. (MI)*	30	42	52	22	13	12
Early migr (MII)**	3	1	0	8	5	13
Asynchronous division	36	15	16	38	22	50
Parallel spindle	18	7	0	27	22	11
Fused spindle	0	7	10	0	0	8
Micronucleus (PII)	6	6	10	1	9	0
Tríade	0	21	0	0	26	2
Number of abnormal cells	77	308	64	85	70	83
Total cells	773	1540	917	846	779	920
%Abnormal cells	10	20	7	10	9	9

*Early migration at metaphase I, **Early migration at metaphase II, PI = prophase I and PII = prophase II

Pandolfi (1998) reports a higher frequency of chromosomes with early migration at meiosis I than at meiosis II and a decline in the frequency of abnormalities during the meiosis phases in *Solanum commersonii* spp. clones.

Parallel spindles in metaphase II and asynchronous division were frequent in clone SCM 60, (96.5% viable pollen grain by staining and 75% by germination). Although the parallel spindle organization is a result of mutations (Hannemann 1999), it does not cause pollen inviability, but rather a non-reduction of the same. Therefore, the analysis of pollen viability showed that of the *S. commersonii commersonii Dun.* and *S. commersonii malmeanum Bitt.* clones analyzed, only SCC 07 and SCM 60 could be used for sexual recombination.

Chromosome pairing was evaluated in clones of *S. commersonii* spp. (Figure 1- C through H). Most of the bivalents observed in the diakinesis were ring-shaped with terminalized chiasma and frequently two of them were rod-shaped, fixed to one of the extremities (Figure 1-C). Clone SCC 07 always had 12 bivalents (Table 3), while clone SCC 100 exhibited 100% of abnormal diakinesis. Aside from the irregularites in chromosome pairing different numbers of bivalents were found (Table 3).

The abnormalities 15 II, 18 II, 19 II and 20 II in clone SCC 100 (Table 3) suggest the possibility that this genotype had been derived from previous hybridizations between plants that produced unreduced and normal pollen. This may have occurred concomitantly with the origin of certain polyploids, with the chromosome non-reduction or endomitosis in a precursor cell of meiosis. In both cases 2n gametes are formed which could in turn form a triploid, when fertilizing a normal gamete (Guerra 1988). In *Solanum*, small chromosomes have a low chiasma frequency (Hermsen 1984), which together with the early terminalizations or the presence of synaptic or desynaptic mutants in prophase I, generate univalents. Independent of the causes of origin of univalent chromosomes, their presence in the meiocytes will generally favor the increased chromosome frequency in early migration at metaphase I or delayed chromosomes in the anaphases. In both cases, they can originate micronuclei in telophase I or meiosis II (Pagliarini 2000). Singh (2003) attributed the presence of univalent and trivalent chromosomes to the reduction in the recombination rate of the homologous chromosomes, and consequently, to an abnormal segregation in the progeny resulting in aneuploids, which in turn lead to fertility reduction (Figure 1-G).

Chromosome alterations at diakinesis, principally the presence of univalent chromosomes, can be one of the causes of male sterility in *Hevea Braziliensis* and *Pisum sativum* (Amma et al. 1990, Nirmala and Kaul 1994). Defani-Scoarize et al. (1995) associated the occurrence of male sterility in maize to the action of meiotic abnormalities of the univalent chromosome type, followed by irregular chromosome segregations. In all genotypes analyzed, these alterations, as well as the tetravalent ring (Figure 1- F), all contributed to the formation of aneuploids, i.e., unbalanced and normally unviable gametes.

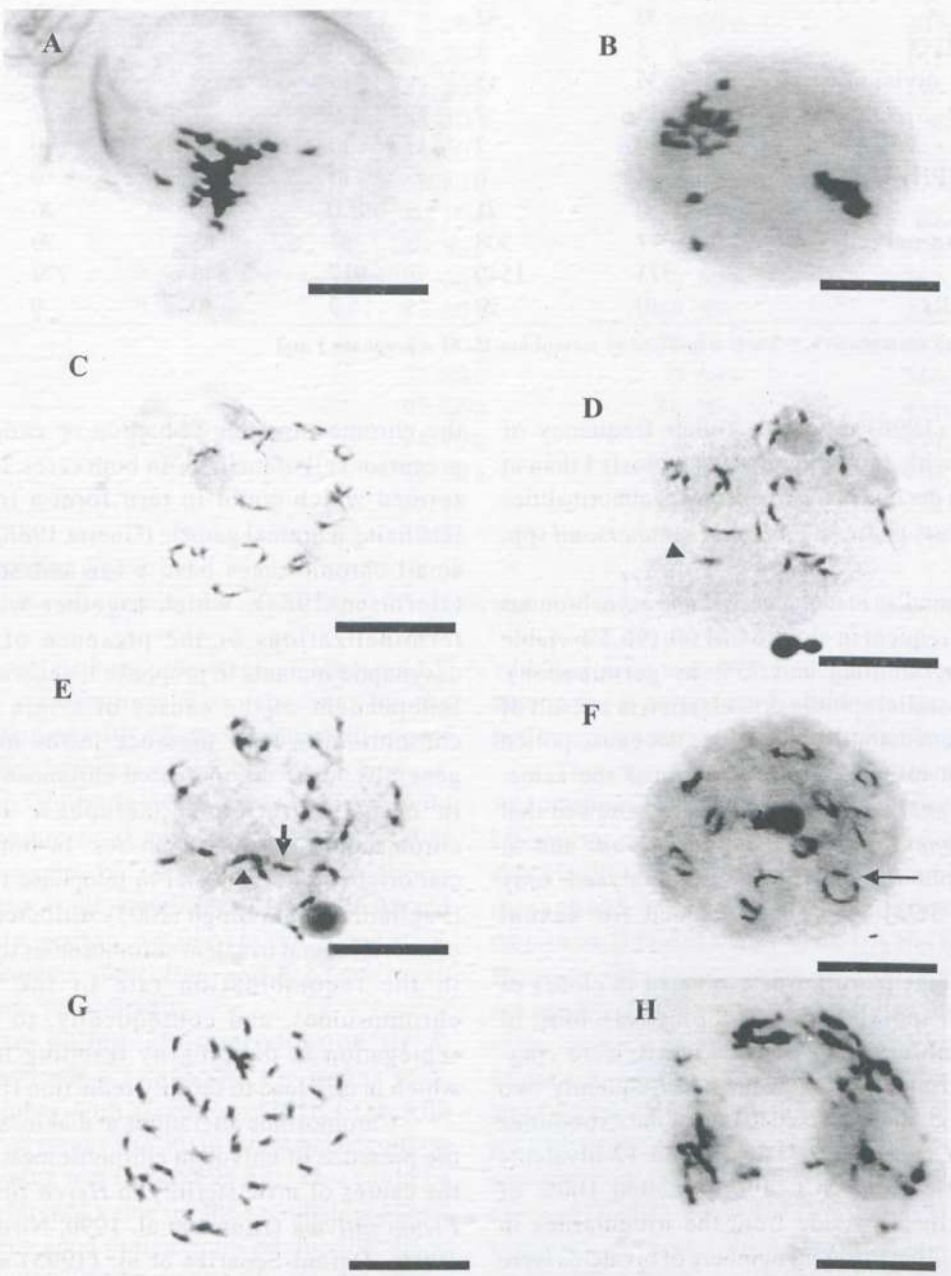

Figure 1. Meiocytes of clone SCC 100 of *S. commersonii commersonii* (A and B). A- metaphase I, chromosomes in early migration; B - asynchronous division and early migration. Meiocytes in diakinesis, of clone SCC 176 of *S. commersonii commersonii* (C through G). C- 12 II.; D- 12 II + 1 I (arrowhead); E-10 II + 1 I (arrow) + 1 III (arrowhead); F- 10 II, 1 IV in translocation ring; G- meiocyte with univalents; H- chromosome fragmentation in clone SCC 100. Bar = 5 μm

Table 3. Frequency of abnormalities (%) in cells at diakinesis of *S. commersonii commersonii Dun.* and *S. commersonii ssp. malmeanum Bitt.* clones and total of cells evaluated

Abnormalities	SCC 07	SCC 100	SCC 176	SCM 57	SCM 60
1. Univalents	0.00	24.00	33.33	5.88	12.50
2. Trivalents	0.00	28.00	16.67	11.76	25.00
3. Tetravalents	0.00	4.00	33.33	0.00	0.00
4. Multivalents	0.00	8.00	0.00	0.00	0.00
5. Translocation ring	0.00	28.00	16.67	5.88	50.00
6. Delayed condensation	0.00	4.00	0.00	0.00	0.00
7. Fragmentation	0.00	4.00	0.00	76.48	12.50
Number of abnormal cells[1]	0.00	14.00	5.00	15.00	4.00
8. Number of cells with abnormal bivalents [2]	0.00	11.00	7.00	3.00	2.00
8.1 – 10 bivalents	0.00	0.00	0.00	0.00	1.00
8.2 – 11 bivalents	0.00	0.00	1.00	2.00	1.00
8.3 – 13 bivalents	0.00	2.00	4.00	1.00	0.00
8.4 – 14 bivalents	0.00	0.00	2.00	0.00	0.00
8.5 – 15 bivalents	0.00	2.00	0.00	0.00	0.00
8.6 – 18 bivalents	0.00	3.00	0.00	0.00	0.00
8.7 – 19 bivalents	0.00	3.00	0.00	0.00	0.00
8.8 – 20 bivalents	0.00	1.00	0.00	0.00	0.00
Number of abnormal cells[1+2]	0.00	25.00	12.00	18.00	6.00
Total cells at diakinesis	30.00	25.00	30.00	32.00	28.00
(%) Abnormal cells	0.00	100.00	40.00	56.25	21.42

[1] Cells with abnormalities 1 to 7; [2] Cells with only abnormal bivalents, with higher or lower numbers than 12 II

Another alteration found in meiosis was chromosome fragmentation. The highest frequency of this abnormality was observed in clone SCM 57 (Table 3 and Figure 1H). Although the appearance of these alterations is not common in plants, chromosome fragmentation had already been observed in maize chromosomes and its occurrence was associated to damages in the DNA reparation mechanisms. Such damages can be due to genetic and environmental factors (Guerra 1988). The appearance of chromosome fragmentation has been attributed to induction by radiation and to the exposure of plants to strong mutagenic chemical agents (Pagliarini 2000). This factor can also lead to an imbalance in the number of chromosomes in the gametes.

CONCLUSIONS

The selection of clones with high male fertility must not be based on the dye method alone. The low pollen viability of the evaluated clones was caused by early migration of the chromosomes at metaphases I and II and alterations in chromosome pairing. Clones SCC 07 and SCM 60 are most suited for crosses.

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Viabilidade do pólen e análise meiótica de *Solanum commersonii commersonii, Solanum commersonii malmeanum* e *Solanum tuberosum* L.

RESUMO - *Abnormalidades meióticas têm dificultado a recombinação sexual em batata, influenciando a produção e taxa de viabilidade do pólen. Neste trabalho foi avaliada a viabilidade do pólen e a meiose de três clones de* Solanum commersonii commersonii *Dun.* (SCC), *dois de* Solanum commersonii malmeanum *Bitt.* (SCM), *sete clones e quatro cultivares de* Solanum

tuberosum *L., objetivando indicar materiais promissores para o melhoramento genético da batata. Migração precoce de cromossomos nas metáfases I e II e alterações no pareamento cromossômico foram as principais causas de inviabilidade do pólen, nos genótipos avaliados. Os clones SCC 07 e SCM 60 são os mais aptos para a realização de recombinação sexual, pois apresentaram alto percentage de grãos de pólen viáveis e baixas freqüências de abnormalidades meióticas.*

Palavras-chave: Batata, meiose, pólen, melhoramento vegetal.

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