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FLOWERING BEHAVIOR AND 2N POLLEN PRODUCTION IN DIHAPLOID *Solanum tuberosum* x *Solanum chacoense* HYBRIDS*

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ABSTRACT

The flowering characteristics of 81 dihaploid *Solanum tuberosum* (2n=2x=24) x *Solanum chacoense* (2n=2x=24) hybrid clones were studied. Two screenhouse experiments were carried out in the Biology Department at "Escola Superior de Agricultura de Lavras" between October and December 1991 (Spring) and March and May 1992 (Fall). The experimental design used was randomized complete blocks with three replications. Each experimental unit (plot) was formed by a single plant. The results showed wide variation among the clones for all the characteristics assessed. Clone flowering was abundant in both periods but more pronounced in the Spring. Clones were found with high 2n pollen frequencies making them promising for 4x - 2x crossings. The frequency and viability of 2n pollen were higher under mild temperatures (Fall). The amount of pollen produced on the anthers was normal and did not show any significant difference between the flowering periods.

INTRODUCTION

The cultivated potato (*Solanum tuberosum* L.) (2n=4x=48) has a narrow genetic base, and many experiments have been carried out to widen it and to create favorable new genetic combinations. The use of wild material from the same species, in this case diploids (2n=2x=24) producers of non-reduced gametes (2n pollen), has resulted in a wide and valuable pool of genetic variability (Mendoza, 1989; Yerk and Peloquin, 1989, 1990). Interspecific crosses are carried out by first obtaining *S. tuberosum* dihaploids (2n=2x=24) which can easily be crossed with similar diploid species to produce diploid hybrids (2n=2x=24). These hybrids are later backcrossed with unrelated cultivars of *S. tuberosum* to produce tetraploid progenies (Iwanaga and Schmiediche, 1989). Thus, it is necessary to obtain hybrids producing non reduced gametes. These 2n gametes, transfer about 80% of the heterozygosity present in their parent hybrid to their progeny (Mok and Peloquin, 1975; Yerk and Peloquin, 1990).

The objective of this work was to assess the flowering behavior and the frequency of 2n pollen production in hybrids from dihaploids of *S. tuberosum* x *S. chacoense*, for their later use in backcrossings with *S. tuberosum* L.

MATERIAL AND METHODS

Eighty one hybrid clones (2n=2x=24) obtained from crosses between dihaploids *S. tuberosum* x *S. chacoense* were assessed. The true seeds were treated for 24 hours with 1500 ppm gibberellic acid, dried in the shade, and sown on plug trays a week later. Approximately 30 days after sowing the plants were transferred to plastic pots to produce tubers. These were harvested roughly 95 days after transplanting and kept in cold storage at 4°C for ninety days. For tuber multiplication the seed potatoes were induced to sprout with gibberellic acid (ACTIVOL) at 10 ppm for about ten minutes.

Planting was carried out in 11 x 18 cm plastic bags and the plants transferred to the field when they were about 15 cm high. Two experiments designed to assess the flowering behavior and the frequency of 2n pollen production were set up, under screenhouse conditions in Lavras between October and December 1991 (Spring) and between March and May 1992 (Fall). The clones were planted in plastic pots (capacity = 3.0 kg) containing organic vegetal substrate for seedlings ("Plantimax Hortaliças").

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A randomized complete block design with three replications was used. Each experimental unit (plot) consisted of a single plant. The beginning of flowering (number of days from germination to the opening of the first flower); the number of inflorescences per plant and of flowers per inflorescence (two countings were made, at the fifth and seventh day after the opening of the first flower); the amount of pollen (grades 1 to 4, corresponding to: 1 = very small amount of pollen, 2 = a little pollen, 3 = normal amount of pollen and 4 = abundance of pollen); the viability and frequency of 2n pollen production (collected three days after flower opening, fixed and stored in 70% alcohol for 24 hours) were determined. The viability and frequency of 2n pollen were determined from samples of approximately 200 pollen grains examined according to the Quinn *et al.* (1974) size classification, stained with 2% acetic carmine and observed at 200X magnification. The formulas used to determine the viability and frequency of 2n pollen were: viability = number of stained 2n pollen grains/number of stained and non stained 2n pollen grains and frequency = number of stained 2n pollen grains/total number of stained pollen grains.

About 20 grains of n pollen and 20 grains of 2n pollen were measured in each slide with the help of a 0.5 OSM ocular at a magnification of 400X. The slides were microphotographed by a Carl Zeiss microscope using Kodak Panatomic X, ASA 32, black and white film.

RESULTS AND DISCUSSION

The flowering of the hybrid clones was generally abundant in both the Spring and Fall experiments. Of the 81 clones assessed, 78 flowered in the Spring experiment but only 53 flowered in the Fall. The variance analysis considered only the clones that flowered during both seasons. The number of days to flowering, number of inflorescences per plant and number of flowers per inflorescence showed great variation among the clones in both the Spring and Fall (Figure 1). It is known that

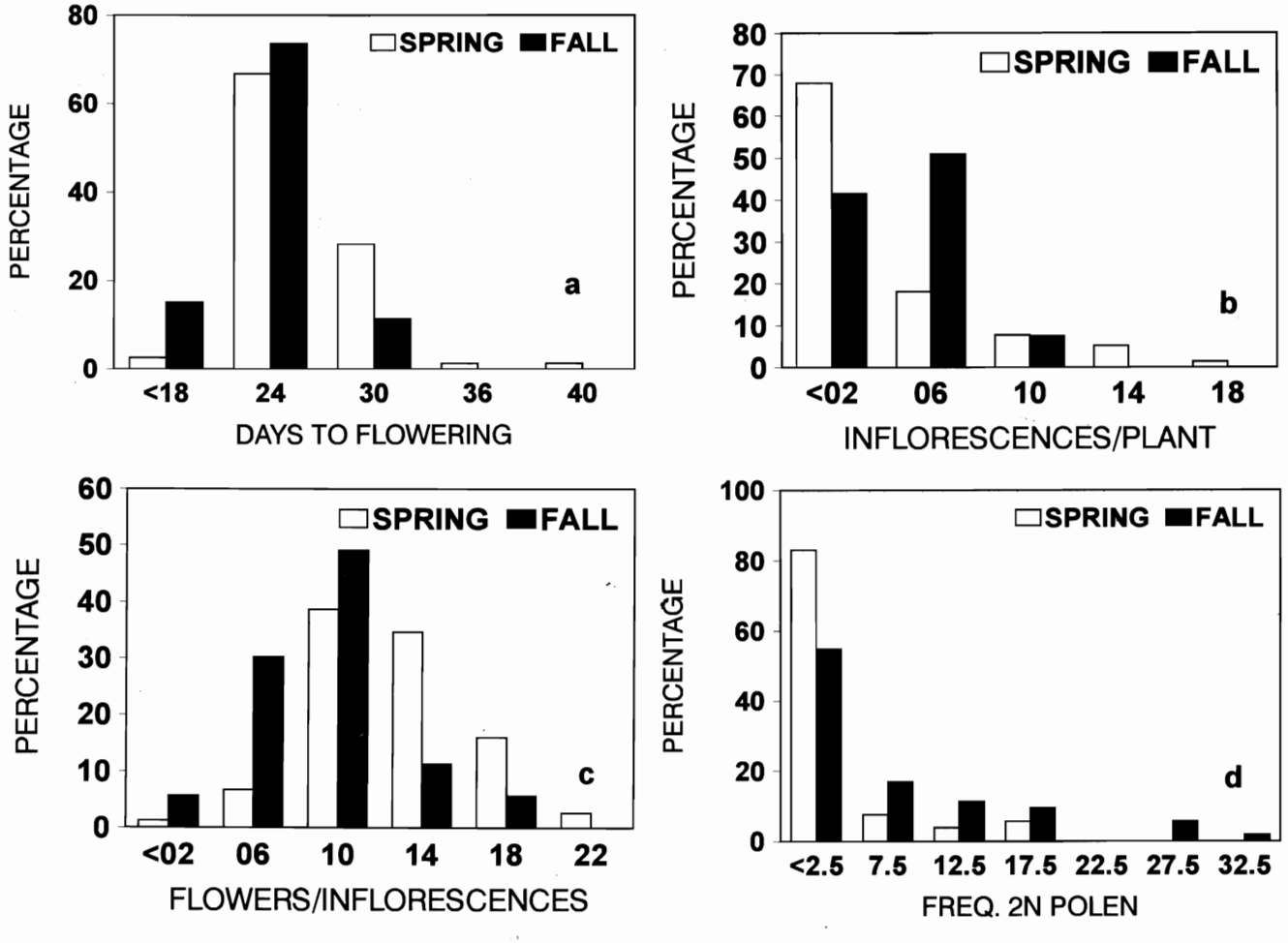

Figure 1 - Frequency distribution of hybrid potato clones relative to the number of days to flowering (a), number of inflorescences per plant (b), number of flowers per inflorescence (c) and 2n pollen frequency (d).

flowering in diploids is generally much more intense than in tetraploids (Carrol and Low, 1975). However, the intensity depends on genetic factors, temperature, photoperiod and environment x genotype interaction (Miller, 1936). In conditions similar to those of this study Pinto and Martins (unpublished results), observed that tetraploid materials produce considerably fewer flowers per plant than diploid materials. The *S. tuberosum* species normally flower under long day conditions while *S. chacoense* flowers even under short photoperiod conditions because it is adapted to near Equator latitudes (Simmonds, 1976). The clones behaved like *S. chacoense*, as the majority of them (65.4%) flowered even under short day conditions in the Fall. Higher temperatures may have contributed to more flowering in the Spring. Turner and Ewing (1988), reported that flowering was more abundant under high temperature conditions for *S. tuberosum* cultivars.

Highly significant differences were observed in the 2n pollen frequency between season (Table I), being lower in the Spring and higher in the Fall (Figure 1d). This suggests that the high temperatures recorded during the Spring experiment were harmful to 2n pollen formation. The great interclonal variability found seems to be an indicator of the extreme susceptibility of the cells to these conditions. It shows that the penetrance of the gene or gene complex controlling the spindle during the meiotic divisions varies with the intensity of intra or extra cell environmental stimuli. However, the abnormality in the fusion can represent the level of intensity of gene expression. Ramanna (1979) argues that the variable expression of the parallel spindles gene (ps) makes the study of the heredity of this trait almost impossible.

Seasons and interaction seasons x clones had highly significant effects on 2n pollen viability (Table I). Pollen viability also seems to be strongly influenced by the environment. In the Spring experiment, probably due to the high temperature observed, 2n pollen viability was lower than in Fall. Hermundstad and Peloquin (1985) found low levels of 2n pollen viability when flowering occurred at high temperatures.

The mean size of n and 2n pollen was 21.9 ± 0.2 µm and 29.6 ± 0.3 µm, respectively (Figure 2). These results agree with those found by Quinn *et al.* (1974). The quantity of pollen produced on the anther did not show significant differences between the flowering seasons (Table I). This indicates that even in situations of shorter photoperiods and milder temperatures, the majority of clones produced a normal amount of pollen, allowing their use in backcrossing with *S. tuberosum*, especially in this period when the 2n pollen frequency is greater and the possibility of success in the crossings increases.

Table I - Summary of the joint analyses of variance for the flowering characteristics of 53 hybrid clones between dihaploids *Solanum tuberosum* x *Solanum chacoense*, assessed in Spring 1991 and Fall 1992.

Sources of variation	df	MS						
		Number of days to flowering	Number of inflorescence/ plant x 10^{-4}	Number of flowers/ inflorescence x 10^{-4}	Frequency of 2n pollen x 10^{-4}	Viability of 2n pollen x 10^{-4}	+Amount of pollen in anther x 10^{-4}	Viability of n pollen x 10^{-4}
Replications/ Blocks	4	36.881	232.819	18.811	45.160	1.897	0.441	0.307
Seasons	1	29.588	8612.146*	3429.363**	10015.796**	50.988**	0.709	6.189**
Clones	52	35.190**	17893.218**	142.901**	816.291**	4.753**	3.732**	4.801**
Clones/ Season 1	52	36.924**	22412.275**	74.875**	406.190**	5.888**	2.138**	3.038**
Clones/ Season 2	52	20.713**	9175.779**	118.804**	820.794**	3.997**	2.477**	2.706**
Clones x Seasons	52	22.447**	13694.936**	50.779**	410.693**	5.131**	0.883**	0.943**
Error	208	8.739	1464.690	24.958	108.104	2.117	0.490	0.462
CV (%)		11.31	18.94	14.72	9.23	1.43	0.86	0.66

+Grades from 1 to 4 (4 most).

*, ** Indicates significance at 5% and 1% probability level for the F test, respectively.

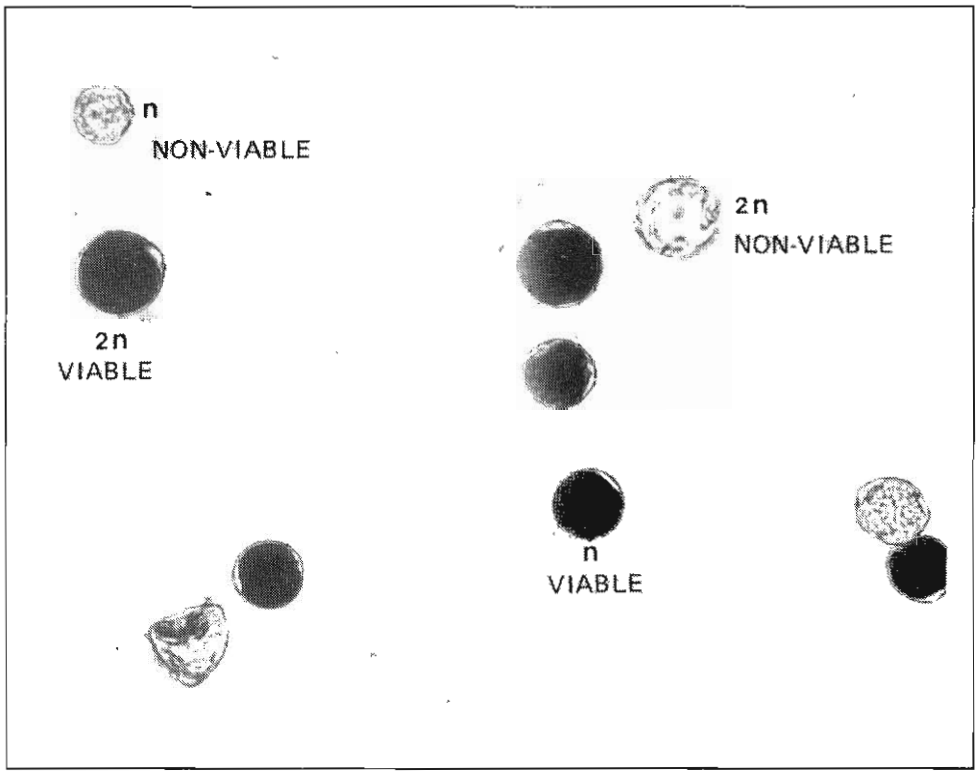

Figure 2 - Samples of n, 2n, viable, and non-viable pollen grains. Magnification of 608X.

RESUMO

Oitenta e um clones híbridos de dihaploides de *Solanum tuberosum* (2n=2x=24) x *Solanum chacoense* (2n=2x=24) foram estudados quanto a caracteres de florescimento. Dois ensaios foram instalados sob condições de telado, no Departamento de Biologia da Escola Superior de Agricultura de Lavras, entre os períodos de outubro a dezembro de 1991 (primavera) e entre março a maio de 1992 (outono). O delineamento experimental adotado foi de blocos casualizados com três repetições, sendo cada parcela constituída por uma planta. Os resultados mostraram ampla variabilidade entre os clones para todos os caracteres avaliados. O florescimento dos clones foi de forma abundante em ambas as épocas sendo que maior número deles floresceu na primavera. Detectou-se clones com freqüências elevadas de pólen 2n o que os torna promissores para a realização de cruzamentos 4x - 2x. A freqüência e a viabilidade do pólen 2n foram mais elevadas em condições de temperaturas mais amenas (outono). A quantidade de pólen produzida nas anteras foi regular e não apresentou diferenças significativas entre as épocas de florescimento.

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