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Somaclones of *C. peruvianus* Mill. (Cactaceae) may Contribute towards the Broadening of the Species Genetic Basis

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Abstract: It is a well known fact that *in vitro* regenerated plants are vulnerable to somaclonal variations and alterations in the floral and reproductive biology of somaclones may have occurred. Current study investigates the floral phenomenon and reproductive characteristics of *C. peruvianus* somaclones since information on the floral and reproductive patterns of somaclones is important to indicated somaclones and/or their descendents for the species's genetic breeding programs. Somaclones flowers opened for one night and closed daily the next morning. Opening and closing of flowers on different somaclones occurred at different times after sunrise. Although pollen reached 100% viability when flowers opened, their viability decreased to 99-98% when pollen was collected the next morning and to 90-89% when collected at 11:00 h Stigma receptivity was positive from 22:20 to 12:30 h the following day. Sugar concentration of nectar averaged 26.78%. Flowers were visited by moths of the Piralidae and Shingidae family. The *Agrius* spp. hawkmoth showed flying activity after 23:00 h and countless bees (*Apis*, *Xylocopa*, *Trigona* spp.) were detected daily from 09:00 to 18:30 h, with peaks at 11:00 and 16:00 h. Although different floral characteristics for flowers and fruit yield in somaclones were reported, the most important factor has been that the phenology of flowering and flower behaviour and pollination type coincided with the characteristics reported for clones and/or wild *C. peruvianus* populations.

Key words: *Cereus peruvianus*, cactus, *mandacaru*, floral phenology, somaclonal variation

Introduction

Cereus peruvianus, popularly known in Brazil as *mandacaru*, is a common ornamental cactus species found in gardens of tropical and subtropical countries. This species is under domestication in Israel where has been cultivated as a fruit crop (Nerd *et al.*, 2002). Gutman *et al.* (2001) have shown that *C. peruvianus* has only a limited genetic base and that further improvement of this crop may require the introduction of additional germplasm during breeding programs. *In vitro* regenerated plants of *C. peruvianus* may contribute towards the broadening of the species genetic basis since somaclonal variation appears to be an important alternative for creation of genetic variability in crops where tissue culture plant regeneration system have been established (Chahal and Gosal, 2002). A considerable number of useful somaclonal variants have been generated from different plant species (Taji *et al.*, 2002) and somaclonal variation has been reported in several crops for both qualitative as well as quantitative traits (Chahal and Gosal, 2002).

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Regenerated plants of *C. peruvianus* obtained from callus tissues (Oliveira *et al.*, 1995) revealed somaclonal variations. Morphological, cytological, biochemical and molecular characters have been the main criteria by which somaclones have been identified. Atypical shoot morphologies and new isozyme patterns occurred in *C. peruvianus* somaclones (Mangolin *et al.*, 1997, 1999; Machado *et al.*, 2000). However, floral and reproductive biology of somaclones can not have been altered. As success of plant breeding programme depends upon that the pollen of somaclones and plants descendents F1 of the somaclones may be used in breeding with clones or wild plants, the current study investigated the floral phenomenon and reproductive characteristics of *C. peruvianus* somaclones since information on the floral and reproductive patterns of somaclones is important to indicated somaclones and/or their descendents for the species's genetic breeding programs.

Materials and Methods

Report in current present study was taken on a somaclone population of *C. peruvianus* maintained at the Experimental Botanic Garden of the State University of Maringá (Maringá PR Brazil; altitude 554.9 m; 23° 25'S; 51° 25' W). Somaclone population with 71 plants has been established on the Experimental Botanic Garden since 1997. Studies were conducted from October 2003 to January 2004 and from October 2004 to February 2005. Table 1 shows mean temperature, relative air humidity and rainfall during the above periods.

Fifteen plants of *C. peruvianus* were used as samples in the present study. Flowers of S3, S9, S71, S48 and S20 somaclones were observed with regard to their daily opening, whereas the 15 somaclones were daily monitored for new flowers during the flowering period during the spring/summer 2004-2005.

Pollen grains from 4 flowers of S3, S9, S48 and S71 somaclones and 2 flowers of S20 and S22 somaclones were tested by propionic carmine for viability (Radford *et al.*, 1974), while stigma receptivity was measured with hydrogen peroxide (20 v) applied to the stigma surface. Sugar concentration in nectar from S9, S20, S21 and S22 somaclones was measured with an Optech K71313 portable refractometer; occurrence of pollinators was also observed in *C. peruvianus* somaclones.

Table 1: Mean temperatures, Relative Air Humidity (RAH) and total rainfall from October 2003 to January 2004 and from October 2004 to February 2005

	October		November		December	
	2003	2004	2003	2004	2003	2004
Mean temperature (°C)	29.4-17.9	27.7-17.2	30.0-19.2	29.1-19.1	30.1-20.4	30.0-19.8
RAH	63	68	60	68	72	69
Total rainfall (mm ³)	106.9	345.6	112.1	231.9	137.8	158.8

Table 1: Continue

	January		February
	2004	2005	2005
Mean temperature (°C)	30.9-20.7	29.0-21.5	30.5-20.4
RAH	69	83	58
Total rainfall (mm ³)	53.1	326.5	16.1

Results and Discussion

Different numbers of flowers were produced by *C. peruvianus* somaclones during the following two periods: October 2003 and January 2004 and October 2004 and February 2005 (Table 2). S2, S4, S12, S19, S21, S22, S23, S30, S33 and S38 produced flowers for the first time during October 2004-February 2005.

Flowers of *C. peruvianus* somaclones opened early in the night and for only one night, closing daily the next morning from 07:40 to 12:00 h. Anthesis started at 19:15-19:30 h and flowers were completely open by 21:30 h. Stigma was laterally or upwardly displaced and after 01:00 the style started to descend slowly placing the open stigma in a central position at 01:30-02:30 h. Whereas flowers on different somaclones started and closed at different times after sunrise, the period during which the flowers closed was longer than that during which the flowers opened.

Tests for pollen viability (N = 600-1490 cells) reached 100% for all flowers when pollen was collected in the evening at the opening of flowers (20:00-20:30 h); viability decreased to 99-98% when pollen was collected the next morning (08:00 h) and to 90-89% when collected at 11:00 h.

Stigma receptivity monitored from 20:00 to 12:30 h of the following morning indicated positive receptivity from 22:20 to 12:30 h in all somaclone flowers.

Sugar concentrations of nectar were 25% (S9), 25.5% (S20), 27% (S21) and 30% (S22), averaging 26.78%, which was similar to that found in a *C. peruvianus* wild population (Silva and Sazima, 1995).

In the Spring-Summer 2004-2005 the flowers were visited by different moths of the Piralidae and Shingidae families from 20:00 h onwards. The *Agrius* spp. hawkmoth showed flying activity after 23:00 h and introduced its proboscis into the nectar chamber while hovering; subsequently it moved forward to the flowers of other plants. Similar pattern of visiting behavior has been reported for the hawkmoth *Manduca rustica* on *Hylocereus costaricensis* (Haber, 1983) and *C. peruvianus* flowers from Southeastern Brazil (Silva and Sazima, 1995). *C. jamacaru* from Northeastern of Brazil, with similar floral attributes, has been reported to be visited by hawkmoths *M. rustica* and *Agrius cingulatus*.

On the other hand, daytime-active honey bees have been also reported to be pollinators and active during the few hours the flowers were open in the late evening or in the early morning (Weiss *et al.*, 1993, 1994; Nerd and Mizrahi, 1997; Mizrahi and Nerd, 1999). Weiss *et al.* (1993) reported that *C. peruvianus* flowers were visited by only day-active insects involved in pollination. They thus conclude that limited pollination or fertilization in open-pollination might be related to the briefness of the bee visits, reduced pollen germination and stigma receptivity during part of the visit period. Honeybees may be also acting to cross-pollinate the flowers in somaclones, since countless bees (*Apis*, *Xylocopa* and principally *Trigona* spp.) were detected daily from 09:00 to 18:30 h, with peaks at 11:00 and 16:00 h.

Table 2: Number of flowers produced by somaclones of *C. peruvianus* from October 2003 to January 2004 and from October 2004 to February 2005

Somaclone															
Period	S2	S3	S4	S9	S12	S19	S20	S21	S22	S23	S30	S33	S38	S48	S71
2003/2004	-	30	-	27	-	-	2	-	-	-	-	-	-	7	6
2004/2005	1	49	3	39	1	21	7	6	10	1	6	2	10	9	8
Total	1	79	3	66	1	21	9	6	10	1	6	2	10	16	14

Fruit development was low in *C. peruvianus* somaclones. In fact, only S3, S9, S19, S21, S22, S30, S38 and S48 produced fruits in the spring-summer period of 2004-2005. Somaclones S3 produced 4 fruits, S9, S19 and S22 produced 3 fruits each, while the others somaclones produced only 2 fruits between October 2004 and February 2005. Characteristics of meiosis behavior in somaclones have been analyzed to deepen knowledge on fruit development. It is well known that pollinators in other cactus species may limit fruit production (Mitchell, 2001; Mothershead and Marquis, 2000; Holland, 2002). Self-incompatibility has been reported for *C. peruvianus* as well as for other night-flowering columnar cactus; fruit was produced by open-pollination and manual cross-pollination, but not by self-pollination (Weiss *et al.*, 1993, 1994; Silva and Sazima, 1995; Casas *et al.*, 1999). In the case of *C. peruvianus* cultivated in Israel, the limited affectivity of bees as pollinators, rather than poor pollen quality, was responsible for lower fruit sets and seed set in open-pollinated flowers (Weiss *et al.*, 1994).

Current research showed that the phenology of flowering and flower behavior as well as fruit yield and pollination type in *C. peruvianus* somaclones agree with the characteristics reported for clones and/or wild *C. peruvianus* populations. The above statement is the most important evidence in our study since the pollen of somaclones and plants of descendents F1 of the somaclones may be used in breeding with clones or wild plants and may contribute towards the broadening of the species's genetic basis. Fruit characteristics such as size, weight and taste of fruits would be analyzed in order to assess somaclones that produce fruit set with potential consumption and commercial interest.

References

- Casas, A., A. Valiente-Banuet, A. Rojas-Martinez and P. Davila, 1999. Reproductive biology and the process of domestication of the columnar cactus *Stenocereus stellatus* in Central Mexico. *Am. J. Bot.*, 86: 534-542.
- Chahal, G.S. and S.S. Gosal, 2002. Tissue Culture in Crop Development. In *Principles and Procedures of Plant Breeding* (Chahal, G.S. and S.S. Gosal, Eds.), Pangbourne, UK: Alpha Science International Ltd., pp: 429-456.
- Gutman, F., D. Bar-Zvi, A. Nerd and Y. Mizrahi, 2001. Molecular typing of *Cereus peruvianus* clones and their genetic relationships with other *Cereus* species evaluated by RAPD analysis. *J. Hortic. Sci. Biotechnol.*, 76: 709-713.
- Haber, W.A., 1983. *Hylocereus costaricensis*. In: *Costa Rica Natural History* (Jazen, D.J., Ed.), Chicago: University Press, pp: 252-253.
- Holland, J.N., 2002. Benefits and costs of mutualism: demographic consequence in a pollinating seed-consumer interaction. *Proc. Royal Soc. London B* 269: 1405-1412.
- Machado, M.F.P.S., C.A. Mangolin and S.A. Oliveira-Collet, 2000. Somatic crossing-over can induce isozyme variation in somaclones of *Cereus peruvianus* Mill. (Cactaceae). *Haseltonia*, 7: 77-80.
- Mangolin, C.A., A.J. Prioli and M.F.P.S. Machado, 1997. Isozyme variability in plants regenerated from calli of *Cereus peruvianus* (Cactaceae). *Biochem. Gen.*, 35: 189-204.
- Mangolin, C.A., L.M.M. Ottoboni and M.F.P.S. Machado, 1999. Two-dimensional electrophoresis of *Cereus peruvianus* (Cactaceae) callus tissue proteins. *Electrophoresis*, 20: 626-629.
- Mitchell, R.J., 2001. Path Analysis: Pollination. In: *Design and Analysis of Ecological Experiments* (Scheiner, S.M. and J. Gurevich, Eds.), New York: Oxford University Press, pp: 217-234.
- Mizrahi, Y. and A. Nerd, 1999. Climbing and Columnar Cacti: New Arid Fruit Crops. In: *Perspectives on New Crop and New Uses* (Janick, J., Ed.), Alexandria, Virginia: Am. Soc. Hortic. Sci., pp: 358-366.

- Mothershead, K. and R.J. Marquis, 2000. Fitness impact of herbivory through indirect effects as plant pollinator interaction in *Oenothera macrocarpa*. *Ecology*, 81: 30-40.
- Nerd, A. and Y. Mizrahi, 1997. Reproductive biology of cactus fruit crops. *Hortic. Rev.*, 18: 321-346.
- Nerd, A., N. Tel-Zur and Y. Mizrahi, 2002. Fruit of Vine and Columnar Cacti. In *Cacti: Biology and Uses* (Nobel, P.S., Ed.), London, England. University of California Press Ltd., pp: 185-197.
- Oliveira, S.A., M.F.P.S. Machado, A.J. Prioli and C.A. Mangolin, 1995. *In vitro* propagation of *Cereus peruvianus* Mill. (Cactaceae). *In vitro Cellular Development of Biology Plant*, 31: 47-50.
- Radford, A.E., 1974. *Vascular Plants Systematic*. Harper and Row, New York.
- Silva, W.R. and M. Sazima, 1995. Hawkmoth pollination in *Cereus peruvianus*, a columnar cactus from southeastern Brazil. *Flora*, 190: 339-343.
- Taji, A., P. Kumar and P. Lakshmanan, 2002. The Origin, Nature and Significance of Variation in Tissue Culture. In: *In vitro Plant Breeding*. Binghamton, New York: Food Products Press An Imprint of The Haworth Press, Inc., pp: 101-109.
- Weiss, J., A. Nerd and Y. Mizrahi, 1993. Development of *Cereus peruvianus* (apple cactus) as a New Crop for the Negev Desert of Israel. In *New Crops* (Janick, J. and J.E. Simon, Eds.), Wiley: New York, pp: 471-486.
- Weiss, J., A. Nerd and Y. Mizrahi, 1994. Flowering and pollination requirements in *Cereus peruvianus* cultivated in Israel. *Israel J. Plant Sci.*, 42: 149-158.