POLLEN GERMINATION IN VITRO OF MEXICAN *CRATAEGUS* SPP.BORYS M.W.,¹ MA. F. SORIA ALATORRE² AND A. SALAZAR B.²¹UPAEP, Agronomía – Recursos Naturales,
21 Sur 1103, 72160 Puebla, Pue., México²Universidad Autónoma Chapingo,
56230 Chapingo, Edo. Méx., México

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ABSTRACT

The germination of pollen in vitro of 42 selections of *Crataegus* belonging to the cultivated group (*C. pubescens* (H.B.K.) Steud. = *C. mexicana* Moc. et Sessé) and wild group (probably *C. nelsoni* Eggleston) was evaluated. The anthers size (length and width) of each selection was also determined. Both attributes were determined by using undehiscent anthers of flowers of the spring flowering period (dry season). The percent of germinated pollen varied from 82.4 to 94.2%, depending upon the selection; both groups of selections gave similar values of germination. The 45.83% of selections of the wild group gave from 88 to 90% of germinated pollen. The selections of cultivated group have been characterized by a wide range of pollen germination. The anther size – length and width – depended upon the selection. The plants of the cultivated group showed a larger size more frequently than those of the wild group. No correlation was found between pollen germination and anthers dimensions. The results were discussed in relation to productivity of fruits trees of the Mexican representatives included in the germplasm collection.

KEY WORDS: pollen germination, in vitro, *Crataegus* spp.

INTRODUCTION

The productivity of *Crataegus* trees in commercial plantations and wild trees varies (Anonymous, 1930; Boboreko, 1974). The percent of parthenocarpic fruits varies, thus the seed production also varies (Borys et al., 1984). The presence of apomixis in cultigens forming commercial plantations and in some selections of *Crataegus* (Nieto Angel et al., 1993) seems to compensate for any problem originating from imperfect pollen or pollen transfer. Low fruits production may be related to a lack of germination capacity of pollen in some species of *Crataegus* (Boboreko, 1974; Campbell et al., 1991). Nevertheless, the pollination, in many cases, conditions the formation of fruits and seeds (Campbell et al., 1991).

Large differences in germination of pollen among *Crataegus* species were stated by Boboreko (1974), e.g., 4.39% in *C. paucispina* Sarg., 95.9% in *C. punctata* f. *aurea* (Ait.) Rehd. and 0% in *C. bretschneideri* C.K. Schneid. The lack of good pollen may be due to the ploidy level (Campbell et al., 1991; Muniyamma and Phipps, 1984) and/or to the reduced size of anthers coupled with triploidy (Muniyamma and Phipps, 1984). The ability of pollen to germinate and pollen yield per flower in some Canadian species were studied stating the presence of a wide variation in both attributes (Dickinson and Phipps, 1986).

The knowledge of germination ability of pollen in *Crataegus* is of interest to the future breeding program planned

among the members of germplasm collection of *Crataegus*. The successful pollination, in part, depends upon the number of pollen grains produced, and the number and size of anthers per flower (Campbell et al., 1991; Dickinson and Phipps, 1986). These attributes vary according to species (Dickinson and Phipps, 1986; Muniyamma and Phipps, 1984). Thus, when autopolliation is the way the fruit setting is to be secured the outcome depends upon the pollen yield and the fertility of pollen.

Autopollination seems to be a frequent phenomenon in *Crataegus* species studied by Boboreko (1974) and in selections evaluated by Nieto Angel et al., (1993), although doubts upon its significance in fruits setting have arisen (Flores Garcia et al., 1991; Nieto Angel et al., 1993). Autopollination resulted in 8% of flowers converted into fruits compared with 63% with open pollination in the spring time study of 5 selections. A similar study conducted with fall flowering (2nd flowering, other selections) gave 64% and 58% of flowers converted into fruits, with autopollination and emasculated flowers, respectively (Nieto Angel et al., 1993). These results seem to suggest the presence of selections with strongly expressed apomixis and a requirement to cross pollination, with some degree of apomixis or the presence of fault pollen.

No data were found concerning the capacity of pollen to germinate and the anthers size in *Crataegus* representatives of Mexico. Thus, the main objectives of this study were to determine the pollen germination and the size – length and width – of anthers in a part of *Crataegus* germplasm collection.

TABLE 1. Pollen germination of *Crataegus* selections.

Selection / Origin		Germination	
		(#)	%
Cultigens			
1	5 Calpan, Pue.	18.84a	94.2
2	13 Huejotzingo, Pue.	18.84a	94.2
3	Batan B, Pue.	18.84a	94.2
4	2 Sn. Pablo, Ixayoc, Méx.	18.80ab	94.0
5	48 Sn. Nicolas Tlaminca, Méx.	18.28abcde	91.4
6	Calpan, Pue.	18.16abcdef	90.8
7	4 Calpan, Pue.	18.12abcdefg	90.6
8	Sn. Nicolas Tlaminca, Méx.	17.92abcdefgh	89.6
9	4 B Caplan, Pue.	17.92abcdefgh	89.6
10	Sn. Nicolas Tlaminca, Méx.	17.84cdefghi	89.2
11	Sn. Nicolas Tlaminca, Méx.	17.80cdefghi	89.0
12	28 Calpan, Pue.	17.76cdefghi	88.8
13	Siden, Méx.	17.32fghijk	86.6
14	Huejotzingo, Pue.	17.00hijk	85.0
15	Pecoso de Tlaminca, Méx.	16.96ijk	84.8
16	12 Pecoso de Tlaminca, Méx.	16.64jk	83.2
17	B Calpan, Pue.	16.56jk	82.8
18	15 Huejotzingo, Pue.	16.48jk	82.4
Wild			
1	22 Chiapas	18.80ab	94.0
2	20 Chiapas	18.56abc	92.8
3	30 Chiapas	18.36abcd	91.8
4	31 Chiapas	18.28abcde	91.4
5	31 Chiapas	18.24abcdef	91.2
6	9 Chiapas	18.16abcdef	90.8
7	27 Chiapas	18.16abcdef	90.8
8	24 Chiapas	18.04abcdefg	90.2
9	23 Chiapas	18.00abcdefg	90.0
10	25 Chiapas	18.00abcdefg	90.0
11	20 Chiapas	17.88bcdefghi	89.4
12	32 Chiapas	17.80cdefghi	89.0
13	26 Chiapas	17.68cdefghi	88.4
14	30 Chiapas	17.64cdefghi	88.2
15	30 Chiapas	17.60defghi	88.0
16	17 Chiapas	17.60defghi	88.0
17	42 Chiapas	17.44defghij	87.2
18	40 Chiapas	17.44defghij	87.2
19	16 Chiapas	17.44defghij	87.2
20	38 Chiapas	17.40efghijk	87.0
21	10 Chiapas	17.40efghijk	87.0
22	37 Chiapas	17.20ghijk	86.0
23	36 Chiapas	17.00hijk	85.0
24	Chiapas s/n	16.56jk	82.8

Méx.; Pue. = State of Mexico and Puebla, respectively.
Values with different letter differ significantly.
Tukey test $\alpha = 0.05$.

MATERIAL AND METHODS

Flowers were collected in *Crataegus* germplasm collection located at the Universidad Autónoma Chapingo (Nieto Angel and Borys, 1991). These included cultigens (originated in the States of Mexico and Puebla) and wild ones (originated in the state of Chiapas). The unopened flowers were collected in the spring period (dry season flowering) for anthers measurement (undehisced anthers) and for pollen germination tests. The flowers, for anthers and pollen extraction, were stored in glazed paper bags at 5°C. Then, the flowers were disinfected with 70% ethyl alcohol and Cloralex at 0.6% for 30 seconds and 15 minutes, respectively. Flushing 4-5 times with sterile water followed. The flowers were used per each selection. The sterilized anthers were kept in sterile Petri dishes for one night to secure pollen grains release. The germination medium consisted of agar, sacharose 5%, boric acid (H₃BO₃) 0.001%, CaSO₄· 2H₂O 0.001%, pH 5.7, t°C = 22-24. The number of germinated pollen was counted 2 hours following pollen sowing. Five locations per Petri dish were evaluated and 5 replications were applied per each selection. Analysis of variance and Tukey test, $\alpha = 0.05$ were used to test the differences among selections.

All the selections were initially classified into *C. pubescens* (H.B.K.) Steud. (Dr. R. Koch, Colegio de Postgraduados, Chapingo, Mexico, cit. by Borys and Vega Cuén, 1984), although Standley (1961) in collecting the wild specimens *in situ*, indicated the presence of *C. nelsoni* Eggleston. The wild group of plants (State of Chiapas) has taken its origin from this site. The data of Nieto Angel et al., (1997) upon the endocarpium morphology indicated that the wild group of selections differed significantly from the cultivated group and casted doubts upon the initial classification. This problem has remained to be solved.

RESULTS AND DISCUSSION

The result of pollen germination are included in Tables 1 and 2. The anthers dimensions include Tables 3, 4, 5 and 7. The results of analysis of variance are listed in Table 6.

Pollen germination

Both groups of selections, those cultivated for fruits or used locally for rootstock production and those of the wild group present a similar range of pollen germination (Table 1) which

TABLE 2. Frequencies of percentages of pollen germination in *Crataegus* selections originated in Chiapas (wild) and in Central States of Mexico (cultigens).

Germination (%)	Number of selections		
	Origin		
	Chiapas (wild)	Mexico + Puebla (cultigens)	
≥ 94	1 (4.17)	4	22.22
91–93	4 (16.67)	1	5.56
88–90	11 (45.83)	7	38.89
85–87	7 (29.17)	2	11.11
82–84	1 (4.17)	4	22.22
Total	24 (100%)	18	(100%)

TABLE 3. Anthers length of *Crataegus* selections.

Selections / Origin		Length (mm)
Cultigens		
1	15 Huejotzingo, Pue.	1.88b
2	48 Sn. Nicolas Tlaminca, Méx.	1.83b
3	5 Calpan, Pue.	1.80b
4	Sn. Nicolas Tlaminca, Méx.	1.71c
5	2 Sn. Pablo Ixayoc, Méx.	1.71c
6	Sn. Nicolas Tlaminca, Méx.	1.65cd
7	Batan B, Pue.	1.60d
8	Calpan, Pue.	1.51e
9	Sn. Nicolas Tlaminca, Méx.	1.49ef
10	13 Huejotzingo, Pue.	1.47efg
11	Pecoso Tlaminca, Méx.	1.46efg
12	B Calpan, Pue.	1.46efg
13	4 B Calpan, Pue.	1.45efgh
14	Siden	1.43efghi
15	12 Pecoso de Tlaminca, Méx.	1.42fghi
16	4 Calpan, Pue.	1.35ijk
17	28 Calpan, Pue.	1.22nopq
18	Huejotzingo, Pue.	1.16pqr
Wild		
1	42 Chiapas	1.97a
2	30B Chiapas	1.66cd
3	30B Chiapas	1.47efg
4	22 Chiapas	1.43efghi
5	17 Chiapas	1.42fghi
6	10 Chiapas	1.41fghij
7	38 Chiapas	1.39ghij
8	31 Chiapas	1.37hijk
9	32 Chiapas	1.34jkl
10	36 Chiapas	1.31klm
11	25 Chiapas	1.30klmn
12	26 Chiapas	1.29klmno
13	9 Chiapas	1.27lmno
14	40 Chiapas	1.25mnop
15	24 Chiapas	1.23mnopq
16	30 Chiapas	1.21opq
17	37 Chiapas	1.18opq
18	20B Chiapas	1.18opq
19	16 Chiapas	1.12pqr
20	20 Chiapas	1.11r
21	Chiapas s/n	1.11r
22	27 Chiapas	0.96s

Values with different letter differ significantly Tukey test $\alpha = 0.05$.

varies from 82.4% to 94.2%. Among the selections, small but significant differences are found. These values are similar to those found by Boboreko (1974) for various species of *Crataegus* collection in Byelorussia.

The interesting feature of the present study lies in the frequency distribution of the pollen germination presented by both groups of selections (Table 2). Those, included in the

TABLE 4. Anthers width of *Crataegus* selections.

Selections / Origin		Width (mm)
Cultigens		
1	Sn. Nicolas Tlaminca, Méx.	0.88a
2	Sn. Nicolas Tlaminca, Méx.	0.87a
3	2 Sn. Pablo Ixayoc, Méx.	0.85ab
4	5 Calpan, Pue.	0.83ab
5	Pecoso de Tlaminca, Pue.	0.79bc
6	4 Calpan, Pue.	0.77cd
7	Batan B, Pue.	0.76cde
8	4B Calpan, Pue.	0.74cdef
9	Siden	0.71efgh
10	48 Sn. Nicolas Tlaminca, Méx.	0.70efgh
11	15 Huejotzingo, Pue.	0.69fghi
12	Sn. Nicolas Tlaminca, Méx.	0.69fghij
13	B Calpan, Pue.	0.67ghijk
14	24 Calpan, Pue.	0.66ghijkl
15	Huejotzingo, Pue.	0.64ijklmn
16	28 Calpan, Pue.	0.64ijklmn
17	Calpan, Pue.	0.64ijklmn
18	12 Pecoso de Tlaminca, Méx.	0.62klmn
19	13 Huejotzingo, Pue.	0.54o
Wild		
1	42 Chiapas	0.83ab
2	31 Chiapas	0.80bc
3	32 Chiapas	0.76cde
4	17 Chiapas	0.74cdef
5	31 Chiapas	0.71defg
6	22 Chiapas	0.69fghi
7	26 Chiapas	0.69fghi
8	25 Chiapas	0.69fghij
9	30B Chiapas	0.68ghijk
10	10 Chiapas	0.67ghijk
11	20B Chiapas	0.66ghijkl
12	23 Chiapas	0.66ghijkl
13	30B Chiapas	0.66ghijkl
14	40 Chiapas	0.66ghijklm
15	20 Chiapas	0.66ghijklmn
16	9 Chiapas	0.65hijklmn
17	36 Chiapas	0.65hijklmn
18	38 Chiapas	0.63jklmn
19	37 Chiapas	0.61lmn
20	16 Chiapas	0.60mn
21	Chiapas s/n	0.60mn
22	30 Chiapas	0.60n
23	27 Chiapas	0.54n

Values with different letter significantly Tukey test $\alpha = 0.05$.

wild group gave 45.83% of selections with 88% to 90% of germinated pollen. Those, originated from the cultivated group gave selections of equal distribution of germination capacity.

The general conclusion can be drawn that the pollen germination does not constitute a limiting factor to the planned bre-

TABLE 5. Frequencies of anthers dimensions in *Crataegus* selections originated in Chiapas (wild) and in ceentral states of Mexico (cultigens).

Dimensions (mm)	Origin				Relations (Ch : C)
	Chiapas		Mexico + Puebla (Cultigens)		
Length					
2–1.80	1		3		
1.79–1.60	1		4		
1.59–1.40	6	8	8	15	0.53
1.39–1.20	10		2		
1.19–1.00	5		1		
≤ 0.99	1	16		3	5.33
Width					
0.89–0.80	2		4		
0.79–0.70	3	5	6	10	0.50
0.69–0.60	17		8		
≤ 0.59	1	18	1	9	2.00

eding program. Faulty pollen in *Crataegus* is frequently bo-
und with triploid or tetraploid species (Campbell et al., 1991).
The high percent of parthenocarpic fruits produced by both
groups of selections is surprising considering the high pollen
germination found. The selection # 9 gives 21% of partheno-
carpic fruits, while those originated from Sn. Nicolas Tlamin-
ca gives from 51.3 to 74.8%; those of the wild group give
from 7.8 to 57.5% of parthenocarpic fruits (Borys et al.,
1984). The causes of parthenocarpic fruits in those selections

are unknown. Embryo abortion at its early stages of develop-
ment may be its cause.
Apromixis may exclude the requirement for cross or auto-
pollination. Two studies with limited selections, using ema-
sculation, open and autopolllination, on limited numbers of
selections do suggest, that apomixis may play the important
role in *Crataegus* fructification in both groups of selections –
the cultigens and of the wild group (Flores Garcia et al.,
1991; Nieto Angel et al., 1993).

Anthers size

The second objective of the present study was to check if
pollen germination is related to linear dimensions of anthers.
The analysis of correlation showed lack of any relation be-
tween these variables. Both the germination and the size of
flower components could be influenced by drought during the
spring flowering, under the Mexican conditions. The unde-
hisced anthers of selections evaluated differed significantly in
both dimensions in each group (Tables 3, 4, 6). The range of
length and width value was similar in both groups.
The differences in length of anthers between some selec-
tions of the “cultigens” group were of special interest, e.g. the
groups 5 and 10 or 16, originated from the same commercial
orchard, but from different trees. Those trees from a pomolo-
gical point of view were equal, presumably. These trees were
selected initially on the basis of difference in the percentage
of seeds in stones (Borys et al., 1984). These selections were
regarded by the hawthorn growers as one horticultural type
(cultigen).
The pollnen yield per flower’s ovule, depended upon the
number of anthers and anthers size (Dickinson and Phipps,
1986). Low pollen yield per flower, combined with low ger-
mination may have influenced the percent of flowers conver-
ted into fruits. In the case of cultigen group of selections the

TABLE 6. Variance analysis of germination of pollen and the anthers dimensions.

Source of variation	Pollen germination			Anthers			
	D.F.	S.S.	Fratio	Length S.S.	Fratio	Width S.S.	Fratio
Selections	41	20.07	15.26**	0.25	236.76**	0.033	59.63**
Reps	4	0.134	0.99n.s.	0.001	1.69n.s.	0.0007	1.37n.s.
Error	164	0.13		0.001		0.0005	

** = significant, P ≥ 0.01; n.s. = not significant.

TABLE 7. Frequency of anthers length/width ratio in *Crataegus* germplasm collection.

Originated in State	f	Range of ratio values Length / width	Mean	Ratio L / W			
				(#)	<2.0 (%)	(#)	>2.0 (%)
Chiapas	24	1.68–2.44	1.98	14	58.33	10	41.66
Mexico	7	1.71–2.61	2.13	3	42.86	4	57.14
Puebla	10	1.71–2.73	2.17	4	40.00	6	60.00

per cent of flowers converted into fruits is lower than in the wild group (Borys, paper in preparation). Moreover, it varied greatly the percentage of seeds formed, in relation to the number of endocarpium per fruit or in relation to stones number in particular selection (Borys et al., 1984). Perhaps, this is somehow related to the pollen yield per ovule as suggested by the data of Dickinson and Phipps (1986). Our data, although of limited number, indicated that our selections of both groups do not vary much in the number of stamens and stigmas (Nieto Anger et al., 1993). Thus, it seemed that the yield of pollen per flower was not limited by this attribute. The size of pollen, its yield per ovule, the relations among the ploidy, anthers size, pollen viability and the presence of seeds remain to be defined in order to infer the presence of apomixis.

The frequency distribution of anthers size (length, width and ratio length/width) presented by both groups of selections suggested the occurrence of a peak of frequency typical for the wild and the cultigen group (Tables 5, 7). Those of the wild group were mostly of smaller size, while those of the cultigen group were mostly of larger size. Anthers size was used as one of the morphological attributes in taxonomic differentiation and in studies of reproductive biology of *Crataegus* (Campbell et al., 1991). Such attributes could be applied in pomological differentiation of a particular selection from a group of plants.

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KIELKOWANIE PYŁKU IN VITRO U MEKSYKAŃSKICH GŁOGÓW (*CRATAEGUS* SPP.)

STRESZCZENIE

Oceniono zdolność kielkowania pyłku in vitro 42 selekcji głogów należących do grupy uprawnej (*Crataegus pubescens* (H.B.K.) Steud. = *C. mexicana* Moc. et Sessé) oraz do grupy dzikich (należących przypuszczalnie do *C. nelsoni* Eggleston). Scharakteryzowano również wielkość pylników każdej selekcji. Obydwie cechy oznaczono w fazie zamkniętego pąka kwiatowego w okresie wiosennego kwitnienia, używając zamkniętych pylników.

Procent skielkowanego pyłku wahał się od 82,4 do 94,2%, zależnie od selekcji; obydwie grupy selekcji dały podobne wartości kielkowania. W grupie dzikich 45,83% selekcji dało 88-90% pyłku skielkowanego. Selekcje z grupy uprawnych dały szeroki rozrzut procentu kielkowania. Wielkość pylników – długość i szerokość – zależała od selekcji. Selekcje z grupy uprawnych miały w wyższym procencie większe rozmiary, a z grupy dzikich mniejsze. Stwierdzono brak korelacji między zdolnością kielkowania a rozmiarami pylników. Uzyskane wyniki stanowią przedmiot dyskusji w odniesieniu do problemu produkcji owoców oraz reprodukcji generatywnej przedstawicieli meksykańskich należących do rodzaju *Crataegus* znajdujących się w kolekcji banku genotypów.

SŁOWA KLUCZOWE: kielkowanie pyłku, in vitro, *Crataegus* spp.