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Milla biflora Cav. – seeds, harvest and germination

MICHAŁ W. BORYS, HELENA LESZCZYŃSKA-BORYS

Universidad Popular Autónoma del Estado de Puebla Agronomía – Recursos Naturales
21 Sur 1103, Còl. Santiago 72160 Puebla, Puebla (México).

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Summary

Milla biflora Cav. is one of six species of this genus reported to be present in Mexico. To determine the present and potential seed production of this plant, the following characteristics were studied: the number of flowers per scape, the frequencies of scape with defined number of flower per scape, the conversion of flowers into capsules, the capsule size, seed numbers, average seed size per capsule. Samplings were taken from the natural stands and evaluation of the respective attributes was done in plants growing in germplasm collection belonging to the University UPAEP.

The number of flowers ranged from 1 to 10 per scape, with 90.6% of scapes giving from 1 to 6 flowers. The capsules were formed from flowers in the following proportions: in scapes with one flower – 13.64%, in scapes with 3 flowers – 48.21%, in scapes with four or more flowers – 60%; 67.80 of capsules gave from 60 to 140 seeds; 45% of seeds had the average size from 1.81 to 2.20 mg. The mean weight of 1000 seeds was 1937.2 mg. The seeds germinated during 7 days in water, in 88 to 100%. The plant emergence from soil sown seeds ranged from 41.4 to100 %. The seeds have not lost the germination ability after 2 years of storage at room conditions.

Key words: flower conversion into capsules, seed size, seed production, capsule size, number of seeds, ornamental plants seeds, germination.

INTRODUCTION

The following species of *Milla Cav.* are present in Mexico: *Milla biflora* Cav., *M. bryanii* I.M. Johnson, *M. delicata* H. Moore, *M. magnifica* H. Moore, *M. mortoniana* H. Moore, *M. oaxacana* Ravenne, *M. rosea* H. Moore (Espejo Serna and López Ferrari, 1993). *Milla biflora* is the most frequently found in natural habitat. According to Bailey (1953) it is „an attractive, spring flowering bulb excellent for cutting and last several days". Reiche (1977) indicates that *M. biflora* constitutes „beautiful component of fields due to its graceful white flowers, which emanate a pleasant delightful aroma during the night'.

The genus *Milla* is an integral part of the flora of xerophytic matorrals of Mexico (Rzedowski, 1991). The geophytes of xerophytic matorrals could form an option to mesophytic flora in the formation of green areas of the dry and hot sites of cities and road sides for regions of dry/wet seasons. This species is not abundant enough to secure sufficient seeds or corms for gardening purposes. Commercial plantations require basic data upon the generative reproduction and seeds basic characteristics. Thus, the aim of this report is to present the results of evaluation of seeds production, characteristics and germination of the species.

MATERIAL AND METHODS

Aleatory sampling of wild stands of plants (Valsequillo, Puebla, Pue.; road side of Puebla – Cholula, Pue.) was done in 1991, 1995 and 1996. All capsules were collected from the collection established at the Germplasm Bank of Bulbous Species in the Universidad Popular Autonoma del Estado de Puebla, in 1995 and 1996. Geographic location of the last is 19° 31′ 30" – 19° 06′42" N and 98° 15′ 06" – 98° 24′ 00" W and approximate elevation of all stands above the sea level 2100-2200 m. The climate of all sites is subhumid with rainfalls in the summer. The precipitations of the driest month lies below 40 mm. The winter precipitations reaching < 5% of the total annual of 798 mm. The temperatures of the coldest month ranging from -3 to 18°C. The wet season, frequently presents spells of dry, hot weather lasting from two to three weeks. This, combined with an evaporation rate exceeding almost three times the precipitations, and sandy volcanic soils, with underlying hardpan results in rapid development of soil water deficit.

Plants in their natural habitat of Valsequillo were scattered in a radii of 800 m; those on the road side were grouped in 100 m distance, approximately. Normally, only one capsule per infrutescence could be collected from those stands. All mature capsules were harvested from plants in the collection (opened capsule or still closed with outside walls of yellow-white coloration and black seeds).

The term „capsule initially" refers to the initial time of capsule growth; only part of such capsules were reaching final maturity. The „mature capsule" refers to those with normal mature seeds reaching harvest maturity (black seeds of normal size). Germination tests included seed samples from a composite sample. The seeds were taken from capsules, taken at random, from different plants of stands from the collection. Fifty seeds or one capsule constituted one replication. Seeds were placed in Petri dishes containing only water, at 18 to 24° C (night/day) and in room light.

Emergence of plants from seeds sown in the soil was evaluated using all seeds from a capsule. This was done to define the number of days from sowing to emergence of plants under field and greenhouse conditions. In order to compare the results of germination tests the variance analysis was done, using the F test (0.05 P). Some regressions were done too. The seed size and the yield of seeds per capsule were measured in 1991, 1995 and 1996. The seed size and weight were measured after 2 months of seeds storing at room temperature (16 to 24° C).

RESULTS AND DISCUSSION

Data reported include results of visual observations, measurements taken from the number of flowers, capsules initially formed and mature capsules harvested, capsule and

seed mass, harvest time and seed main features, seed germination. Other horticultural attributes, e.g. size of flower and flowering will be reported elsewhere.

Visual observations upon capsule development

Observations conducted upon pollination revealed lack of visiting insects or birds. This question merits special study. The formation of capsules and seeds seems to occur by autopollination or night insects. The production of aroma during night indicates this last possibility.

The capsule growth was largely dependent upon the soil water conditions at anthesis and during the following first week. Under above mentioned edaphic and air climatic conditions (shallow sandy soil, spells of dry, hot weather) the capsule stopped growing, the perianth tube presented discoloration and started to dry with final abscission of the entire structure. If in such conditions some seeds were formed, then, the capsule size and the seed size were strongly reduced (0.33 mg/seed). Seeds of these sizes did not germinate.

The dry, hot weather and clear sky, at the time the capsules were maturing, might cause large losses in seeds due to sudden opening of capsules. Thus, it was necessary to cut the capsules at the very early day hours, when the capsules were still closed, when their external color was changing to clear-yellow-whitish and when the capsule-cover have started to open.

The yield components of seeds

The main components studied of seed yield were: the number of flowers per inflorescence present and converted into capsules, the capsule size, the number of seeds per capsule and the seed size. Two components, which were not measured: the number of scapes per corm and the corm size are currently under study.

Flower number per inflorescence

The flower number per inflorescence depended upon the site and the year (Table 1). Six flowers per scape in natural habitats seems to be the highest observed by botanists (Rzedowski, 1991; Reiche, 1977). Also Bailey (1958) reported a range of flowers usually to be from 1 to 5. In wild stands of *Milla biflora* of Valsequillo and valdillo only one specimen with 10 flowers was found in 1991. The number of flowers per inflorescence found in the Collection ranged from 1 to 10 per scape, with 90.6% of scapes giving from 1 to 6 flowers (Table 3).

Transformations of flowers into capsules

This component of seeds yield depends primary upon the presence of pollen transported by insects or birds. No data is available upon pollen transporting organisms. During the day we have never seen insects or birds visiting flowers of *Milla biflora*.

The data presented in Table 1, 2, 3 indicate that the percentage of flowers having been converted into capsules is rather low and may be influenced by habitat and year. The data of Table 2 indicate that the flower convertion into capsules is concentrated to a short time. There was 30% rise in the number of capsules initially set in two weeks.

Table 3 indicate that under clean cultivation (plants in the collection) the scapes had higher number of flowers than it was reported by Bailey (1958), Rzedowski

(1991) or Reiche (1977). More interesting was that the increase of flower number per scape was not accompanied by the increased percentage of flowers transformed into capsules. The critical, limiting, value seems to be 3-4 flowers per scape with 48-61% flowers converted into mature capsules. Further increase in the number of flowers per inflorescence did not improve the convertion of flowers into capsules.

Table 1

Convertion of flowers into capsules.

Origen of samples	Number of inflorerescences observed	Number of flowers formed	Flowers converted into capsules (%)	Average number of flowers per scape
1995				
Valdillo Collection	48 48	128 103	39.07 23.30	2.67 2.15
1996				
Collection	267	1007	54.02	3,8

Table 2

Number of flowers and capsules initially set and observed at two dates.

	19.VII.96	7.VIII.96	Increase
Inflorescences number	167	267	100
Flowers number	778	1007	229
Capsules number	187	544	357
Flowers converted into capsules (%)	24	54	30

Table 3

Tramsofrmation of flowers into mature capsules as dependent on the number of flowers per inflorescence. Collection 1996.

Flowers per inflorescence	Number of inflorescence	Flowers formed total	Flowers transformed into capsules (%)
1	22	22	13.64
2	48	96	32.29
3	68	195	48.21
4	49	196	61.23
5	30	150	52.00
6	25	150	60.67
7	13	91	67.03
8	4	32	62.50
9	5	45	62.22
10	3	30	60.00

From the data included in Table 1 and 2 it may be concluded that the flower convertion into capsules depends upon the site and the year of observation. Elimination of competing plants (grasses, shrubs) in the collection can explain the higher convertion of flowers into capsules. This, however, did not improve the percentage of flowers converted into capsules in scapes of higher than 3 to 4 flowers per scape. The only explanation which could be offered at present is that the limiting factor constitute the low soil water. It was observed that under high temperature, dry air and low soil water the stigma was dry, which normally prevents pollen germination.

Seeds yield per capsule

The next component of seed yield constitute the number of seeds per capsule. This attribute varies from a minimum of 20, maximum 180 and an average of 82 seeds per capsule in a sample of 115 capsules collected in 1996. The frequency of capsules with variable seed number is illustrated by Fig.l. The seed number per capsule depends principally upon capsule size. These relations illustrate respective regressions (Table 4).

Table 4

Regressions related to some seeds yield components[1)].

	R^2	r
1. Seeds number per capsule (y) function of:		
a) capsule weight with seeds (PCS) $y = 0.0695 + 0.0019$ PCS	0.524	0.724
b) capsule weight without seeds (PC) $y = 0.0171 + 0.0017$ PC	0.524	0.736
2. Weight of one seed (y) function of		
a) seeds number of capsule (SC) $y = 94.55 - 26.68$ SC	0.720	0.85
b) capsule weight with seeds (PCS) $y = 0.24 - 0.05$ PCS	0.32	0.57
c) capsule weight without seeds (PC) $y = 0.18 - 0.05$ P	0.45	0.67
3. Number of germinated seeds (y) function of		
a) seeds number of a capsule (SC) $y = 7.76 + 1.18$ SC	0.62	0.79
4. Percent of germinated seeds (y) function of		
a) seeds number in a capsule $y = 123.24 - 83.60$ SC	0.40	0.64

[1)]1991 Data

Seed mass

Plants of wild habitat, sampled in 1991 in Valsequillo, gave one capsule with seeds of 0.74 mg minimum average mass, 3.06 mg maximum and an average mass of seeds of all capsules 1.35 mg. The average seed mass taken from 52 capsules from plants of the germplasm collection, (1996 harvest), was 2.0 mg; the minimum size was 0.33 mg and maximum 3.3 mg per seed. The frequency of seed mass/capsule in this sample is presented in Fig. 2.

The seed mass per capsule is influenced by the seed number per capsule and capsule weight, (regressions 2 a, b, c; Table 4). These data suggest that there exists a competition for some internal factors between the seeds and the capsule affecting the seed quality (seed size).

Weight of one thousand seed, the seeds number in 1 g and 1 cm^3 samples are listed in Table 5 with respective values of standard deviations.

Table 5

Weight of one thousand seeds of one seed and number of seeds in 1 g and 1 cm^3.

	Weight of 1000 seeds (mg)	Seeds number in 1g	1cm^3	Mean weight mg/seed
Minimum	1643.9	477	92	1.68
Maximum	2061.8	591	115	2.10
Mean	1937.2	536	104	1.88
Standard deviation (SD)	134.9	39.8	36.3	0.14

Data of 14 samples taken from 220 g of seeds harvested from plants forming the Collection.

Seed harvest

The harvest time of capsules was restricted in 1996 to two weeks (9.08 to 22.08, with 57% of capsules harvested between 13 and 20.08).

Potential of productivity is visualized by the range of seed number per capsule, which oscillate between 20 and 181 (Fig.l), capsule number per scape (theoretically 10 capsules) and scapes number per corm (unknown value). We got in 1996 44 g, equivalent to 22000 seeds, per 3.2 m^2 of plant stands (distances among plants can not be given due to the grouping of corms). Some data on seed yield are listed in Table 6.

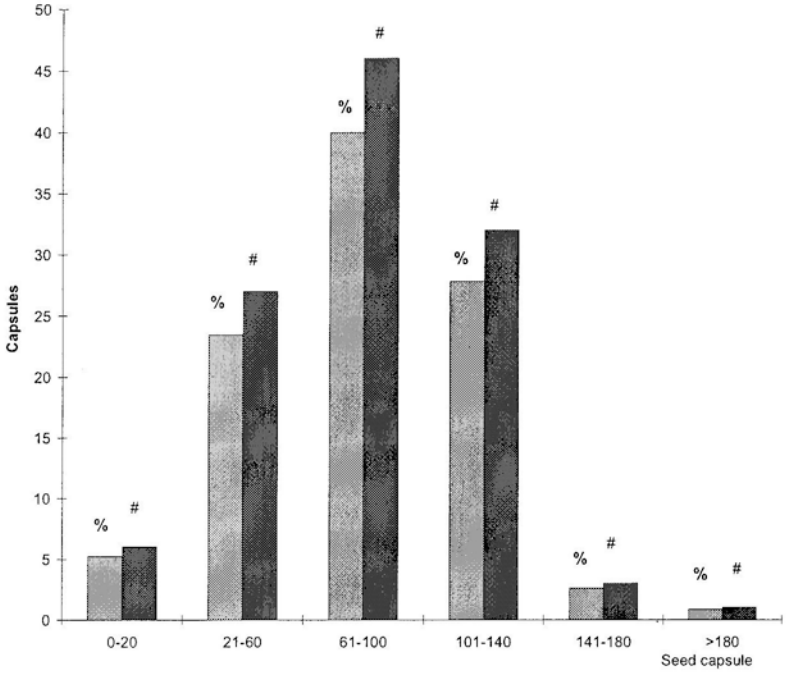

Fig. 1. Frequency of capsules with seed number per capsule

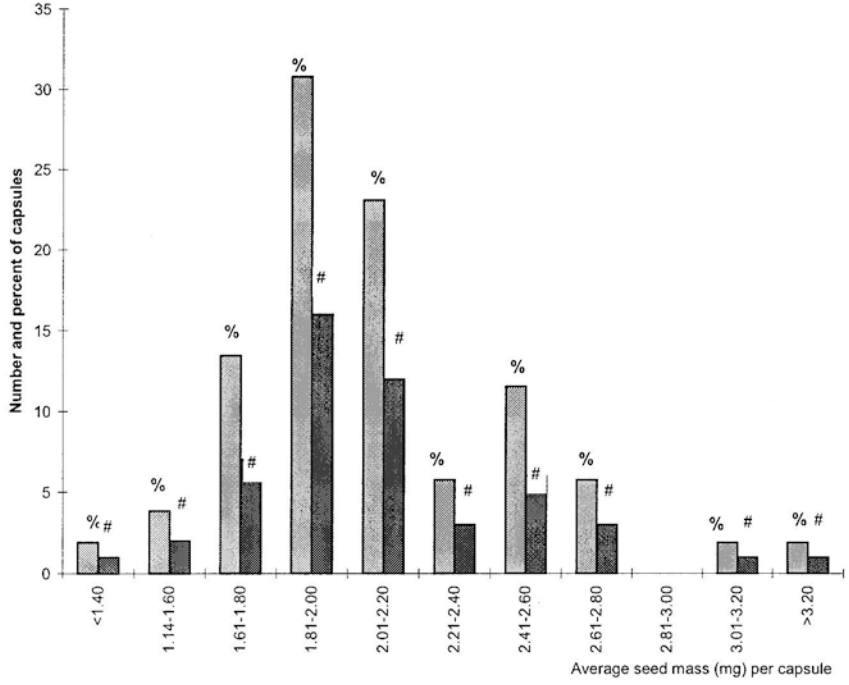

Fig. 2. Frequency of capsules with the averege seed mass (mg).

Table 6
Estimation of seed yield

Capsules harvested	Total mass capsules with seeds (mg)	Seed mass (%)	Total number of seeds	Seed number per capsule
31	9523.6	72.4	3342	107.8
18	4100.6	57.6	1209	67.2
74	14384.2	64.7	4580	61.9
63	16633.1	50.1	4099	65.1
12	2921.0	63.4	912	76.0
18	3847.3	61.0	1528	84.9
27	5770.9	61.0	1889	69.9
94	14849.0	63.0	4467	47.5
Total	72029.8		22026	580.3
Average		69.0		72.6

Seed germination and seedlings emergence

Seeds do not present dormancy and can be sown directly after the harvest of capsules. The data available indicate that they may be stored for 2 years at ordinary room conditions (temperature 14 to 24° C, day light and 60% relative humidity) without any loss in germination capacity.Tests with seeds harvested in 1995 and 1996, in Petri dishes, in water, at room temperature gave during 7 days from 88 to 100% of germinated seeds (Table 7). The number and per-cent of germinated seeds depended on the number of seeds per capsule. It is illustrated by regressions 3 and 4 (Table 4). Thus, this attribute should be taken into account when studying seeds quality as a function of capsule distribution on infrutescence or when studying the process of capsules maturation as a function of time.

Table 7
Seed germination

Collection number[1)]	Number of seeds sown on 24.01.96	Germination 1.02.96 (%)	Number of seeds sown on 7.10.96	Germination 14.10.96 (%)
	1995 harvest[1)]		1996 harvest[2)]	
1	70	98.57	50	96.0
4	16	93.75	50	100.0
13	50	98.00	50	100.0
24	150	96.00	50	98.0
26	74	100.00	50	96.0
29	112	88.39	50	94.0
78	65	92.31		

[1)] All seeds from one capslue of each collection were sown. Germination test conducted in water in Petri dishes, at room temperature, in the light.

[2)] From compound samples of 10.0 g of seeds of various plants.

The seeds were sown in open-field conditions, at a depth of 1 to 1.5 cm, in sandy, permeable, volcanic, sandy soil. After watering, the surface was covered with carton-paper till the first plants emerged. The emergence (19.66 to 56.7%) occurred aproximately in 3 weeks (Table 8).

Table 8

Plant emergence from seeds sown under open field condition in volcanic soil. The seeds from 1995 harvest.

Collection number	Number of seeds sown on 22.03.96	Emergence	
		after 17 days	after 24 days
		(%)	
47	42	26.19	35.71
48	44	9.09	29.55
50	45	13.33	42.22
52	109	20.18	36.70
53	117	1.71	19.66
54	57	28.82	52.63
56	40	7.50	37.50
57	97	7.22	56.70
58	55	9.09	36.36
60	116	7.76	43.96
Average	72	9.85	39.09

[1] All seeds from one capslue were sown of each collection at 1 cm depht at ambient temperature (15/25°C). Soil pH 5.8.

Table 9

Emergence of seedlings from seeds sown in the soil in greenhouse conditions 1991.

Average seed mass per capsule (mg)	Number of seeds sown[1]	Seedling Emergence (%)
0.740	70	41.4
0.742	62	66.1
0.780	98	58.2
0.792	76	85.5
1.019	69	78.3
1.194	49	89.8
1.206	67	71.6
1.369	55	78.2
1.454	50	76.0
1.600	65	90.8
2.182	23	91.3
3.060	20	100.3

Each lot of seeds originated from one capsule.

The seeds sown on the greenhouse bench (1991) in a substrate composed of volcanic sand and forest organic matter (1:2), using seeds of variable size showed an emergence range from 41.4 to 100% (Table 9). The data presented in Table 9 suggest

that the seeds with the mass smaller than 0.74 mg should not be used for commercial purposes due to low percentage of emerged plants (seeds of 0.33 mg did not germinate in water).

Sowing seeds in an unsterilized substrate, high in organic matter should be avoided, especially when germination runs in low greenhouse temperature (below 10°C) from December to February. This may result in high losses of seedlings from root patogens. Only from 1 to 19.6% of plants were secured per 500 seeds sown in 23 cases.

This paper summarizes basic information upon some aspects of seed production and seed quality of *Milla biflora*. The generative propagation is a basic method of reproduction of this species and therefore it seemed important to find all negative influences which could affect the commercial production of seeds. We expect, that with the data presented in the present paper a part of unknown factors may be eliminated. Corms produced from seeds may contribute to wider introduction of this interesting plant to gardening and landscaping.

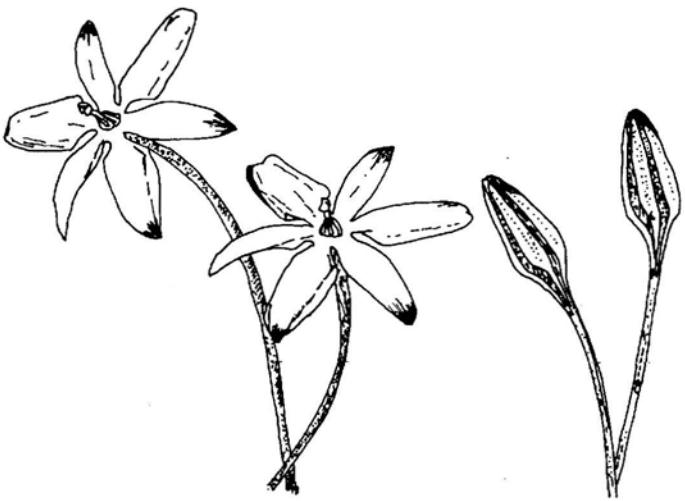

Fig. 3 Flower buds and open flowers of *Milla biflora* Cav.

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Milla biflora Cav. - nasiona, zbiór, kiełkowanie

Streszczenie

Milla biflora Cav. jest jednym z sześciu gatunkow z tego rodzaju występujących w Meksyku. W celu określenia aktualnej i potencjalnej produkcji nasion przeprowadzono następujace obserwacje: ilość kwiatów na pędzie, częstotliwość występowania pędów z określoną ilością kwiatów, rozwój torebek nasiennych z kwiatów, ilość nasion w torebce i średnia wielkość nasion w torebce nasiennej. Próbki pobierano ze środowiska naturalnego oraz z kolekcji. Przeprowadzono ocenę wszystkich powyższych cech na roślinach znajdujących się w kolekcji germoplazmy roślin bulwiastych.

Ilość kwiatów na pędzie wynosiła od 1 do 10; 90.6% pędów tworzyło 1 do 6 kwiatów. Zamiana kwiatów na torebki nasienne wystapiła u 13.6% pędów z 1 kwiatem, u 48.2% pędów z 3 kwiatami i u 60% pędów z 4 lub więcej kwiatami. 67.8% torebek nasiennych dało po 60 do 140 nasion. Średnia masa 45% nasion wynosiła od 1.81 do 2.2 mg. Średnia masa 1000 nasion wynosiła 1937.2 mg. Nasiona kiełkowały w wodzie w ciągu 7 dni w 88 do 100%. Kiełkowanie nasion wysianych do gleby wynosiło od 41.4 do 100%. Nasiona mogą być przechowywane w warunkach laboratorium 2 lata bez strat w sile kiełkowania.