Adv. Hort. Sci., 2018 32(4): 549-556 DOI: 10.13128/ahs-23504

AHS
Advances in Horticultural Science

Flower development and pollen vitality of *Moringa oleifera* Lam. grown in a humid temperate climatic condition

S. Radice [1], E. Giordani [2 (*)]

[1] *Department of Plant Physiology, FAyCAUM - CONICET, Machado 914, University of Morón, Argentina.*

[2] *Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.*

Key words: fertility, flower anatomy, microsporogenesis.

(*) **Corresponding author:**
edgardo.giordani@unifi.it

Citation:
RADICE S., GIORDANI E., 2018 - *Flower development and pollen vitality of Moringa oleifera Lam. grown in a humid temperate climatic condition*. - Adv. Hort. Sci., 32(4): 549-556

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 26 June 2018
Accepted for publication 28 September 2018

Abstract: *Moringa oleifera* is a tropical tree cultivated in many countries. This species has acquired a great importance in human nutrition and it was recently indicated as a "novel food" by the European Commission. Recently, moringa plants have been introduced in humid temperate climatic areas, among which Moreno (Buenos Aires Province - Argentina). In such area, the cultivation is possible for the production of leaves, but plants need protection during winter time in order to overcome damages due to low temperatures and hence to produce capsules and seeds. The main objective of this research was to study flower morphology and anatomy of *M. oleifera*, as well as microsporogenesis and viability of pollen grains of plants cultivated in Moreno in comparison with those produced in a humid sub-tropical climatic area of Argentina (San Miguel de Tucumán). Flowers grown in the temperate environment resulted similar for morphological parameters to those observed in the sub-tropical environment. Nevertheless, pollen grain fertility depended directly on air temperature and it was negatively affected by the lower temperatures registered in the temperate site. According to the observed results, pollen viability increases with mean monthly temperatures above 16°C.

1. Introduction

Moringa oleifera Lam. (moringa) is a multipurpose small to medium-sized, evergreen or deciduous tree, native to northern India, Pakistan and Nepal. It has a spreading open crown with drooping, fragile branches, feathery foliage with tripinnate leaves, and a thick corky whitish bark (Marcu, 2005). *M. oleifera* is utilised as animal fodder and employed in human nutrition due to its healthy properties (Fuglie, 1999; Palada and Chang, 2003; Ganatra *et al.*, 2012; Paula *et al.*, 2017), as well as in the production of fuel (Foidl *et al.*, 2001), water sanitation (Wilson, 1992; Lekgau, 2009; Padilla *et al.*, 2012). Moringa leaves are considered a "novel food" by the European Commission, so confirming their valuable properties in terms of energy, nutrients, proteins and minerals, as reported by several authors (Atawodi *et al.*, 2010; Tende *et al.*, 2011; Yameogo

et al., 2011; Gopalakrishnan *et al*., 2016; Vats and Gupta, 2017). Araujo *et al.* (2016) highlighted also the importance of *M. oleifera* in regions characterized by desertification and water deficit.

Moringa cultivation is expanding all over the world, including in climatic areas, which differ noticeably from those of its tropical origin. Recently some experiments showed the feasibility of cultivating moringa in the humid temperate climatic conditions of Buenos Aires Province (Argentina) for leaf production. Leaf extracts from trees grown in that conditions showed higher phenol content and antioxidant activity than those obtained from plants cultivated in typical tropical climates (Arena and Radice, 2016). Nevertheless, flower differentiation, anthesis and fertility resulted negatively altered and the production of pods and seeds, both of them important source of nutrients, was very low. The main objective of this research was to study the effect of air temperature on flower morphology and anatomy of *M. oleifera*, as well as on the microsporogenesis and pollen grain viability, observed on trees cultivated at Moreno (Buenos Aires Province) in comparison with those grown in San Miguel de Tucumán (Argentina), the first characterized by a humid temperate climate and the latter by a humid subtropical environment.

2. Materials and Methods

Plant material

All plants were obtained from the same seed lot. Homogeneous seedlings (n= 10) were grown in soil and in open air and cultivated in San Miguel de Tucumán (26° 49'59.00" S, 65° 13'00" W, elevation 456 m asl), while another similar set of seedlings (n=10) was planted in Moreno (34° 39' 0" S, 58° 47' 0" W, elevation 14 m asl) in 25 l plastic pots under a glasshouse from April to September. Successively pots were placed in open air. Moreno has a humid temperate climate, with an average temperature of 23.4°C in January and 10.0°C in winter time (June); San Miguel de Tucumán, has humid subtropical climate (19.4°C the average annual temperature) with a hot and long summer and mild and dry winter. The precipitation pattern is monsoonal with an average of 997 mm (climate-data.org). A set of monthly air temperature parameters is reported in figure 1.

Flower morphology

Flowers (*n = 100*) on different phenological stages were observed on both groups of plants, and samples were collected monthly for further observations from September to December. Button flower collected were used fresh and fixed in FAA (formaldehyde, 100 ml; ethyl alcohol, 500 ml; acetic acid, 50 ml; distilled water, 350 ml).

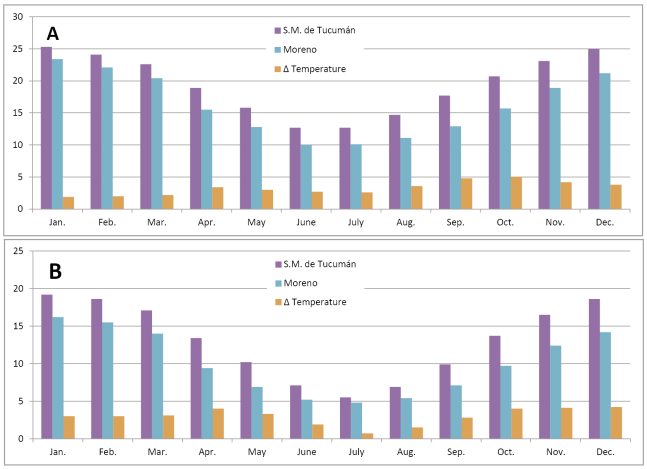

Fig. 1 - Mean (A) and average minimum (B) air monthly temperatures (°C) in San Miguel de Tucumán and Moreno locations.

Light microscopy

Button flowers (*n= 10*) were immediately frozen to -25°C and embedded in a medium consisting of polyethylene glycol and polyvinyl alcohol. Successively they were cut frozen by microtome inside the cryostat. Histologic slices were cut at 5 to 10 µm.

A set of ten button flowers were dehydrated in an ethanol series and embedded in Spurr's resin. Thin sections (75-90 nm thick) were stained with uranyl acetate and lead citrate. Sections were observed with a Leica DM 2500 microscope.

Fluorescent microscopy

Flowers in anthesis phase (*n= 50*) fixed in FAA were shaved with distilled water and softened with NaOH (8N) as described by Martin (1959). Then, they were stained with aniline blue to study pollen tube growth. Squash material was observed by a Leica microscope (DM 2500) using fluorescence with excitation filter BP: 450-490.

Scanning electron microscopy (SEM)

Button flowers fixed in FAA (*n= 10*) were dehydrated in an ethanol series and critical point-dried with liquid CO_2 was employed. Then it was sputter-coated with gold-palladium (40% gold and 60% palladium) for 3 minutes. Samples were observed with Philips XL30 SEM.

Pollen viability

Pollen viability was performed with fluorescent microscopy according to Radice and Arena (2016) on fresh anthers taken from button flowers of two localities (Moreno and San Miguel de Tucumán). Pollen evaluation was expressed in percentage. To determine the statistical significance of the hypothesis the chi-squared test (χ^2) was used.

3. Results

Flower development

Flower development starts with the appearance of the flower on a clustered inflorescence (Fig. 2). These first buds are green reddish and about 1mm long (Fig. 2A). They grow up to about 10mm and turn to white greenish (Fig. 2A). Anthesis takes place sequentially among the flowers of the inflorescence (Fig. 2B). At anthesis, flower shows a zygomorphy symmetry. The larger transversal petal is bent upwards, while the others are reflexed downwards together with the sepals (Fig. 2C). Anthers are yellow and no dehiscent; flowers have odour and nectar in this phase.

As the flower develops, anthers change colour to dark yellow (Fig. 2C) and finally to brown (Fig. 2D). It was observed that 1-3 anthers were not developed in flowers collected from Moreno field. Pistil, which at the time of the anthesis is below the anthers, continues to grow until it protrudes several millimetres above the androecium (Fig. 2C). Finally, petals wither and fall, while the ovary enlarges and turns to reddish colour regardless its fertilisation (Fig. 2E).

Flower structure

Moringa oleifera plants grown in Moreno and San Miguel de Tucumán experimental fields developed flowers with average values of ≈5 petals, ≈6 sepals, ≈6 stamens, ≈5 staminoides and 22 ovules (data not showed). In the observed flowers, the unique largest petal (referred as “primordium petal”) stands right; the others are folded (Fig. 2C). Female part of the flower shows the complete pistil with the style and a hairy ovary (Fig. 3A). Ovary is tricarpelar and ovules are located in parietal placentation (Fig. 3B). Stigma is just a hole (Fig. 3C). Some nectarostomata surround the gynophore (Fig. 4). Glandular hairs, with the function of expelling the nectar, are present on the nectarostomata surface (Fig. 4A). Nectarostomata produces nectar in sub epidermal cells, that accumulate it and transfer it through the intercellular spaces (Fig. 4B).

Frozen section of a button flower just before the anthesis phase allows to appreciate the state of the

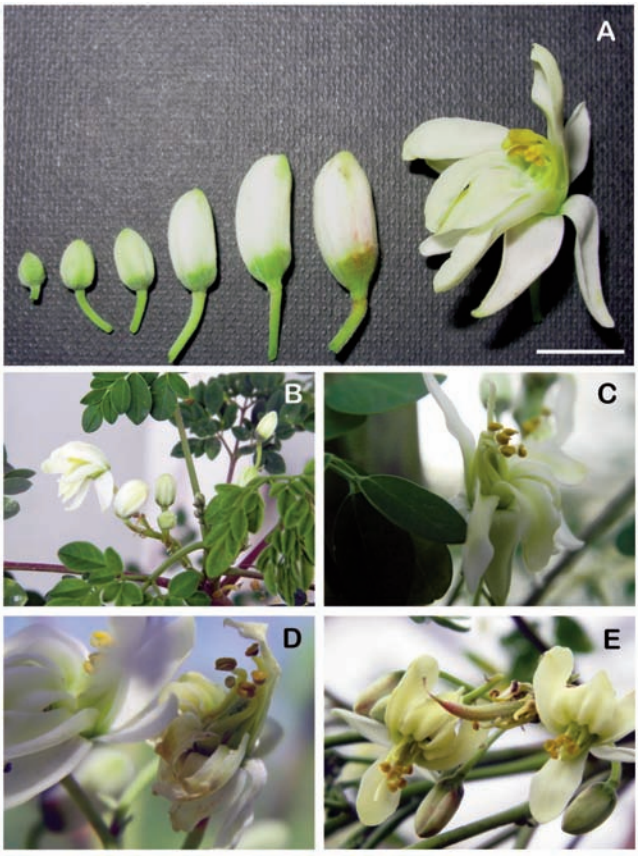

Fig. 2 - Flower development of *Moringa oleifera* Lam. A, from button flower to anthesis. B, beginning of anthesis. C, anthesis; D, flower senescence; E, pod formation. Bar = 1 cm.

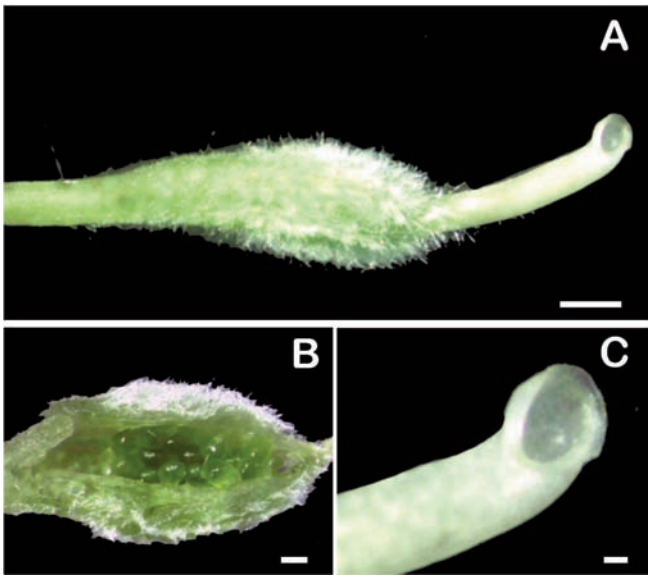

Fig. 3 - Pistil of *Moringa oleifera* Lam. A, external view of the pistil; B, internal view of the ovary with ovules; C, detail of the stigma. Bars = A-C, 1mm.

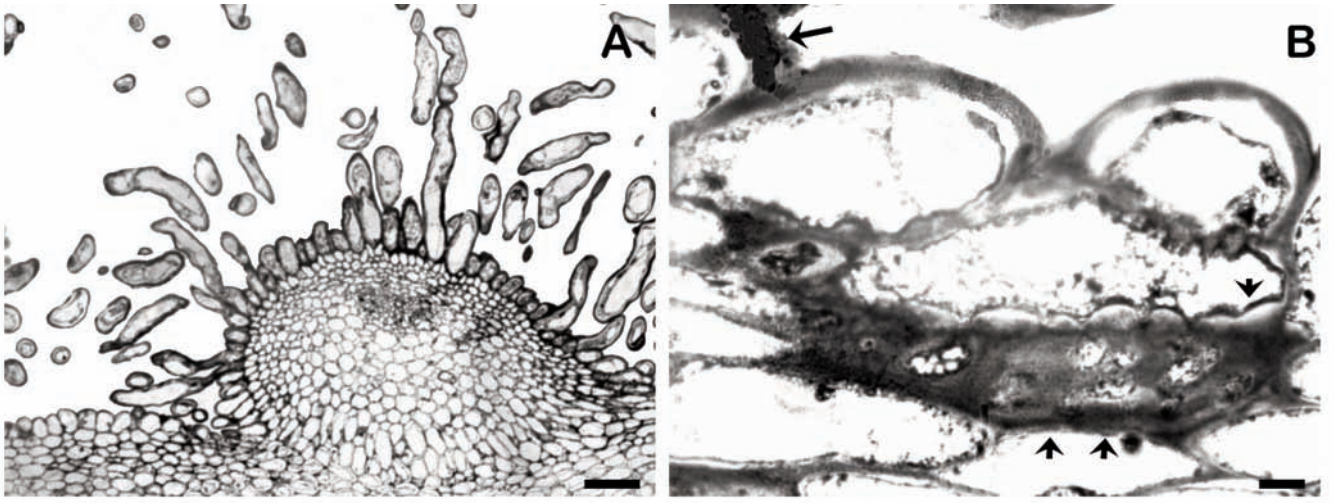

Fig. 4 - Nectary of *Moringa oleifera* Lam. (light micrographs). A, longitudinal section of the nectary; B, detail of the nectar cumulated in the intercellular spaces (arrows). Bars = A, 100 µm; B, 10 µm.

structures and their normal coloration (Fig. 5). In fact, it is possible to see anthers with pollen grains already formed wrapped in a yellow substance similar to sporopollenin (Fig. 5B). Pistil shows developing ovules attached to the carpelar wall (Fig. 5A). Finally, all internal organs are enveloped by sepals and petals (Fig. 5A).

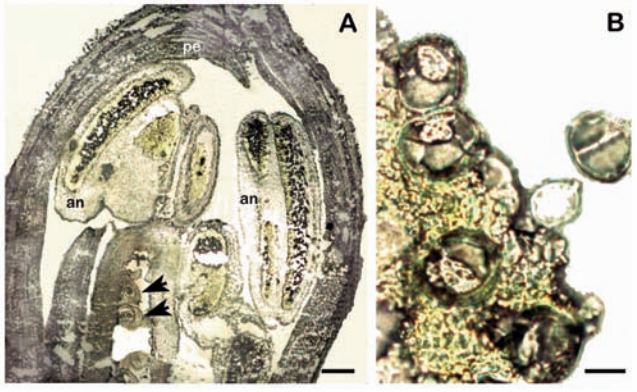

Fig. 5 - Flower in pre anthesis phase of *Moringa oleifera* Lam. (frozen section). A, Longitudinal section of flower with petals (pe), anthers (an) and ovary with ovules (arrows); B, mature pollen grain surrounded by sporopollenin. Bars = A, 10 cm; B, 10 µm.

Flowers studied by SEM showed there is no defined stigmata structure. Pistil is coronate by a smooth cell structure as a continuation of the style (Fig. 6A). The internal cavity of the ovary is covered with hairs (Fig. 6C) and ovules adhere to their walls on the connection of two carpels. Ovules appear to be campylotropous (Fig. 6D).

Microsporogenesis

Button flowers from 1mm to 10 mm (Fig. 2A) have been used to observe different steps of pollen grain formation. Different stages of pollen grain differentiation were observed: microsporocytes mother cells, tetrads, microsporocytes and mature pollen (Fig. 7). Development was very fast and it accomplished in less than one week.

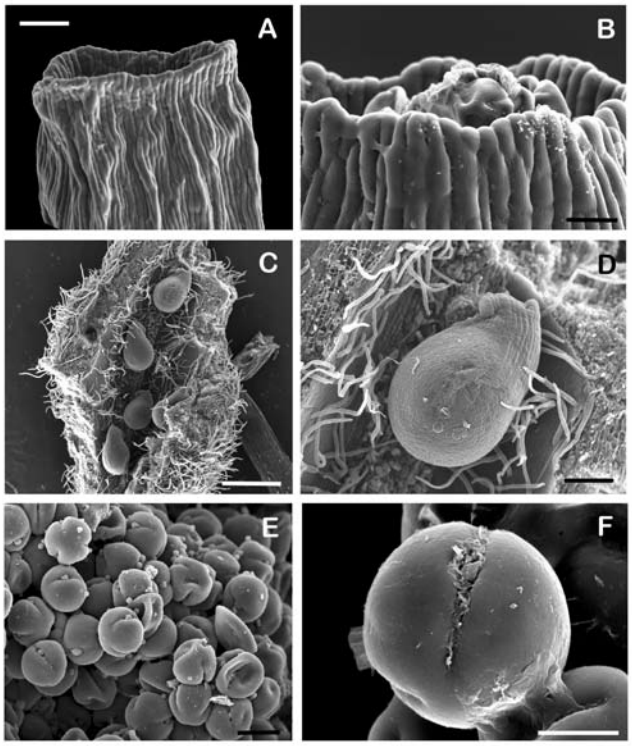

Fig. 6 - SEM micrographs of *Moringa oleifera* Lam pistil. A-B, style and stigma; B, detail of the stigma with pollen grain; C, ovarian cavity with ovules; D, detail of an ovule; E-F, pollen grains. Bars = A, 50 µm; B, E, 20 µm; C, 500 µm; D, 100 µm; F, 10 µm.

Moringa flowers have monothecal anthers. Button flowers shorter than 5 mm contains inside the just formed pollen sac microspore mother cells (Fig. 7A). These cells show a big and visible nucleus and a very dense cytoplasm indicating a high activity.

During this stage, the last inner layer of the anther wall corresponds to tapetal cells that are formed by large and binucleate cells (Fig. 7A).

As the buds lengthen, more advanced stages of microsporogenesis are observed. In fact, it was observed the tetrad (Fig. 7B) and then the release of microsporocites (Fig. 7C). At this point, tapetum degrades (Fig. 7C). The young microspores show a central nucleus and a vacuolated cytoplasm.

The last stage shows mature pollen grains and free orbicules (Fig. 7D). When mature pollen grains are formed, tapetum disappears completely. Mature pollen grains measure about 20 µm. It is possible to observe the exine of the grains well formed and the cytoplasm of the vegetative cell with a lot of amyloplasts (Fig. 7D).

Pollen viability

It was possible to differentiate green, red and orange yellowish pollen grains corresponding to viable, non-viable and sub viable pollen grains respectively.

Pollen viability among flowers at different dates (Table 1) showed great variations on flowers collected in Moreno; on the contrary, no differences were observed between flowers collected from San Miguel de Tucumán throughout the study period (Table 2). Moreno flowers showed a very low percentage of viable pollen grains respect to San Miguel de Tucumán flowers during September to November. Although the number of pollen grains per anther in

Table 1 - Viability of pollen grains collected on different months and different locality

Source	Month	Viable	Non Viable	Sub-viable
Moreno	September	10 c	79 a	11 a
Moreno	October	28 c	53 a	19 a
Moreno	November	48 b	32 b	20 a
Moreno	December	74 a	3 c	23 a
S.M.Tucumán	September	68 a	22 b	10 a
S.M.Tucumán	October	63 a	25 b	12 a
S.M.Tucumán	November	70 a	22 b	8 a
S.M.Tucumán	December	69 a	28 b	13 a

Values with different letters between the same column are significant different. Tukey (p≤0.05).

Table 2 - Viability of pollen grains collected on September from anthers of the same flower

Source	Anther	Viable	Non Viable	Sub-viable
Moreno	1-1	10 a	84 a	6 a
Moreno	1-2	7 a	91 a	2 a
Moreno	1-3	10 a	82 a	8 a

Values are expressed on percentage. Values with different letters between the same column are significant different. Tukey (p≤0.05).

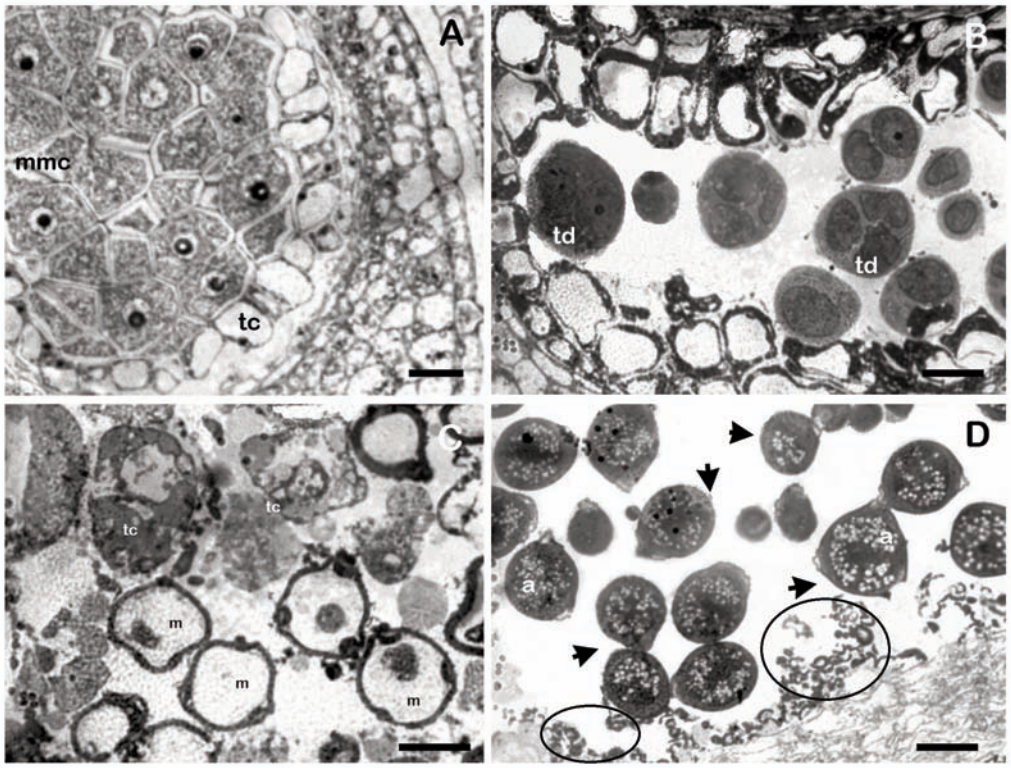

Fig.. 7 - Microsporogenesis of *Moringa oleifera* Lam (light micrographs). microsporangium with conspicuous microspore mother cells (mmc) and tapethal cells (tc); B, tetrads (td); C, microporocytes free (m) and tapetal cells (tc) in metabolization phase; D, mature pollen grains (arrows) with amyloplast (a) in the cytoplasm and orbicules (circles). Bars = A 10 µm; B-D, 20 µm.

the flowers was not evaluated, it was observed that flowers collected from San Miguel de Tucumán had more amount of pollen grain in each anther. Some anthers of Moreno flowers contained immature pollen and a viscous substance. Additionally, according to the harvesting period of the flowers, some anthers developed only dead pollen grains.

According to these results, a more detailed analysis of pollen viability was performed between pollen grains derived from different anthers of the same flower and between anthers from different flowers collected from Moreno trial. Viability of pollen grains of different anthers collected from the same flower was not statistically different (Table 2), while viability of pollen from different flowers of different trees collected on the same date resulted significantly different between flowers (Table 3). Viable pollen grains were contained on great proportion in some flowers but scarce in others, with a random distribution among trees.

Table 3 - Viability of pollen grains from different flowers of the same plant

Source	Flower	Viable	Non viable	Sub-viable
Moreno	1 A	7 b	78 ab	15 a
Moreno	1 B	6 b	82 a	12 a
Moreno	1 C	18 a	67 b	15 a
Moreno	1 D	2 b	95 a	3 b
Moreno	1 E	15 a	72 b	13 a

Values are expressed on percentage. Values with different letters between the same column are significant different. Tukey ($p \leq 0.05$).

Pollination

During anthesis, pistils both with or without germinated pollen were observed. When pollination occurs, pollen grains fall freely into the stigma cavity and then germinate (Fig. 6B). In effect, pistils treated by Martin technique showed that a mass of pollen grains is housed in the cavity and that many pollen tubes germinated (Fig. 8A) and later on reached the ovary and fertilized the ovules (Fig. 8B).

Pollination was rarely observed on flowers collected from Moreno during spring time (September to November) but it was very frequent in summer time. On the contrary, it was observed that flowers collected on San Miguel de Tucumán were profusely pollinated in both periods and fruit production was continuous throughout the year. Fruit production on Moreno plants started in summer until May, while it resulted continuous in San Miguel de Tucumán.

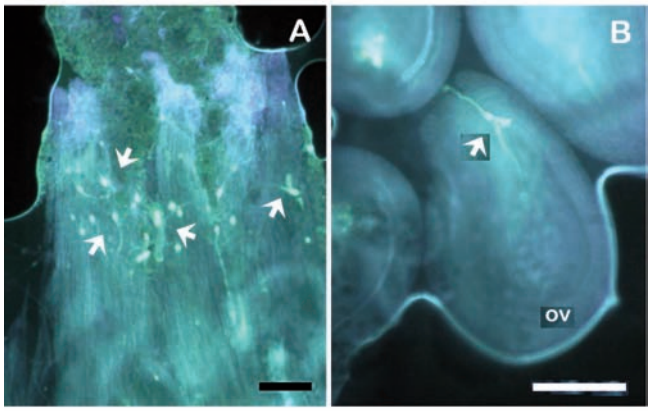

Fig. 8 - Fertilization of *Moringa oleifera* Lam (fluorescent light micrographs). A, pollen tube growing toward the style (arrows); B, ovules (ov) and pollen tube crossing the micropile and entering the embryo sac (arrows). Bars = A-B, 100 μm.

4. Discussion and Conclusions

Flower morphology and anatomical structure observed in flowers collected from Moreno field and San Miguel de Tucumán did not show significant difference with those described in literature (Ramachandran *et al.,* 1980). Furthermore, flowers from the two localities studied in Argentina have a normal external and internal development.

Frozen sections allowed to see the internal anatomy with its natural colorations and to show more clearly the presence of orbicules, that, in the traditional cuts, appear very confused because of the different colorations applied. On the other hand, flowers studied by SEM allowed clarifying some concepts. In fact, it was clearly showed how pollen germination begins even in the absence of any connection to any structure of the stigma or style. Bhattacharya and Mandal (2004) found that some extra proteins and esterases contribute towards the stigmatic receptivity; furthermore, the occurrence of intraovarian trichomes, which is not widespread in the angiosperms, could facilitate the growth of the pollen tubes. This assessment made by Dickison (1993) is based on that the trichomes functionally resemble obturators.

Pollen grain formation seems to be very variable depending on the geographical location and the time of year. In fact, Muhl *et al.* (2011) showed that low temperature regime induces flowering but provokes low pollen viability and this statement would explain the results obtained in Moreno spring flowers, thus confirming that pollen grain viability is affected by air temperature. In fact, the lowest values of pollen

grain viability were observed on September and October when mean temperatures were lower than 16°C. Additionally, the higher percentage of pollen viability observed in San Miguel de Tucuman is consistent with the air temperatures registered in that location, which resulted about 4/5°C higher than those observed in Moreno (Fig. 1).

The difference of the quality of pollen grains collected in spring and summer could be related to the amount of orbicules or Ubisch bodies. Studies on Ubisch body formation in *Brachypodium* support the evidence that they are formed in the tapetum and are involved in exine synthesis (Sharma *et al*., 2014). Actually, during spring time, anthers with mature pollen grains brings many orbicules. This fact suggests that when the exine was not well formed the quantity of dead pollen was important.

On the other hand, pollen viability seems to have an influence on the efficiency of pollination. As directly observed, all plants were very visited by insects during blooming on both experimental locations, but pollination was rarely observed in Moreno flowers, while it was very frequent in flowers collected in San Miguel de Tucumán.

Although tropical climates are those considered ideal for *M. oleifera* according to Muhl *et al.* (2011), good results obtained with San Miguel de Tucumán flowers confirm that sub-tropical climates are also suitable for this species. Taking into account reproductive functions, and namely the microsporogenesis, Moreno environment seems to be just below the threshold of good temperature regime for *M. oleifera* during spring time despite the climatological predictions made by Falasca and Bernabé (2008).

In conclusion, the reported results demonstrate the possibility of moringa cultivation unusual non tropical climates. Flowers of the trees grown in Moreno field were normal from the morphological point of view only in some periods of the year and the quality of their development was related to air temperatures. Plants grown on San Miguel de Tucumán have an un-interrupted production of flowers despite not being in a tropical climate. On the other hand, moringa cultivated in marginal areas, as the locality of Moreno can be considered, could offer important advantages.

Acknowledgements

The authors thank Ing. Oscar Dantur for the collection of plant material in San Miguel de Tucumán and Mrs. Isabel Farías for her assistance with the histology. This research was supported by IFUND - University of Florence project.

References

ARAÚJO M., SANTOS C., COSTA M., MOUTINHO-PEREIRA J., CORREIA C., DIAS M.C., 2016 - *Plasticity of young* Moringa oleifera *L. plants to face water deficit and UVB radiation challenges*. - J. Photochem. Photobiol., B. Biol., 162: 278-285.

ARENA M.E., RADICE S., 2016 - *Seasonal variation in leaf growth and antioxidant content of* Moringa oleifera *cultivated at Buenos Aires, Argentina*. - Int. J. Agric. Biol., 18: 719-725.

ATAWODI S.E., ATAWODI J.C., IDAKWO G.A., PFUNDSTEIN B., HAUBNER R., WURTELE G., OWEN R.W., 2010 - *Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of* Moringa oleifera *Lam.* - J. of Medic. Food, 13(3): 710-716.

BHATTACHARYA A., MANDAL S., 2004 - *Pollination, pollen germination and stigma receptivity in* Moringa oleifera *Lam.* - Grana, 43: 48-56.

DICKISON WC., 1993 - *Floral anatomy of the Styracaceae, including observations on intra-ovarian trichomes.* - Bot. J. Linn. Soc., 112: 223-255.

FALASCA S., BERNABÉ M.A., 2008 - *Potenciales usos y delimitación del área de cultivo de* Moringa oleifera *en Argentina.* - Revista Virtual Redesma, 2(1).

FOIDL N., HARINDER P.S., BECKER K., 2001 - *Potentiel du* Moringa oleifera *pour les besoins agricoles et industriels*, pp. 45-78. - In: FUGLIE L.J. (ed.) *L'arbre de la vie. Les multiples usages du Moringa*. - CTA et CWS, Dakar, Senegal.

FUGLIE L.J., 1999 - *The miracle tree:* Moringa oleifera, *natural nutrition for the tropics.* - Church World Service, Dakar, Senegal.

GANATRA T.J., JOSHI UMANG H., BHALODIA PAYAL N., DESAI TUSHARBINDU R., 2012 - *A panoramic view on pharmacocnostic, pharmacological, nutritional, therapeutic and prophylactic value of* Moringa oleifera *Lam.* - Int. Res. J. Pharm., 3(6): 1-7.

GOPALAKRISHNAN L., DORIYA K., KUMARA D.S., 2016 - Moringa oleifera *A review on nutritive importance and its medicinal applicatio*n. - Food Sci. Human Wellness, 5: 49-56.

LEKGAU S., 2009 - Moringa oleifera *a tree giving life to rural communities.* - National Agricultural Marketing Council (NAMC), Pretoria, South Africa.

MARCU M.G., 2005 - *Miracle tree*. - KOS Health Publications, La Canada, CA (USA).

MARTIN F.W., 1959 - *Staining and observing pollen tubes in the style by means of fluorescence*. - Stain Technology, 34: 125-128.

MUHL Q.E., DU TOIT E.S., ROBBERTSE P.J., 2011 - *Adaptability of* Moringa oleifera *Lam. (Horseradish) tree seedlings to three temperature regimes.* - Am. J. Plant Sci., 2: 776-780.

PADILLA C., FRAGA N., SUÁREZ M., 2012 - *Effect of the soaking time of moringa* (Moringa oleifera) *seeds on the germination and growth indicators of the plant*. - Cuban. J. Agric. Sci., 46 (4): 419-421.

PALADA M.C., CHANG L.C., 2003 - *Suggested cultural practices for Moringa*. - International Cooperator' Guide, Asian Vegetable Research and Development Center, AVRDC, Pub., 3-545.

PAULA P.C., SOUSA D.O.B., OLIVEIRA J.T.A, CARVALHO A.F.U, ALVES B.G.T, PEREIRA M.L., FARIAS D.F., VIANA M.P., SANTOS F.A., MARIAS T.C., VASCONCELOS I.M., 2017 - *A protein isolate from* Moringa oleifera *leaves has hypoglycemic and antioxidant effects in alloxan-induced diabetic mice*. - Molecules, 22: 271.

RADICE S., ARENA M.E., 2016 - *Characterization and evaluation of* Berberis microphylla G. Forst *pollen grains*. - Adv. Hort. Sci., 30(1): 31-37.

RAMACHANDRAN C., PETER K.V., GOPALAKRISHNAN P.K., 1980 - *Drumstick* (Moringa oleifera): *a multipurpose Indian vegetable*. - Econ. Bot., 34: 276-283.

SHARMA A., SINGH M., BHALLA P., 2014 - *Cytochemistry of pollen development in* Brachypodium distachyon. - Plant Syst. Evol., 300(7): 1639-1648.

TENDE J.A., EZEKIEL I., DIKKO A.A.U., GOJI A.D.T., 2011 - *Effect of ethanolic leaves extract of* Moringa oleifera *on blood glucose levels of streptozotocin-induced diabetes and normoglycemic Wistar rats*. - Br. J. Pharmacol. Toxicol., 3: 1-4.

VATS S., GUPTA T., 2017 - *Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of* Moringa oleifera *Lam. from Rajasthan, India*. - Physiol. Mol. Biol. Plants, 23(1): 239-248.

WILSON G., 1992 - Moringa oleifera (*the kelor tree*). - Agrovision Publishing, Mansfield, Queensland, Australia.

YAMEOGO C.W., BENGALY M.D., SAVADOGO A., NIKIEMA P.A., TRAORE S.A., 2011 - *Determination of chemical composition and nutritional values of* Moringa oleifera *leaves*. - Pakistan J. Nutr., 10(3): 264-268.