Different factors involved in the low fruit set of mango (*Mangifera indica*)

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Abstract

Mango (*Mangifera indica*) is a tropical fruit tree originated in Southeastern Asia, which is cultivated in regions with tropical and subtropical climates worldwide. Mango cultivation has significantly increased in the last decades, often spreading to areas where environmental conditions are not the most favorable for optimal fruit set. In this work, we describe the reproductive biology of mango in the coastal areas of Southern Spain, the furthest region from the equator where mango is produced commercially at a significant scale. As in other regions, mango trees produced several inflorescences per tree with hundreds to thousands of male and hermaphrodite flowers, depending on the cultivar, but only a very small percentage of the flowers are able to set fruits due to a massive drop of flowers and fruitlets. Although about half of the flowers in the inflorescence are male flowers and, consequently, contribute to this massive drop, a high proportion of the hermaphrodite flowers were not receptive to pollen germination. In receptive flowers, the characterization of the pollen tube pathway within the pistil showed chalazogamy. A detailed study of the ovules indicates that a high proportion of them showed degenerated embryo sacs, both before and after fertilization. Paternity analysis with molecular markers showed a high proportion of embryos resulting from self-pollination in orchards planted with a single cultivar. However, without pollination restrictions, the offspring shows a higher proportion of genotypes resulting from cross-pollination, suggesting preferential cross-fertilization. The variable response of this parameter, together to the variable proportion of hermaphrodite/male flower ratio, also obtained in response to environmental conditions, suggests a reproductive plasticity in this species.

Keywords: reproductive biology, pollen tube, pollen germination, ovule, paternity.

INTRODUCTION

Mango (*Mangifera indica* L.) is an evergreen tropical tree native from Southeastern Asia, where it was domesticated thousands of years ago, and still remains as an important crop. Mango cultivation has expanded from Asia to all tropical and subtropical regions of the world. Currently, mango is one of the five most economically important fruit crops together with bananas, oranges, grapes and apples, with an annual production over 40 million tons (FAO, 2016). Increased mango cultivation and interest in this fruit in the last decades has resulted in its cultivation in areas where climatic conditions are not the best for optimal mango flower development and fruit set. An example is Southern Spain, the furthest region from the equator with a significant commercial mango production with a current extension of more than 3000 ha (Magrama, 2015).

Mango is a member of the Anacardiaceae, a family that includes other economically important crops as pistachio (*Pistacia vera* L.), cashew (*Anacardium occidentale* L.), ambarella (*Spondias dulcis* Frost.) or yellow and purple mombins (*Spondias mombin* L. and *Spondias purpurea* L., respectively). The genus *Mangifera* is composed of 70 species (Kostermans and Bompard, 1993) of which some of them, in addition to mango, are of local commercial importance (Bompard, 1993; Bally et al., 2009). However, despite the economic importance

of this crop, knowledge on the reproductive biology of this species is scarce. In this work the main factors limiting fruit set under the environmental conditions of Southern Spain are studied with the aim to understand and try to reduce the bottlenecks that limit fruit set in this species.

MATERIALS AND METHODS

Plant Material

Experiments were carried out in four mango cultivars, 'Osteen', 'Kent', 'Keitt' and 'Kensington', maintained in a mango germplasm collection at the IHSM la Mayora, Málaga (36°45'32"N; 4°2'25"W).

Phenology studies

To follow the pattern of flower opening, inflorescences were labelled and each day all the opened flowers were collected. Flower type (hermaphrodite or staminate), and flower position in the inflorescence (apical, medium and basal) was recorded. Also flower/fruit drop was controlled in another set of inflorescences by collecting daily the dropped flowers.

Microscopy

To study pollen tube kinetics, hermaphrodite flowers were collected in the field, carried to the laboratory and hand pollinated. Then, flowers were fixed in FAA at four hour intervals. Pistils were stained with 0.005% aniline blue in 0.15 M K_3PO_4 (O'Brien and McCully, 1981) and squashed. A Leica DM LB2 (Leica, Wetzlar, Germany) epifluorescent microscope with a 340-380 excitation filter and LP 425 barrier filter was used. The number of pollen tubes in the stigma and in the upper, middle and basal sections of the style was counted. Pollen tube kinetics at the style was evaluated as the percentage of the style length traversed by the longest pollen tube.

The pollen tube pathway in the ovary was studied in pistils hand-pollinated and daily fixed over five days in 2.5% glutaraldehyde in 0.03 M phosphate buffer (Sabatini et al., 1963), dehydrated in an ethanol series, and embedded in Technovic 7100 (Kulzer, Friedrichsdorf, Germany). Later, samples were sectioned at 2µm and stained with periodic acid-Schiff's reagent (PAS) (Feder and O'Brien, 1968) and observed with a brightfield microscope.

Pollen number, viability and germination

To record the number of pollen grains per anther, anthers were collected from 'Osteen', 'Kent' and 'Keitt' genotypes. Both male and hermaphrodite flowers were used. The anthers were fixed in 3:1 (v1/v2) ethanol/acetic acid. Then, 20 anthers were placed in 100µL distilled water with a drop of soap and transferred to a 1.5mL microtube where they were pressed to the microtube walls with a pellet pestle. The number of pollen grains was counted with the help of a haemocytometer, counting pollen grains under a brightfield microscope in the diagonal squares.

To evaluate pollen viability and *in vitro* germination, flowers were collected from 'Osteen', 'Kent' and 'Keitt' before anther dehiscence from 9 to 11a.m. Flowers were kept in the laboratory at room temperature and high relative humidity until anther dehiscence. Then, anthers were removed and placed on 35 mm Petri dishes containing 2mL of liquid germination medium following a modification of Sukhvibul et al. (2000), changing the concentration of sucrose and polyethylene glycol. To examine pollen viability, a drop of fluorescein diacetate at 2mg/mL was added to the germination medium (Heslop-Harrison and Heslop-Harrison, 1970). Then, Petri dishes were observed under a fluorescent microscope. To evaluate pollen germination, other Petri dishes with pollen and the germination medium were incubated 24 hours, after the number of germinated pollen grains was counted. Pollen was considered as germinated when the length of the pollen tube was longer than the grain diameter.

DNA extraction and paternity analysis

To perform paternity analyses, mango ripe fruits were collected in two orchards: a germplasm collection with different genotypes that could act as pollen donors and a commercial orchard, with two opposite single-variety blocks. Then, mango fruits were opened and embryos dissected. DNA from the embryos was extracted using the method reported by Hormaza (2002). PCR amplifications were performed according to Viruel et al. (2005), using the following temperatures: a step of one minute at 94°C followed by 35 cycles of 30s at 94°C, 30s at 50°C of annealing temperature and an extension step of five minutes at 72°C. PCR amplified fragments were analysed by capillary electrophoresis in a CEQ™ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). LMMA6, LMMA7, LMMA8, LMMA9, LMMA10, LMMA11 and LMMA15 primers were used for paternity analysis in the multivarietal orchard and LMMA9, LMMA10, LMMA11 in the monovarietal orchard (Viruel et al., 2005). Paternity analyses were performed with Cervus 3.0 software (Marshall et al., 1998; Kalinowski et al., 2007).

RESULTS AND DISCUSSION

Reproductive biology in mango was studied with the aim to elucidate the main bottlenecks that limit fruit set in this crop. The results obtained show that different factors, such as the proportion of hermaphrodite flowers in the inflorescence, the development of the reproductive female structures, the interaction between the female sporophyte and the male gametophytes, pollen performance and fruit development, are involved in fruit set in mango.

Andromonoecy in mango

Mango inflorescences are composed thirsoids with a high number of hermaphrodite and staminate flowers. The proportion of both types of flowers depends on the genotype and the environmental conditions, especially temperature (Singh, 1954; Singh et al., 1966; Abourayya et al., 2011). Results obtained with 'Kent' (n=20 inflorescences) showed that approximately 50% of the flowers are staminate (staminate $258 \pm 29,66$; hermaphrodites $242 \pm 21,77$). Consequently, less than half of the flowers in an inflorescence can produce fruits. A greater proportion of hermaphrodite flowers were located at the top of the inflorescence (63%).

Pollen performance

Mango male and hermaphrodite flowers have one or two anthers per flower depending on the genotype (Mukherjee, 1997). Mango pollen grains measure approximately 25µm in diameter. The results show differences in the number of pollen grains per anther, depending on the cultivars, but within the range recorded for other cultivars in other growing areas (Gehrke-Vélez et al., 2011). The highest numbers were recorded in 'Kent' and the lowest in 'Osteen'. No differences were found between the number of pollen grains in the two types of flowers: hermaphrodite or staminate. The number of pollen grains ranged from 578 to 981 in male flowers and from 625 to 1266 in hermaphrodite flowers. Pollen viability was high and pollen germinated properly in the three cultivars: 'Osteen' (97%; 52%), 'Kent' (97%; 53%), and 'Keitt' (94%; 68%).

Pollen-pistil interaction

Hermaphrodite mango flowers have a short style and only one anatropous ovule per flower (Robbertse et al., 1986). Following pollination, pollen grains germinated and pollen tubes grew through the transmitting tissue of the style, where a reduction in pollen tube number was observed. In most circumstances, just one pollen tube reached the base of the style 24 hours after pollination. The pollen tube then faced the *ponticulus*, a structure that bridges the ovary and the ovule (Joel and Eisenstein, 1980), entered the ovule through the chalaza (Maheshwari, 1950), bordered the embryo sac, and penetrated in the basal area of the embryo sac where the synergids and egg cell are located. Following fertilization, a good proportion of the ovules degenerated, but degeneration was also observed in unfertilized ovules.

Preferential cross-fertilization

Mango fruit drop due to a putative incompatibility system has been proposed and supported by several authors (Singh et al., 1962; Mukherjee et al., 1968; Sharma and Singh, 1970; Ram et al., 1976; Gehrke-Vélez et al., 2012). To check if an incompatibility system is operating in the varieties studied in this work, a paternity analysis was performed using microsatellite markers in fruits harvested in a multivarietal and in a monovarietal orchard. Paternity analysis with 'Osteen' and 'Kent' in the multivarietal orchard showed a high outcrossing rate, 95% and 52%, respectively. However, in the monovarietal orchard, in which two opposite single variety blocks were planted, one with 'Osteen' and the other with 'Keitt', only 21% and 24% of outcrossing rate was obtained, respectively. These results point out that when foreign pollen is available outcrossing is favoured, but self-fertilization is still possible.

CONCLUSIONS

Different factors can play a role in limiting fruit set in mango. First, only a small flower percentage has capacity to produce fruits in the inflorescence, reducing the likelihood of success. Of the pollen grains that arrive to the stigma, few can reach the ovule inside the ovary. Finally, there is a preference for cross-fertilization. A better control of these and other factors could reduce the strong flower and fruit drop in this species helping to increase final yield.

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Literature cited

- Abourayya, M.S., Kassim, N.E., El-Sheikh, M.H., and Rakha, A.M. (2011). Comparative study between inflorescences characteristics, pollen viability, germination and dimensions of Tommy Atkins, Kent and Keitt mango cultivars. *Life Sci. J.* 8, 100-105.
- Bally, I.S.E., Lu, P., and Johnson, P.R. (2009). Mango breeding. In *Breeding plantation tree crops: Tropical species*, S.M. Jain and P.M. Priyadarshan, eds. (New York, NY, USA: Springer), p. 3-50.
- Bompard, J. (1993). The genus *Mangifera* re-discovered: the potential contribution of wild species to mango cultivation. *Acta Hortic.* 341, 69-77 <http://dx.doi.org/10.17660/ActaHortic.1993.341.5>
- Feder, N., and O'Brien, T.P. (1968). Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123-142.
- Food and Agricultural Organization. (2016). www.fao.org.
- Gehrke-Vélez, M.R., Castillo-Vera, A., Ruiz-Bello, C., and Moreno-Martínez, J.L. (2011). Viabilidad y germinación del polen en mango (*Mangifera indica* L.) cv. Ataulfo. *Interciencia* 36, 378-385.
- Gehrke-Vélez, M.R., Castillo-Vera, A., Ruiz-Bello, C., Moreno-Martínez, J.L., and Moreno-Basurto, G. (2012). Delayed self-incompatibility causes morphological alterations and crop reduction in 'Ataulfo' mango (*Mangifera indica* L.). *New Zeal. J. Crop Hort.* 40, 215-227 <http://dx.doi.org/10.1080/01140671.2011.632423>
- Heslop-Harrison, J., and Heslop-Harrison, Y. (1970). Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technol.* 45, 115-120 <http://dx.doi.org/10.3109/10520297009085351>
- Hormaza, J.I. (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theor. Appl. Genet.* 104, 321-328 <http://dx.doi.org/10.1007/s001220100684>
- Joel, D.M., and Eisenstein, D. (1980). A bridge between the ovule and ovary wall in *Mangifera indica* L. (Anacardiaceae). *Acta Bot. Neerl.* 29, 203-206 <http://dx.doi.org/10.1111/j.1438-8677.1980.tb00377.x>

- Kalinowski, S.T., Taper, M.L., and Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16, 1099-1106 <http://dx.doi.org/10.1111/j.1365-294X.2007.03089.x>
- Kostermans, A.J.G.H., and Bompard, J.M., eds. (1993). *The mangoes: their botany, nomenclature, horticulture and utilization*. (London, UK: Academic Press), pp. 250.
- Magrama. Ministerio de Agricultura, Alimentación y Medio Ambiente. (2015). www.magrama.gob.es
- Maheshwari, P. ed. (1950). *An introduction to the embryology of angiosperms*. (New York, USA: McGraw- Hill), pp. 476.
- Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7, 639-655 <http://dx.doi.org/10.1046/j.1365-294X.1998.00374.x>
- Mukherjee, S.K. (1997). Introduction: botany and importance. In *The Mango: botany, production and uses*, R.E. Litz, ed. (Wallingford, Oxon: CAB International), p.1-19.
- Mukherjee, S.K., Singh, R.N., Majumder, P.K., and Sharma, D. K. (1968). Present position regarding breeding of mango (*Mangifera indica* L.) in India. *Euphytica* 17, 462-467 <http://dx.doi.org/10.1007/BF00056248>
- O'Brien, T.P., and McCully, M.E., eds. (1981). *The study of plant structure: Principles and select methods*. (Melbourne: Termacarphy Pty. LTD), pp. 46.
- Ram, S., Bist, L.D., Lakhanpal, S.C., and Jamwal, I. S. (1976). Search of suitable pollinizers for mango cultivars. *Acta Hort.* 57, 253-264 <http://dx.doi.org/10.17660/ActaHortic.1976.57.31>
- Robbertse, P.J., Teichman, I.V., and Rensburg, H.J.V. (1986). A reevaluation of the structure of the mango ovule in comparison with those of few other Anacardiaceae species. *S. Afr. J. Bot.* 52, 17-24.
- Sabatini, D.D., Bensch, K., and Barnett, R.J. (1963). Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* 17, 19-58 <http://dx.doi.org/10.1083/jcb.17.1.19>
- Sharma, D., and Singh, R. (1970). Self-incompatibility in mango (*Mangifera indica* L.). *Hortic. Res.* 10, 108-18.
- Singh, R.N. (1954). Sex ratio and fruit setting in mango (*Mangifera indica* L.). *Science*, 119, 389-390 <http://dx.doi.org/10.1126/science.119.3090.389-a>
- Singh, R.N., Majumder, P.K., and Sharma, D.K. (1966). Sex-expression in mango (*Mangifera indica* L.) with reference to prevailing temperature. *J. Am. Soc. Hor. Sci.* 89, 228-230.
- Singh, R.N., Sharma, D.K., and Majumdar, P.K. (1962). Self-incompatibility in mango (*Mangifera indica* L.) var. Deshehari. *Curr. Sci. India* 31, 209-209.
- Sukhvilul, N., Whiley, A.W., Vithanage, V., Smith, M.K., Doogan, V.J., and Hetherington, S.E. (2000). Effect of temperature on pollen germination and pollen tube growth of four cultivars of mango (*Mangifera indica* L.). *J. Hort. Sci. Biotech.* 75, 214-222 <http://dx.doi.org/10.1080/14620316.2000.11511226>
- Viruel, M.A., Escribano, P., Barbieri, M., Ferri, M., and Hormaza, J.I. (2005). Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Molec. Breeding*, 15, 383-393 <http://dx.doi.org/10.1007/s11032-004-7982-x>