African & Mediterranean AGRICULTURAL JOURNAL AL AWAMIA

Ait Aabd N. et al. (2022). AFRIMED AJ –Al Awamia (135). p. 64-83

Floral structure and pollinizer tree as reliable factors for cross-breeding system of *Argania spinosa*

Ait Aabd Naima [(1)], Tahiri Abdelghani [(1)], Qessaoui Redouan [(1)], Mimouni Abdelaziz [(1)] and Bouharroud Rachid [(1)]

naima.aitaabd@inra.ma

1: National Institute of Agronomic Research, CRRA Agadir, Morocco

Abstract

In most breeding programs the pollen donor parents (pollinizers) are characterized by a strong flowering rate and very low fruit set, even after hand pollination. Hence, the notion of pollinizers in the argane species was born, first mentioned, checked, documented and confirmed like in the other conventional tree species. Since the argane tree is completely self-incompatible, the presence of compatible pollinizers is necessary for the fruit set. Indeed, pollinizers need to be selected accurately based on the synchronization of bloom periods and compatibility to receiver trees (females). The pollinizer is essential in any breeding program and as well as for any new orchard plantations. The current study was carried out on 13 argane genotypes including two pollinizers. The flowering period, bloom phenology, floral structure and fruit set of crossed genotypes were monitored, illustrated and a season phenogram was established. The pollen viability and germination were also evaluated. Concerning compatibility trials, the hand pollination, using two selected pollinizers pollens, was compared to open pollination. Then, the compatibility system was monitored and evaluated through the index of self-incompatibility. It has been observed that the flowering periods are genotype depending, that there is one to three blooms during the two years study (2018-2019) and that the argane tree is a tristylous species (Mesostylous, brevistylous and longistylous flowers). The *in vitro* tests showed that the pollen originated from crossed genotypes were viable and able to germinate. The cross-compatibility rate depends on cross associations and it varies from 39 to 84 %. It was observed, for the first time, that all compatible pollinizers have metaxenic effects of pollen on argane fruit.

Keywords: Argane tree, breeding program, pollinizers, self-incompatible, crossing diallel programs, compatibility, metaxenia.

Structure florale et arbre pollinisateur comme facteurs fiables pour le système de croisement d'*Argania spinosa*

Résumé

Au cours des programmes d’amélioration génétique, les parents donneurs de pollen (pollinisateurs) se caractérisent par une forte intensité florale et une très faible nouaison, même après une pollinisation manuelle. C’est ainsi que la notion de pollinisateurs pour l'espèce d'arganier est née, d'abord évoquée, vérifiée, documentée et confirmée comme pour d'autres espèces d'arbres. L'arganier étant totalement auto-incompatible, la présence de pollinisateurs compatibles est nécessaire à la nouaison. Ces pollinisateurs doivent être sélectionnés avec précision en fonction de la synchronisation des périodes de floraison et de la compatibilité avec les arbres récepteurs (femelles). Le pollinisateur est essentiel aussi bien dans tout programme d’amélioration génétique que pour les plantations de nouveaux vergers. La présente étude a été menée sur 13 génotypes d'arganier dont deux pollinisateurs. La période de floraison, la phénologie de la floraison, la structure florale et la nouaison des génotypes croisés ont été suivies, illustrées et un phénogramme de saison a été établi. La viabilité et la germination du pollen ont également été évaluées. En matière de compatibilité, la pollinisation manuelle utilisant deux pollinisateurs sélectionnés a été comparée à la pollinisation libre. Ensuite, le système de compatibilité a été surveillé et évalué à travers l'indice d'auto-incompatibilité. Il a été constaté que les périodes de floraison dépendent du génotype, qu’il y a eu une à trois période de floraisons au cours des deux années d'étude (2018-2019) et que l'arganier est une espèce tristyle (fleurs mésostyleuses, brévistyles et longistyles). Les tests in vitro ont montré que le pollen issu de génotypes croisés était viable et capable de germer. Le taux de compatibilité croisée dépend des associations croisées et il varie de 39 à 84 %. Il a été observé, pour la première fois, que pour des pollinisateurs compatibles, des effets métaxéniques du pollen sur les fruits de l'arganier se sont produits.

Mots clés : Arganier, Amélioration génétique, pollinisateurs, auto-incompatibilité, Croisements diallèles, compatibilité, Métaxénie.

البنية الزهرية و الشجرة الملقحة كعوامل موثوقة في نظام التهجين لشجرة الأركان

نعيمة أيت عابد. عبد الغني الطاهري. رضوان قساوي . عبد العزيز. ميموني. رشيد بو هرود

ملخص

لقد لوحظ من خلال دراسات التحسين الوراثي لشجرة الأركان، أن الأشجار المنتجة لحبوب اللقاح (الملقحات) تتميز بمعدل إزهار قوي وإثمار منخفض، و لو بعد التلقيح اليدوي. لذلك وُلد مفهوم الملقحات لذى شجرة الأركان، وتم ذكره أولاً وفحصه وتوثيقه وتأكيده كسائر أنواع الأشجار التقليدية الأخرى. نظرًا لأن شجرة الأركان غير متوافقة تمامًا مع ذاتها، فإن وجود الملقحات المتوافقة أمر ضروري للإثمار. لهذا، يجب اختيار الملقحات بدقة بناءً على تزامن فترات الإزهار والتوافق مع الأشجار المستقبلة لحبوب اللقاح (الإناث). فالملقح ضروري في أي برنامج تحسين وأي زارعة بساتين جديدة. أجريت الدراسة الحالية على 13 نمط جيني من الأركان متضمنة اثنين من الملقحات. تم رصد فترة الإزهار، فينولوجية الإزهار، وبنية الزهرة واثمار الأنماط الوراثية المهجنة، وتم وضع مخطط ظاهري للموسم. كما تم تقييم صلاحية حبوب اللقاح والإنبات. فيما يخص اختبار التوافق، تمت مقارنة التلقيح اليدوي بالتلقيح المفتوح دلك باستخدام اثنين من حبوب اللقاح المختارة. بعد ذلك، تمت مراقبة وتقييم نظام التوافق من خلال مؤشر عدم التوافق الذاتي. لقد لوحظ أن فترات الإزهار تعتمد على النمط الجيني و أن هناك واحدة إلى ثلاثة فترات إزهار خلال الدراسة التي استمرت لعامين (2018-2019) و أن شجرة الأركان هي من الأنواع الثلاثية الأزهار (أزهار ميزوستيليوس، قصيرة ، وطويلة الشكل). أظهرت الاختبارات في المختبر أن حبوب اللقاح التي نشأت من أنماط وراثية مهجنة كانت قابلة للحياة وقادرة على الإنبات. يعتمد معدل التوافق المتبادل على الارتباطات المتقاطعة ويتراوح من 39 إلى 84٪. وقد لوحظ، لأول مرة، أن هناك تأثيرات لحبوب اللقاح على فاكهة الأركان لدى كل الملقحات المتوافقة.

الكلمات المفتاحية: شجرة الأركان، التحسين الوراثي، الملقحات، عدم التوافق الذاتي، تهجينات تبادلية نصفية، التوافق، تاثيرات حبوب اللقاح.

Introduction

The argane tree (*Argania spinosa* (L.) Skeels, a unique representative of the sapotaceae family in Morocco, is one of the most important oil seed plants in the world. It was previously called *Sideroxylon spinosum*, then *Argania sideroxylon*.. It is widely distributed in arid and sub arid climates of the Southwest region of Morocco, while other three small size relics populations are in the North at Oued Grou (close to Rabat), Northeast at Beni Snassen and South at Guelmim (Ehrig, 1974; Prendergast and Walker, 1992) covering over 900,000 ha (HCEFLCD, 2012).

Several studies characterized the great genetic diversity of the argane tree (Msanda, 1993 ; El Mousadik and Petit, 1996; Ait Aabd et al., 2013 ; Ait Aabd et al., 2014 ; Yatrib et al., 2015 ; Ait Aabd et al., 2019) and its geographical distribution (from sea level to 1400-1500 meters altitude). There is an intensive demand at international market level, and policy makers in Morocco are multiplying efforts for the conservation of this endemic and endangered species. A national agency (Called ANDZOA = National Agency for the Development of Oasis Areas and the Argane Tree) was therefore created to ensure the argan tree protection and its sustainable use . A planting program of 50 000 Ha was also developed and initiated in accordance with the new Moroccan agricultural strategy " GENERATION GREEN 2020-2030".

The argane tree is a perennial and monoecious tree with hermaphrodite flowers. The flowers are protogynous, the style emerging from the flower before anthesis. Several works have been published on the floral biology of argane trees, flowering periods, pollination and fruit maturation (Perrot, 1907 ; Nerd et al., 1998; Belmouden and Bani-Aameur, 1995; Benlahbil, 2003; Ait Aabd et al., 2019; Ajerrar et al., 2020). The pollination of the argane tree is allogamous, and needs a vector for pollination either by insects (Ajerrar et al., 2020) or by the wind. This vector is also called a pollinator. The pollinizer term used in this study is simply for the tree that provides viable, abundant and compatible pollens to female flowers. The pollinizer refers therefore to pollen donor. The knowledge in flowering, pollination and fertilization in the argane tree are still very limited. Further research is needed on the appropriate mode of argan tree reproduction te be advised for the domestication and fruit set improvement in Argane orchards.

The synchronization of the flowering period, the availability of compatible pollen grains, or compatible trees, the presence of vectors to transfer pollen to the stigma, are essential criteria for the success of fruit production. The argane tree is characterized by a high flowering intensity , but the fruit set rate is very low, due to the massive and premature fall of the flowers at the beginning of the bloom. Reasons for this flower fall in the argane tree are still not clear. For fruit trees (almond, olive, citrus...), the most important factor in fruit set and fertilization is compatibility (Ortega and Dicenta, 2004). Self-incompatibility or incomplete flowers pollination is one of the main causes of fruit drop (Boöković and Tobutt 2001), and plays a major role in reducing yield for many fruit trees. Compatibility and incompatibility in the argane tree should therefore be studied to have necessary information for the improvement of fruit yield.

There are several methods to study the compatibility or incompatibility of different cultivars and to determine their suitable pollinizers (Ortega and Dicenta, 2004; Mousavi et al., 2014). Among these methods, the controlled pollination allows to estimate the performance of various cultivars in the orchard, and it is therefore recommended to determine the appropriate pollinizers (Rasouli et al., 2009). In order to improve the knowledge of the compatibility mechanisms of the argane tree, the compatibility was studied. The goal of the present work is to study the compatibility of two identified pollinizers with some selected argane genotypes. The concept of pollinizers in the argane tree species originated from this study which aims mainly the confirmation of this concept through appropriate tests. Thus, the current study aims to check different flowering patterns among different genotypes after morphological characterization in the orchard, to test the cross and self-incompatibility of pollination of this monoecious species with hermaphrodite flowers, to describe the polymorphism of the argane tree flowers and finally to test if cross-pollination leads to large fruit size than self-pollination (metaxenic effect).

Materials and methods

Orchard design

The experiments were carried out in a 2 ha argane orchard of the Experimental Station Melk Zhar (30.0434N; -9.55635W; alt. 100 m) of the INRA Regional Centre of Agadir. This orchard has been planted in 2010 at a density of 8 x 6 m (Fig. 1). The trees are elite individuals selected from the natural area of argane. All trees were drip irrigated simultaneously and at the same frequency and dose.

Figure 1. Argane tree orchard located at Melk Zhar Experimental Station.

Plant materials

This experiment was carried out during the seasons 2018 and 2019. Two promising pollinizers genotypes, labeled INRA-62 and INRA-142, as pollen donor parent were used to pollinate eleven genotypes of argane trees. The INRA-62 pollinizer was crossed to seven genotypes (INRA-139, INRA-135, INRA-109, INRA-98, INRA-79, INRA-65, INRA-49), while the INRA-142 pollinizer was crossed to six trees (INRA-132, INRA-107, INRA-98, INRA-65, INRA-75, INRA-54). Morphological characteristics (tree height, trunk number, diameter of trunk at collar, number of the branches at a collar and tree circumference), flowering and fruiting period, self-cross-compatibility and fruit set of the donor parent (or pollinizers) and the eleven female parents (pollen receivers) were recorded (Table 1, Fig. 2, 3 and 4).

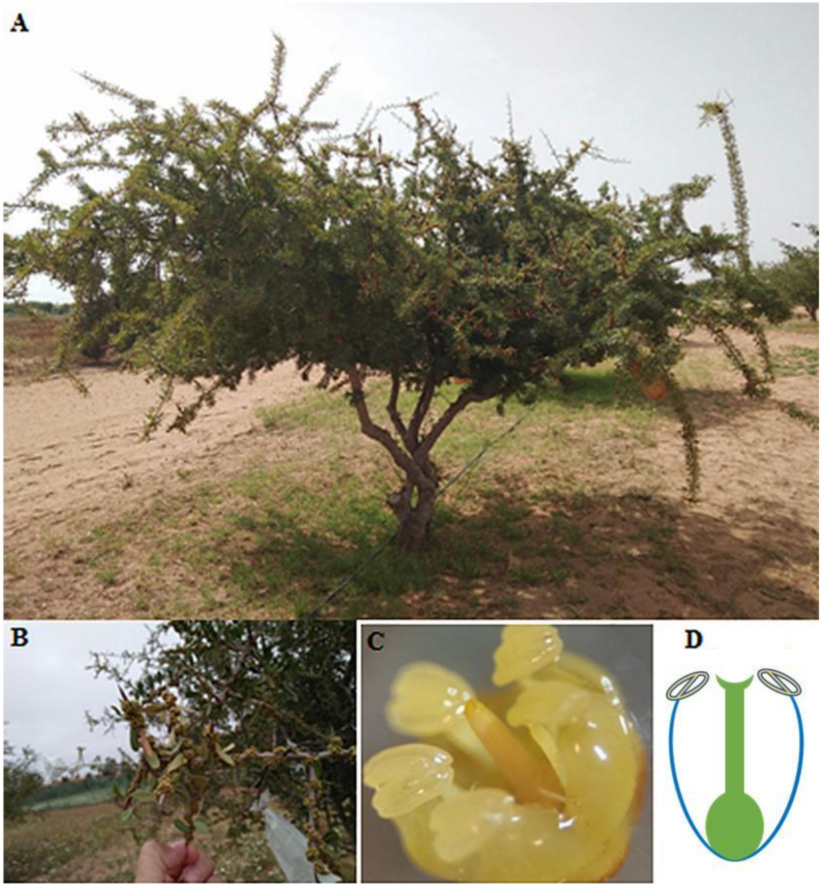

Figure 2. Morphological variability in domesticated argan pollinizer: **A** (tree morphology of domesticated pollinizer; INRA-142), **B** (large number of flower buds per branch), **C-D** (flower morphology of pollinizer).

Flowering period and bloom phenology

The flowering period of the parent trees was observed over the two successive years (2018-2019), according to the following stages: beginning of bloom (first flower bud), full bloom (open flowers), and end of bloom (total petals fall). The observations were collected from each parent according to three periods per year: from February to April (bloom once a year), from June to July (bloom twice a year) and all the year (for a single genotype that blooms three times a year). Season phenograms of flowering

were then established for all evaluated trees based on the collected data of the bloom period (Fig. 3 and Fig. 4).

Pollination

Pollination was peformed in March-April of 2018–2019. The flower buds were bagged before anthesis to prevent pollination with alien pollen and hand-pollinated with pollen collected from the two mentioned pollinizer trees, after emasculation.

The flowers of the eleven receiver trees were assigned randomly to one of three treatments: cross-pollination, self-pollination and no-pollination. Branch with a large number of flowers of donor and receiver parents were bagged before and after anthesis. In the morning, from 07:30 to 10:30 h, before anther dehiscence, the donor pollens were collected and flowers of the receiver parents were emasculated then pollinated. Thus, pollinated flowers were counted, labeled and bagged. To ensure pollination, flowers were pollinated again with donor pollen 24 h later. Open pollination was considered as a control treatment, although cross (hand-pollination) and self-pollination were used for the study of compatibility treatment. For each hand-pollination treatment, 676 flowers were emasculated and pollinated. For self-pollination treatment, 1130 flowers were bagged without emasculation. The compatibility system was evaluated through analysis of crossing diallel programs and through the index of self-incompatibility (ISI). So, the ISI ratio was calculated between the percentage of fruit set resulting from hand-self-pollination over that from hand-cross-pollination. As a result, the species is considered as a self-compatible (SC) when ISI is over 0.25, and as a self-incompatible (SI) when ISI is less than 0.25 (Bawa, 1974). The fruit set for all types of pollination was recorded 30 days after the end of bloom (initial fruit set) and was expressed as a percentage.

Floral polymorphism and study of pollinizers and floral morphs effects on fruit set and fruit weight

The floral structure was observed in the field and the laboratory, to determine the floral polymorphisms and the influence of the different morphs in the argane tree (different heights of stigmas and anthers) on the fruit set and fruit weight,. During the bloom period (March-June / 2018), the flowers were collected at the late balloon stage, fixed in Carnoy's solution 3:1 (absolute ethanol, glacial acetic acid) and stored in ethanol 70%. Different anthers and stigma positions were observed and illustrated. Photos have been taken under the binocular magnifier (x2, x4) which allowed individual identification of flowers.

Pollen viability test

The mature flower buds were bagged for the pollen viability analysis. On the day of anthesis, the flowers were collected and dissected to recover the anthers in the laboratory. The viable pollens were estimated by placing and crushing a single anther on a glass slide followed by staining with hematoxylin and eosin solution for 15 min. Finally, the pollen grains were collected and counted under a microscope. Pollen grains with bright red stains were categorized as viable, pink stain as semi-sterile and unstained as sterile (Prasad et al., 2006).

Pollen germination *in vitro*

Branches with flowers in the mature stage were collected for each tree, during the bloom period of March-April 2018. Pollen have been released from the anthers and sown in Petri dishes containing a solidified germination medium containing 1% agar, 20% sucrose, 200 ppm of Calcium Chloride ($CaCl_2$) and 75 ppm of Boric acid (H_3BO_3), and then incubated in the dark at 30 ° C for 20 h (Benlahbil and Bani-Aameur, 2002). A pollen grain was considered as germinated when the pollen tube was longer than the length of the pollen grain. The observations were performed under a microscope coupled to a digital camera for illustration.

Fruit set

The number of fruit set was recorded at the beginning of the fruit maturation stage. So, the number of fertilized flowers was calculated one month after pollination. The initial and final fruit setting was followed directly after the end of the crosses. Therefore, the set for all types of pollination was recorded 30 days after the end of the flowering and expressed as a percentage to deduce the most compatible genotypes. Intra and inter-compatibility were determined as a percentage of fruit set and the number of fruits developed after successful fertilization in response to self and cross-pollination.

Data analysis

All statistical analyses were perormed using Statistica V.12 software package. The following parameters were evaluated: mean, minimum value, maximum value, standard deviation (SD) and coefficient of variation (CV %).

Results

Agro-morphological characterization

The descriptive analysis of the morphological data is presented in Table 1. The overall average values for the height of the trees vary from 2.67 to 5.20 m, with a minimum value of 2.60 observed for INRA-142 (pollinizer) and INRA-107 (receiver tree). All trees monitored in this study have an upright shape except for INRA-75 which has a semi-weeping shape with a very broad leaf, typical spindle and very easy to break fruit. The number of trunks per tree is relatively similar, except for INRA-132 which showed three trunks.

The collar diameter of the 13 studied trees varied according to the number of ramifications, which ranged from 1 to 3 per tree, and their circumference presented a range from 9,33 to 16.66 m.

Table 1. Morphological characteristics of two pollinizers (INRA-142; INRA-62) and 11 receiver argan genotypes.

Tree reference	Tree form	Tree height (m)	Number of trunk	Collar diameter (cm)	Number of branches	Tree circumference (m)
INRA-62	draw up	3.20	1	37	2	12.00
INRA-142	draw up	2.60	1	56	2	12.66
INRA-49	draw up	5.00	1	61	6	16.66
INRA-54	draw up	3.55	1	51	4	10.66
INRA-75	Semi-weeping	3.80	1	63	5	14.00
INRA-79	draw up	3.25	1	44	3	9.33
INRA-98	draw up	3.95	1	62	3	14.33
INRA-109	draw up	3.50	1	37	2	12.00
INRA-132	draw up	3.80	3	76	4	11.33
INRA-135	draw up	2.95	1	44	2	12.00
INRA-139	draw up	3.60	1	62	3	14.00
INRA-107	draw up	2.60	1	36	3	9.33
INRA-65	draw up	4.35	1	62	2	14.66

Flowering periods

The current study showed that there are several types of argane trees according to their flowering periods (Fig. 3, Fig. 4). Figure 4 shows there are Argane trees that bloom once a year (INRA-139, INRA-109, INRA-98, INRA-79, INRA-65 INRA-49), argane trees that bloom twice a year over a more or less extensive period overlapping between March-May and June-July (INRA-135, INRA-132, INRA-107 and INRA-54) and argane trees that bloom throughout the year (INRA-75).

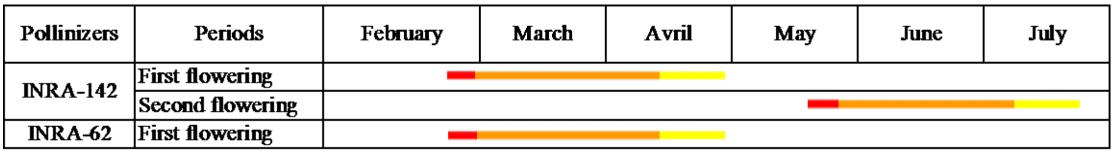

Figure 3. Flowering period of two argane pollinizer. Full bloom is showed in orange color.

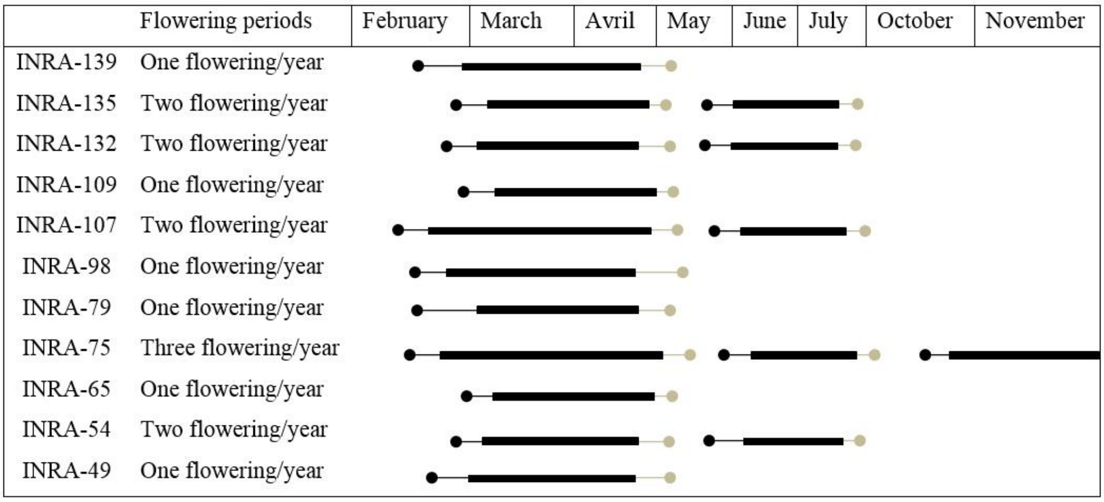

Figure 4. Flowering Diagram of the 11 argane genotypes crossed with pollinizers. Full bloom is showed in dark color.

Concerning the floral polymorphisms and the different morphs in the argane tree, different positions of the anthers and stigma have been observed. Three morphs were identified in the argane tree: Mesostylous flower has a style of intermediate length and long and short stamina, longistylous flower has long style and intermediate and short stamina and their brevistylous has a short style and stamens of intermediate and long length (Fig. 5).

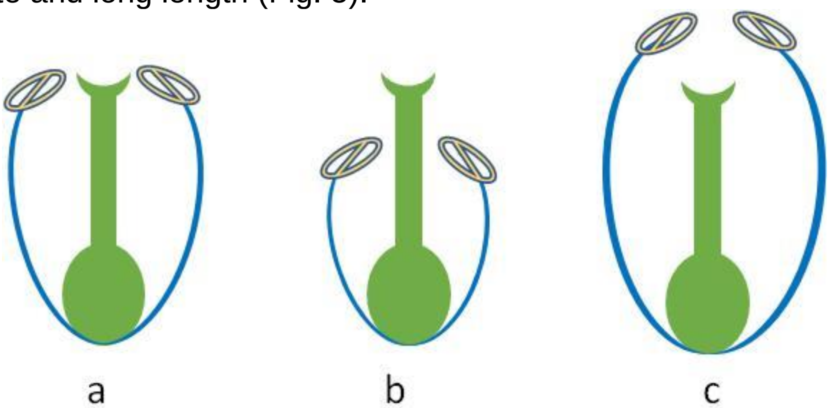

Figure 5. The heterostyly observed in the argane tree. (**a**): Mesostylous (**b**): Longistylous and (**c**): Brevistylous flower.

The argane female reproductive system is made up of a super gynaecium with a conical style ending in a stigma. The pubescent and superior ovary is surmounted by a short and conical style, also protruding from the stamina or vice versa (Fig. 5). The ovary is made of 2 to 5 welded and uniovulate carpels. The 5 carpel gynaecium was rare. The ovaries are tiny and located on the central column of the gynaeceum. The placentation is of the axile type. The floral formula is: (5S) + [(5P) +5St+5E à (10E)]+ (1C) à (5C) (Fig. 6).

Figure 6. Floral morphology of argane with a flower of 10 (a), 8 (b) and 5 (c) anthers.

Viability and *in vitro* pollen germination

The viability of the pollen assessed by staining technique showed that the pollens of trees crossed were viable and able to germinate.. The germination of pollens on an agar medium is illustrated in Fig. 7.

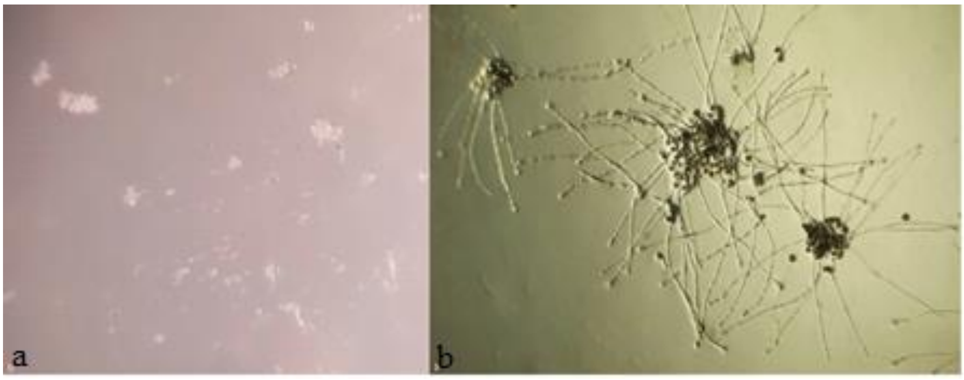

Figure 7. *In vitro* pollen germination test, (a) absence of development of the pollen tube germinated, (b) pollen germination with tube growth on a germination medium (1% agar and 20% sucrose)

Compatibility study

To determine the nature of the breeding system, the index of self-incompatibility (ISI) was calculated and the results show that the ISI of the 11 receiver parents was less than 0.25 and all trees were self-incompatible (Table 2). Also, cross combinations between the two pollinizers and these 11 receiver parents were very efficient. The cross-compatibility rate often depends on cross associations and varies from 38.71 % to 84.38 %. Five combinations have results of less than 50 % for pollinizer INRA-62

and all combinations or crossing with pollinizer INRA-142 showed that pollinizer compatibility varied from 50 to 84 %.

Table 2. Fruit set of cross-pollination of two pollinizers with 11 argane genotypes.

Pollinizers	Receiver genotypes	floral morphs	Flowers number		Fruits number		Rate of fruit set		Nature of breeding system
			Cross/p.	Self*/p.	Cross/p.	Self/p.	Cross/p.	Self/p.	
INRA-62	INRA-139	Mst	66	115	30	0	45.45	0	SI
	INRA-135	Bst	62	9	24	0	38.71	0	SI
	INRA-109	Bst	14	168	8	0	57.14	0	SI
	INRA-79	Mst	36	15	24	0	66.67	0	SI
	INRA-65	Mst	79	64	39	1	49.37	1.56	SI
	INRA-49	Mst	47	107	21	0	44.68	0	SI
	INRA-98	Bst	101	109	50	0	49.50	0	SI
INRA-142	INRA-132	Bst	49	107	34	0	69.39	0	SI
	INRA-107	Mst	19	139	10	0	52.63	0	SI
	INRA-98	Bst	31	109	25	0	80.65	0	SI
	INRA-75	Mst	35	37	25	0	71.43	0	SI
	INRA-65	Mst	64	24	54	0	84.38	0	SI
	INRA-54	Bst	73	127	38	2	52.05	1.57	SI

(*): Number of flower self-pollinated (female parent), p: pollination, SI: self-incompatible, Mst: mesostylous, Bst: brevistylous.

Fruit set and metaxenic pollen effect on the fruit size

Although most cross combinations resulted in fruits, but low fruit number has arrived at full maturity . The number of fruit sets differs from one genotype to another and the response of trees via the same pollen differs from one tree to another. The presence of trees with high bloom potential and compatibility in the orchard is very effective for argane tree's productivity. Hence, in 13 combinations that interested this study (Table 2), the difference was highly significant in fruit size between cross pollination “pollinizers and cross-pollinated female parents” and open pollination, comparing the fruit from cross-pollination in each combination (♂INRA-142*INRA-98♀), (♂INRA-142*INRA-65♀), (♂INRA-62*INRA-98♀), (♂INRA-62*INRA-65♀) (Fig. 8).

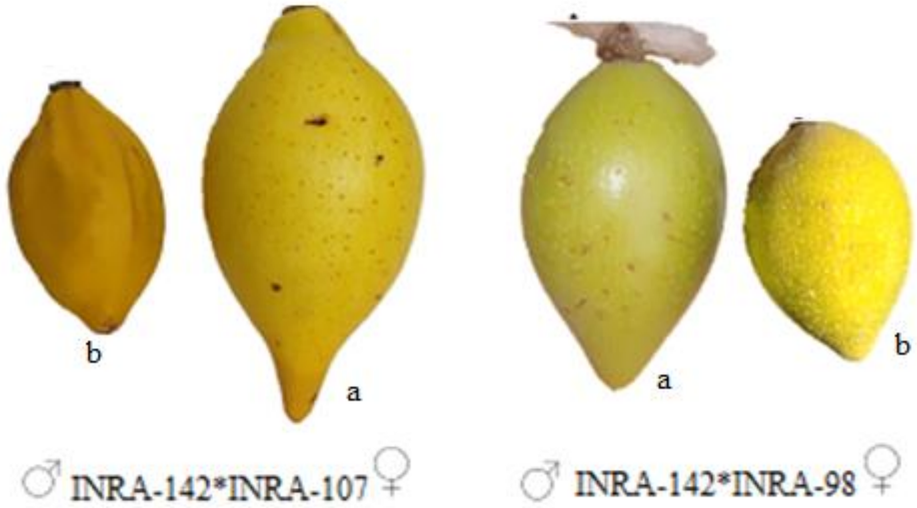

Figure 8. A careful choice of compatible pollinizers significantly improves the size of fruits in argane tree, (a): fruit from cross-pollination (♂INRA-142*♀INRA-98, and (♂INRA-142*♀INRA-107), (b): fruit from open pollination.

Discussion

Currently, there are scarce data regarding the pollen quality and the pollen tube growth rate during pollination (self-pollination and cross-pollination).

The pollen germination rate and the development of the pollen tube are important for fertilization. Thus, a low pollen germination rate can cause fruit set failure due to degradation of the ovum before the pollen tube reaches the ovary (Mellenthin et al., 1972 ; Therios et al., 1985). The argane tree is a monoecious species, with hermaphrodite flowers (Perrot, 1907), alone or grouped in glomeruli that appear at the base of the leaves or on the nodes of mature branches (Perrot, 1907 ; Bani-Aameur, 2002). Each glomerulus can contain up to 15 flowers. The calyx is made up of 5 free sepals (dialysepal) and a corolla with 5 free petals (dialypetal) (Bani-Aameur, 2000).

The majority of flowering plants show polymorphisms in floral morphology. For *A. spinosa*, tristylous polymorphism with three flower forms (Mesostylous, longistylous and brevistylous) was observed as for other wild species (Nettancourt, 2001). However, for *A. spinosa* differences in style length and anther positioning influenced the interaction between the pollen and the pistil then the pollination compatibility. The morph phenotype is genetically linked to genes responsible for a unique system of self-incompatibility. Indeed, the pollen from a flower on one morph cannot fertilize another flower of the same morph. These findings of polymorphisms in floral morphology factors play an important role in the compatibility mechanism of argane tree pollination.

Based on the results of controlled pollination, it can be concluded that argane trees are considered self-incompatible and cross-pollination is very important. Vulnerable pollination is a limiting factor in plant production in various regions (Alizadeh et al., 2009). Thus, choosing a particular pollinizer for particular female parents is currently

a new topic of research for argane fruit production under arganiculture programs. Currently, there is a scarce of data regarding the effect of genetically different pollen sources on argane fruit (size or weight, color, biochemical composition...). This study aimed to evaluate the effect of two pollinizers (pollen source) INRA-142 and INRA-62 on fruit size and weight and their ability to affect the phenotype of directly developed fruits from the pollinated flowers; more precisely, these characters can present metaxenia effect on the fruits. This metaxenia effect is for the first time evaluated for argane tree. Moreover, a comparison of the fruit characters (fruit size, color, number of almonds per seed) between open pollination and cross-pollination showed either a decrease or increase of the character depending on the source of pollen for the INRA-142 and INRA-62 pollinizers. While, the shape of the fruit and the maturation cycle are controlled by female parents, knowing that the argane trees cycle to collect ripe fruits varies from 9 to 15 months.

Based on the findings of the current study, it can be concluded that the genetically different pollen sources affected the weight and size of the argane fruit, its shape, color, and developmental timing, depending on the genetic background of the mother or female parents. The differential effects of fertilization with various pollen sources on the fruit size, shape, color, or the chemical composition of seeds and fruits is defined as xenia effect (Denney, 1992). However, we find another term expressed as “metaxenia” (Swingle, 1982). For scientists who study “xenia”, this term is used to describe direct pollen effects on (embryo and endosperm) and “metaxenia” for the effects on maternal-plant tissue, and is applied to fruit in which fleshy carpel and accessory tissues are more economically important. The current study, with four different pollination treatments applied to 11 argane genotypes revealed the differences between open-pollination and cross-pollination for most fruit traits (size, color, shape, time of fruit maturity), and suggest the influence of the pollinizer (effect of pollen source) on fruit size, shape, time of fruit ripe. More researches are still needed to identify suitable maternal/pollen-parent combinations that will realize some of the potentials of argane pollen parents to increase productivity. Consequently, further investigation will resume after the growth of the obtained descendants from different crossings of the current study.

Conclusion

Synchronization of flowering is a major factor in the hybridization potential in the argane tree. The availability of genotypes with high flowering potential (pollinizer) and compatibility is a very desired matter, taking into account also good climatic conditions and the presence of vectors for good pollination. This study showed that at an agronomic level, artificial pollination is feasible for successful crosses at the argane tree and the plantation of pollinizer in the orchard could be valuable to improve yield and quality. Identification of compatible genotypes provides an important baseline for successfully transferring traits of interest from selected elite genotypes to Argane cropping system. The controlled pollination technique demonstrated during this study will serve as the basis for the future breeding programs of the argane tree and fruit productivity. These results from diallel crosses could be also useful in the screening of the vigorous parent with desired traits to be improved for argane orchard planning.

Acknowledgements

This research was funded by the National Institute of Agricultural Research (INRA-Morocco) within the framework of the argane scientific research program. The authors would like to thank Bouzoubaa Z. for collecting and installing the plant materials and Boujghagh M. for providing expertise and technical support.

References

Ait Aabd N., Bouharroud R., Tahiri A., Wifaya A., Mimouni A. and El Mousadik A., (2019). Genetic Diversity, Conservation and Breeding of Argan Tree (*Argania spinosa* L. Skeels). J.M. Al-Khayri et al., (eds.), Advances in Plant Breeding Strategies: Nut and Beverage Crops. p. 31–56. https://doi.org/10.1007/978-3-030-23112-5_2.

Ait Aabd N., El Asbahani A., El Alem Y., El Finti A., Msanda F. and El Mousadik, A. (2013). Variation in oil content and fatty acid compositionin preselected argan trees with morphological characters and geographical localization. Mediterranean journal nutrition metabolism 6. p. 217–225. https://doi.org/10.1007/s12349-013-0134-2(2013).

Ait Aabd N., Msanda F., El Mousadik A. (2014). Evaluation of variability in argan oil content through different environments and preselection of elite genotypes. Euphytica 195(2). p.157–167. https://doi.org/10.1007/s10681-013-0969-0.

Ajerrar A., Akroud H., Ait Aabd N., Qessaoui R., Amarraque A., Lahmyd H., Zaafrani M., Chebli B., Mayad H. and Bouharroud R.(2020). Pollination system and effective pollinators of *Argania spinosa* (L. Skeels). Journal of the Saudi Society of Agricultural Sciences. https://doi.org/10.1016/j.jssas.2020.04.002 .

Alizadeh S., Arzani K., Imani A. (2009). Determination of effective pollination period in late flowering almond (*Prunus dulcis* Mill.) Shahroud 12 in Karaj climate conditions. Iranian Journal of Horticultural Science 40. p. 1–7.

Bani-Aameur F.(2000). Phenological Phases of Argania spinosa (L.) Skeels Flower. Forest Genet 7. p. 333–338.

Bani-Aameur F. (2002). *Argania spinosa* (L.) Skeels flowering phenology. *Genetics resources and crop evolution* 49. p. 11–19.
https://doi.org/10.1023/A:1013831015120.

Barrett S.C.H. (1992). Heterostylous genetic polymorphism: model systems for evolutionary analysis. In: Barrett, S.C.H. (Ed.), Evolution and Function of Heterostyly p. 1–29. Springer-Verlag, Heidelberg; https://doi.org/10.1007/978-3-642-86656-2_1.

Bawa K.S. (1974). Breeding systems of tree species of a lowland tropical community. Evolution 28. p. 85–92; doi:10.1111/j.1558-5646.1974.tb00729.x.

Belmouden S. and Bani-Aameur F. (1995). Contribution à l'étude de l'anthèse et de la viabilité du grain de pollen. Proceedings of the International Symposium of the Forest Facing Desertification: Case of Arganeraies. Faculty of Sciences of Agadir. p. 57–62.

Benlahbil S. (2003). Pollinisation Naturelle et Artificielle de l’Arganier (*Argania spinose* (L.) Skeels). PhD Thesis, Ibn Zohr University, Morocco. 157p.

Benlahbil S. and Bani-Aameur F.(2002). *In-vitro* germination pollen of argan (*Argania spinosa* (L.) Skeels. Acta Botanica Gallica 150 (2). p. 231–238. https://doi.org/10.1080/12538078.2003.10515421.

Ben-Njima N., Sociasi Company R. (1995). Characterization of some self-compatible almonds. I. Pollen tube growth. HortScience 30. p.318–320.

Bolat I. and Pirlak L. (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. Turkish Journal of Agriculture and Forestry 23. p.383–388.

Boöković R. and Tobutt K.R. (2001). Genotyping cherry cultivars assigned to incompatibility groups by analyzing stylar ribonucleases. Theoretical and Applied Genetics 103. p. 475–485. https://doi.org/10.1007/PL00002906.

Burgos L., Berenguer T. and Egea J. (1993). Self- and cross-compatibility among apricot cultivars. HortScience 28. p. 148–150. https://doi.org/10.21273/HORTSCI.28.2.148.
Cerović R. and Ružić, D.(1992). Pollen tube growth in sour cherry (*Prunus cerasus* L.) at different temperatures. Journal of Horticultural Sciences 67. p. 333–340. https://doi.org/10.1080/00221589.1992.11516256.
Cruzan M.B. (1990). Pollen-pollen and pollen-style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). American Journal of Botany 77. p. 116–122. DOI: 10.2307/2444799.
Dafni A. and Firmage D. (2000). Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Systematics and Evolution 222. p. 113-132. https://doi.org/10.1007/BF00984098.
Dafni A., Kevan P.G. and Husband B.C. (2005). Practical Pollination Biology, ed. Enviroquest Ltd., Cambridge, Canada.
Denney J.O.(1992). Xenia includes metaxenia. *HortScience* 27. p. 722–728. https://doi.org/10.21273/HORTSCI.27.7.722.
Dicenta F., Ortega, E., Cánovas, J.A. and Egea, J.(2008). Self-pollination vs. cross-pollination in almond: pollen tube growth: fruit set and fruit characteristics. Plant Breeding 121. p. 163–167. https://doi.org/10.1046/j.1439-0523.2002.00689.x.
Egea J. and Burgos, L. (1996). Detecting cross-incompatibility of three North American apricot cultivars and establishing the first incompatibility group in apricot. Journal of the American Society for Horticultural Science 121(6). p. 1002–1005. https://doi.org/10.21273/JASHS.121.6.1002.
Ehrig F.R. (1974). The Argan Nature, Ecology and Economic Significance of a Tertiary Relic in Morocco. Gotha: Klett-Perthes.
El Mousadik A. and Petit R.J.(1996). High level of genetic differentiation for allelic richness among population of argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. Theor Applied Genetics 92. p. 832–839. https://doi.org/10.1007/BF00221895.
Focke W.O. (1881). Die Pflanzen Mischinge: ein Beitrag zur Biologie Der Gewächse. Berlin: Borntraeger. p. 510.
High Commision for Water, Forests and Desertification Control (HCEFLCD)., 2012. Update of the National Program against Desertification (PAD-LCD): Adaptation to Areas Specificity.
Linskens H.F. (1986). Recognition during the progamic phase. In: Cresti, M., Dallai, R. (Eds.), Biology of Reproduction and Cell Motility in Plants and Animals. University of Siena, Siena. p. 21–32.
Mellenthin W.M., Wang C.Y. and Wang S.Y.(1972). Influence of temperature on pollen tube growth and initial fruit development in ‘d’Anjou’ pear. *HortScience* 7. p. 557–559.
Mouhaddab J., Msanda F., Filali-Maltouf A., Belkadi B., Ferradouss A., El Modafar, C., Ibnsouda Koraichi S. and El Mousadik A. (2017). Using microsatellite markers to map genetic diversity and population structure of an endangered Moroccan endemic tree (*Argania spinose* L. Skeels) and development of a core collection. Plant Genet 10. p. 51–59. https://doi.org/10.1016/j.plgene.2017.05.008.

Mousavi S., Hosseini Mazinani M., Arzani K., Ydollahi A., Pandolfi S., Baldoni L. and Mariotti R. (2014). Molecular and morphological characterization of Golestan (Iran) olive ecotypes provides evidence for the presence of promising genotypes. Genetic Resources and Crop Evolution 61. p. 775–785. https://doi.org/10.1007/s10722-013-0071-4.

Msanda F. (1993). Ecologie et cartographie des groupements végétaux d'Anzi (Anti-Atlas Occidental, Maroc) et contribution à l'étude de la diversité génétique de l'arganier (*Argania spinosa* (L.) Skeels). Thèse, University of Joseph Fourrier, Grenoble, France.

Nerd A., Irijimovich V. and Mizrahi Y.(1998). Phenology, breeding system and fruit development of Argan (*Argania spinosa*, Sapotaceae) cultivated in Israel. Economic Botany 52(2). p. 161–167 https://doi.org/10.1007/BF02861204.

Nettancourt D.(2001). Incompatibility and Incongruity in Wild and Cultivated Plants. (springer) doi:10.1007/978-3-662-04502-2.

Nikolić D. and Milatović D. (2010). Examining self-compatibility in plum (Prunus domestica L.) by fluorescence microscopy. *Genetika* 42(2). p. 387-396. https://doi.org/10.2298/GENSR1002387N.

Ortega E. and Dicenta, F.(2004). Suitability of four methods to identify self-compatible seedlings in an almond breeding programme. Journal of Horticultural Science and Biotechnology 79. p. 747–753.
https://doi.org/10.1080/14620316.2004.11511837.

Ortega E., Egea J., Cánovas J.A. and Dicenta F.(2002). Pollen tube dynamics following half- and fully-compatible pollinations in self-compatible almond cultivars. Sexual Plant Reproduction 15. p. 47–51. https://doi.org/10.1007/s00497-002-0137-5.

Perrot E.M.(1907). L'Argan. In Les Végétales Utiles de l'Afrique Tropicale Française. p. 127-158 (Challamel), Paris (1907).

Prasad P.V.V., Boote K.J., Allen J.L.H., Sheehy J.E. and Thomas J.M.G.(2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Research 95. p. 398–411. https://doi.org/10.1016/j.fcr.2005.04.008.

Prendergast H.D.V. and Walker C.C.(1992). "The Argan: Multipurpose Tree of Morocco." Curtis's Bot. Mag. 9 (2). p. 75–85.

Rasouli M., Fattahi Moghadam M.R., Zamani Z., Imani A. and Ebadi A.(2009). Study of compatibility and additional pollination of Supernova cultivar with pollen of different almond cultivars. Iranian Journal of Horticultural Science 40. p. 61–70.

Rezazadeh R., Hassanzadeh H., Hosseini Y., Karami Y. and Williams R.R. (2013). Influence of pollen source on fruit production of date palm (*Phoenix dactylifera* L.) cv. barhi in humid coastal regions of southern Iran. Scientia Horticulturae 160. p. 182–188. https://doi.org/10.1016/j.scienta.2013.05.038.

Riazi G., Khanizadeh S. and Rahemi M., (1995). The effect of various pollen grains on growth and development of Pistacia vera L nuts. *HortScience* 30(4). p. 886–887. https://doi.org/10.21273/HORTSCI.30.4.886G.

Richards, J.H. and Barrett, S.C.H. (1992). The development of heterostyly. In: Barrett, S.C.H. (Ed.), Evolotion and Fuction of Heterostyly. p. 85–127.

Sanchez-Perez R., Arrazola G., Martin M.L., Grane N. and Dicenta F. (2012). Influence of the pollinizer in the amygdalin content of almonds. Scientia Horticulturae 139. p. 62–65. https://doi.org/10.1016/j.scienta.2012.02.028.

Selak VG., Perica S., Goreta Ban S. and Radunić M. (2011). Reproductive success after self-pollination and cross-pollination of olive cultivars in Croatia. *HortScience* 46 (2). p. 186–191. https://doi.org/10.21273/HORTSCI.46.2.186.

Shivanna, K.R., 2003. Pollen development, in: Shivanna, K.R. (Ed.), Pollen Biology and Biotechnology (Science Publishers, USA).

Steven D.J., Marco Balducci G.M. and Shuttleworth, A. (2019). Hawkmoth pollination of the orchid *Habenaria clavata*: mechanical wing guides, floral scent and electroantennography. Biological Journal of the Linnean Society 129(1). p. 213–226. https://doi.org/10.1093/biolinnean/blz165.

Swingle W.T. (1928). Metaxenia in the date Palm: Possibly a Hormone Action by the Embryo or Endosperm, Journal of Heredity 19(6). p. 257–268. https://doi.org/10.1093/oxfordjournals.jhered.a102996.

Therios I.N., Tsirakoglou V.M. and Dimassi-Therous K.N. (1985). Physiological aspects of pistachio (*Pistacia vera* L.) pollen germination. *Riv. Ortoflorofruit Italiana* 3. p. 161–170. https://www.jstor.org/stable/42878737.

Yatrib C., Belkadi B., Pakhrou O., Alami M., Medraoui L., El Mousadik A., Ferradous A., Msanda F., El Modafar C., Ibn Souda-Kouraichi S. and Filali-Maltouf A. (2015). Assessment of genetic diversity of *Argania spinosa* L. growing in arid and semi-arid areas of Morocco as revealed by inter-simple sequence repeats. Journal of Agricultural Science and Technology 5. p. 336–346. https://doi.org/10.17265/2161-6264/2015.05.005.