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FINAL REPORT

Submitted to the office of the Science Advisor
U.S. Agency for International Development

Title: **Improvement of Pistachio Nut Production in Arid Zones**

Principal Investigator: Avi Golan (Goldhirsh)
Principal coinvestigator: Abilio Mendes Gaspar

Grantee Institution:
The Jacob Blaustein Institute for Desert Research
Ben-Gurion University of The Negev
Sede Boqer Campus 84993 Israel

Project Number: C5-224
Grant Number: DPE-5544-G-SS-7013-00

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3 Executive Summary

This project presents a unique combination of agrotechnology and biotechnology for the introduction of a crop tree (Pistachio) to an arid zone and research on its reproductive biology. The following objectives were set: 1. To introduce and establish experimental pistachio orchards in arid zones of Portugal where it has not been cultivated before. 2. To investigate biological aspects of low fertility and productivity of pistachio under arid conditions. Major progress was made towards achieving these objectives. Pistachio (*Pistacia vera* L.) was introduced to Portugal and six experimental orchards were established. In the course of establishing the new experimental orchards Israeli experts in pistachio orchard agrotechnology and nursery technics went to Portugal, guided and assisted the portuguese colieagues in all operations. Scientific meetings were held throughout the research. This has culminated in a workshop on "Intensive Agriculture in Arid and Semi-Arid Zones" held in Portugal in 1988. Rootstock and grafted seedlings and drip irrigation systems were transferred from Israel to Portugal to initiate the experimental sites. Then, filling-up of gaps, germination trials, budding and grafting were carried out by the Portuguese team.

The questions regarding low fertility and the effect of salinity on pistachio productivity were studied using mature trees. Two polypeptides, a 32 KDa and a 27 KDa, which coincide with reproductive bud development were identified, isolated and monoclonal antibodies produced against them. These polypeptides and their monoclonal antibodies may serve as molecular markers for horticultural research on the effect of environmental, hormonal and chemical treatments on floral induction, differentiation, and bud dormancy in pistachio and other deciduous fruit trees. Continuous exchange of information on experimental progress was maintained throughout the project and was presented in the semi-annual reports.

4 Research Objectives

The *Pistacia vera* L. (common name pistachio) is a deciduous, dioecious species which adapts to desert conditions. It produces a nutritious nut and its production does not meet market demand. Therefore, we believe that the pistachio offers a unique advantage as a crop and a source of income to many of the less developed countries with vast arid zones. The overall aim of this project is to increase productivity of pistachio nuts in arid zones by focusing research on the following aspects: 1. Introduction and establishment of experimental pistachio orchards in arid zones of Portugal where it has not been cultivated before. In the course of establishing the experimental orchards selection was made for rootstocks and scions, methods of budding and grafting were examined. 2. Biological aspects involved in low fertility of pistachio. We have set out to search for molecular markers associated with reproduction in pistachio.

A common feature of the female inflorescence of pistachio is that many of its flowers do not produce seeds. A major limitation to pistachio cultivation is the requirement for winter chilling for bud dormancy break and normal development. Chilling requirement is a genetic trait, and selection has been made for low chilling requirement. However, the mechanism of bud dormancy development and release in pistachio as well as in other deciduous tree crops is only vaguely known. As a dioecious species the time of anthesis and the duration of flowering is of extreme importance. Under natural conditions the bloom period of male seedlings often precedes that of female seedlings. Therefore, artificial pollinating is practiced in pistachio orchards, and the effect of storage on the quality of the pollen studied. An alternative approach to overlap male and female flowering was to advance flowering by spraying with winter oil sprays.

An interesting innovative breakthrough obtained in this project was the identification and purification of a 32 KDa polypeptide (Ibp32) from pistachio male inflorescence buds. It accumulates during floral induction, remains in the

buds during the dormancy period and disappears during flowering. Using antibodies produced against this polypeptide, we detected an immunologically related protein of 27 KDa (Ibp27) in female buds. This polypeptide was present in about 70% of the female trees tested (37 trees). None of the eight male trees tested so far contained the Ibp27 in its inflorescence buds. The genetics of these polypeptides and their subcellular location(s) are not known, although preliminary experiments suggest association with the reproductive zone of the bud. The sexual linkage is now under investigation because of the basic interest in plant sexual determination and potential practical use of these markers for development of a diagnostic procedure for early gender determination in pistachio.

It may be significant that Ibp32 is synthesized during early development of the bud and remains apparently unchanged during bud dormancy, disappearing during inflorescence opening. Could it be involved in dormancy regulation? For the moment Ibp32 may provide an important molecular marker for male flower development in pistachio and potentially in other trees.

5 Methods and Results

The experimental sites in Portugal are at Ingra-Lameira and Benafim Grande, located in the south of Portugal. Revilheira, Elvas and Lameirões in the sout-east and Vilariça in the north-east. Drip irrigation was introduced in all the sites. Germination trials were carried-out with seeds of several *Pistacia* species (*P. vera*, *P.* atlantica, *P. integerrima*, *P. palaestina* and *P. terebinthus*). These species were also compared for rootstocks characteristics in trials at Ingra-Lameira and Lameirões. Seeds of *P. vera* gave best germination when a "rag doll" was used, i.e. when they were wrapped in a sterilized burlap roll kept constantly moist and maintained at an optimal temperature approx. 26 ^{0}C, rather than 15 to 20 ^{0}C, which was reported by other growers. After germination and development of a radicle longer than 1 cm, the seedlings were shifted to trays (60x40x8 cm) with a mesh base. The seedlings were let to develop in these trays until they reached a

length of 15 to 20 cm with a well ramified root system. Then they were transplanted to the field. The germination of *P. atlantica* seeds was relatively easy in wet and sterilized media like burlap rolls, petri dishes with hydrophilous cotton or with agar media. The germination of *P. terebinthus, P. Palaestina and P. integerrima* seeds was more difficult. This has led to trials to test the effects of several reagents and treatments known to affect germination, e.g. fungicides, calcium hypochlorite, sulphuric acid, GA_3 and hot water. These treatments were given at several concentrations and times to improve germination.

The rootstocks were budded and grafted with budsticks of several varieties obtained from Israel, Portugal, Spain and Italy. The methods used were "chip budding", "shield budding", "wedge graft" and "veneer crown graft". Best initial unions were obtained with the "chip budding". However, in many cases there was wilting after a few weeks of budding and/or grafting.

The following varieties were tested as scions:

1. From the Avdat Run-off Farm, Israel: Avdat, Kerman, Larnaka, Nazareth and Chico.
2. From the Estação Nacional de Fruticultura Vieira Natividade, Portugal: Mateur, Safeed, 25-A and 40-A.
3. From Centre Agropecuari Mas Bové, Spain: Ghiandolara, Kerman, Ashoury, Lathwardy, M-38, Sfax, Joley, M-11, Capuccia, Batoury, Larnaka, Boundoky, El Guetar, Aegina, M-37, M-B, Cerasola, Peters, M-36, Bronte, M-C, Red Jalap, Bianca, White Ouleimy, Ajamy, Mateur, Avidon, and Ask.
4. From Istituto Sperimentale per la Frutticoltura, Italy: Chico, Ask, Larnaka, Bronte, Red Aleppo, Kerman, Enk, Naz, Irak and Rashtii.

In the course of the project there were two visits of the PI (A. Golan) in Portugal and two visits of the cooperating Portuguese scientists (A.M. Gaspar and M. Mota) in Israel. In addition, Israeli experts in pistachio agrotechnics (D. Mazigh) and nursery technics (J. Maurice) were sent to Portugal to assist in establishing the experimental sites and at the budding and grafting stages. Over 2000 rootstocks

and grafted seedlings were shipped via air-mail from Israel to Portugal. It takes 5 to 8 years until the seedlings begin to flower then the orchards will be fully set for production. A general current view of the experimental sites at Lameirões and Elvas are shown in figures 1, 2 and 3.

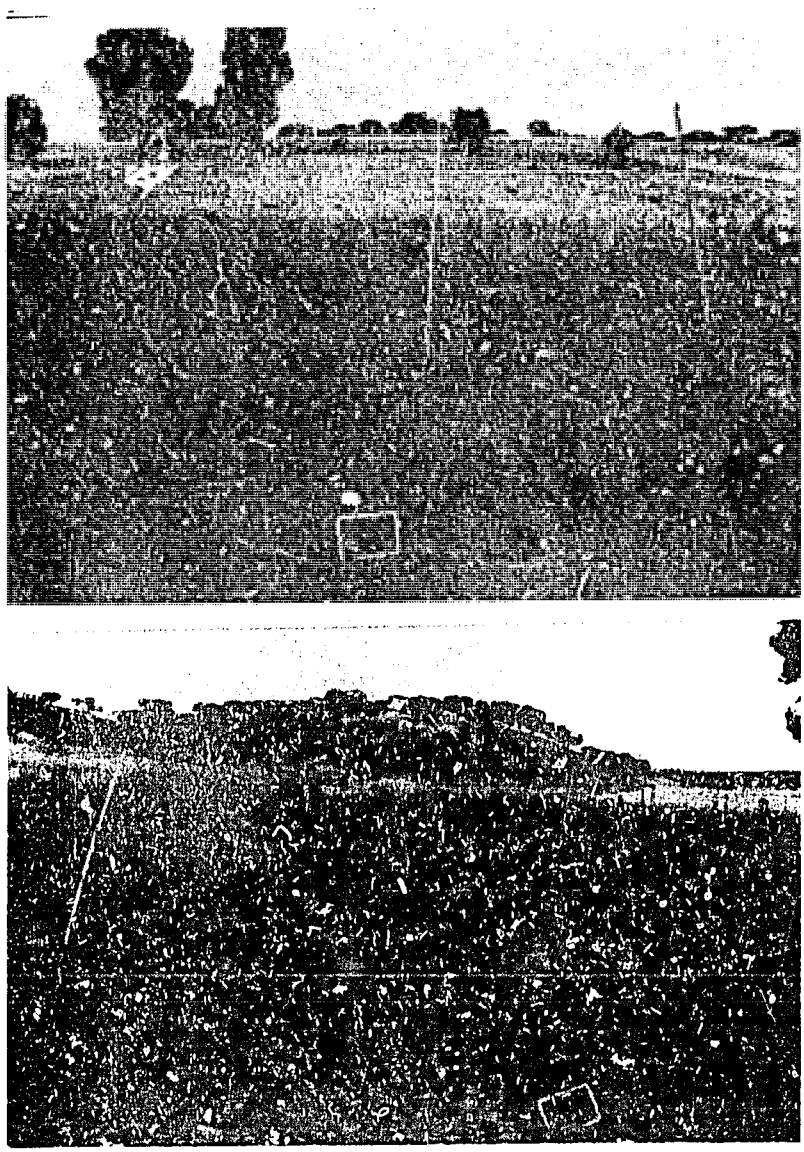

Figure 1: General views of the experimental pistachio plots at Lameirões, Portugal. Trial of varieties - 18; Trial of rootstock x varieties - 28.

Figure 2: Pistachio trees at Elvas in a trial of varieties. Kerman - 6; Kerman grafted (Shield) on July 29, 1991 - 7; Ashoury grafted (cleft) on April 23, 1991 - 8; Ashoury grafted (chip) on June 20, 1991 - 9.

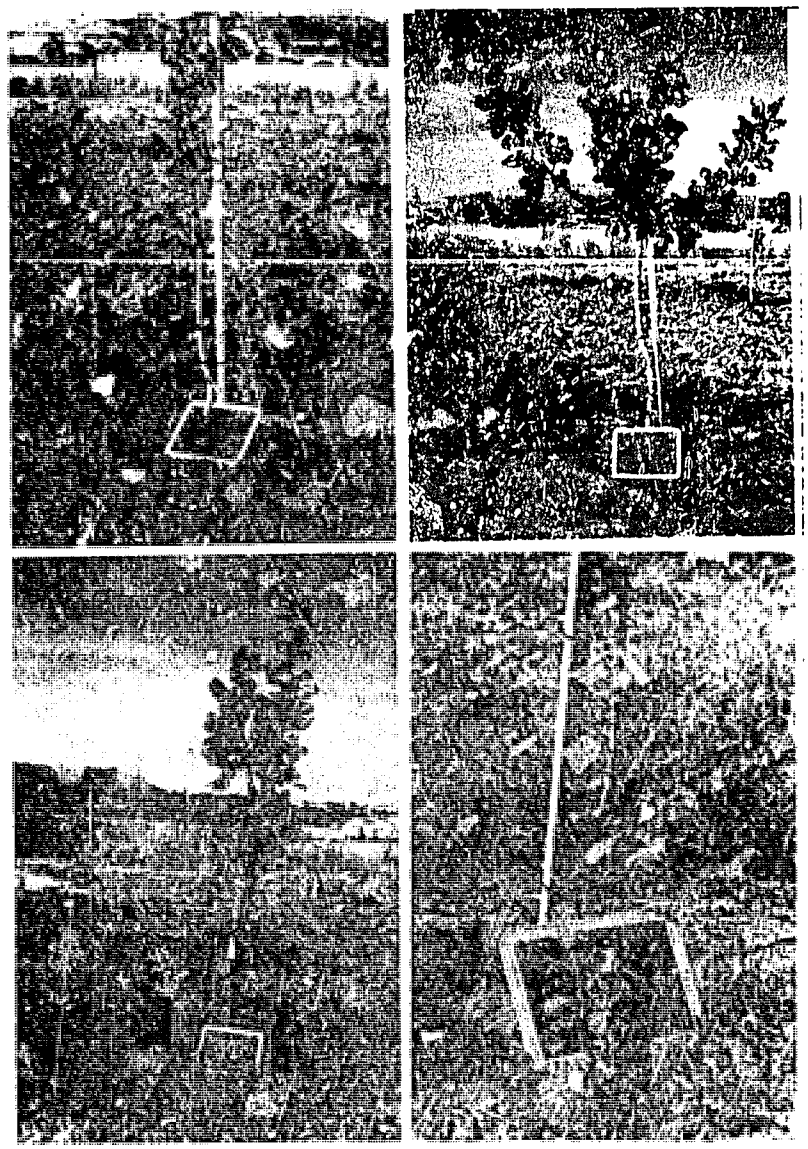

Figure 3: Pistachio trees at Elvas in a trial of varieties. Sfax grafted (chip) on June 20, 1991 - 10; Aegina grafted (cleft) April 30, 1990 - 11; 25-A (male) grafted (cleft) on April 30, 1990 -12; Larnaka grafted (shield) on July 29, 1990 - 13.

The research was productive for Portuguese and Israeli laboratories. We have progressed in our understanding of what is required for the establishment of pistachio orchards in an arid zone and we have a better knowledge of the

reproductive biology of pistachio. Furthermore, the molecular markers identified in the pistachio may have a wider use in studies on the reproductive biology of deciduous fruit trees and aid in their introduction to arid zones.
The results are presented under the scientific papers published or in preparation for publication. Because of the diversity of research and different stages of completeness, it is impossible to attempt to present it as a single paper.

Publications

1. Gaspar, M. A. 1989. The pistachio as a study case for crop trees in arid zones. In " Agricultura intensiva em zonas áridas e semi-áridas". Proceedings of a Luso-Israeli workshop held in Portugal, March 1988, pp. 75-87.
2. Golan-Goldhirsh, A. 1989. The pistachio as a study case for crop trees in arid zones. In " Agricultura intensiva em zonas áridas e semi-áridas". Proceedings of a Luso-Israeli workshop held in Portugal, March 1988, pp. 69-74.
3. Golan-Goldhirsh, A., Heimer, Y. M., Cohen, Z. and Lips, S.H. 1991. Pistachio nut production in the Negev. In "Advances in Desert Arid Land Technology and Development", Vol. 5, pp. 385-395, Hardwood Academic Publishers, New York. (It was accepted for publication before the beginning of this project).
4. Golan-Goldhirsh, A., Schmidhalter, U., Muller, M. and Oertli, J.J. 1991. Germination of *Pistacia vera* L. pollen *in vitro* in liquid medium. Sex. Plant Rep. 4:182-187.
5. Golan-Goldhirsh, A., Laslo-Cohen, E. and Lips, H.S. 1991. Microscopic aspects and protein composition during microsporogenesis in *Pistacia vera* L. (Submitted).
6. Golan-Goldhirsh, A. and Zelig, E. 1991. Polypeptides associated with sexual development of *Pistacia vera* L. inflorescence bud (Submitted).

WORKSHOP LUSO-ISRAELITA SOBRE A AGRICULTURA INTENSIVA EM ZONAS ÁRIDAS E SEMI-ÁRIDAS

FARO – 24 a 26 de Março de 1988
OEIRAS – 28 e 29 de Março de 1988

FARO
- A Agricultura Portuguesa e o seu Enquadramento Comunitário
- O Algarve – seus Recursos e sua Agricultura
- Aproveitamentos Agrícolas Alternativos nas Zonas Áridas
- Gestão dos Recursos Hídricos

OEIRAS
- Relações Planta-Ambiente em Condições de Aridez
- Novas Técnicas de Propagação de Plantas
- Novas Culturas para Regiões Mediterrânicas
- Organização e Intercâmbio na Investigação Agrária

Lisboa, 1989

Na protecção do ambiente podia haver exp orações especializadas agrícolas e silvicultura que se dedicam à preservação da paisagem, à conservação dos s, à várias formas da Agro-Silvo-Pastorícia e por fim também à reflorestação. es trabalhos podem ser praticados como actividade principal ou como activi-'e complementar.

Eu sei que muitas questões sobre mudança das funções da agricultura ainda ão abertas à discussão, mas o estado tem que formular normas e tem que ter a ıgem de *nvestir os subsídios públicos a longo prazo mais na protecção co-nente*. No futuro devia só entrar uma pequena parte dos subsídios públicos na ução agrária, onde o ponto principal devia ser a investigação e a introdução de as culturas. A maioria da produção agrária devia poder-se financiar propriamente ıvés da venda dos seus produtos no mercado sem subvenções.

Conclusões e Recomendações

Tem que haver cuidado quando se transfer experiências dum país para o outro, que históricamente e culturalmente e assim também o comportamento das soas é diferente. Por outro lado podem experiências boas ou más do estrangei-ıudar para não fazer grandes erros no próprio país.

A colaboração internacional na investigação agrária é um bom instrumento do rcâmbio de experiências pelos dois lados. Ainda há muita coisa a fazer no envolvimento da agricultura algarvia, eu só reclamo:

- o inventário dos recursos das águas subterrâneas;
- a intensificação na horticultura, fruticultura e floricultura;
- a diversificação das culturas e a investigação com plantas novas para fins ıicos (matérias-primas renováveis);
- a planificação das redes de rega;
- a organização das associações de beneficiários e utilizadores;
- a expansão da investigação agrária com apoio da Universidade do Algarve ıo também
- os problemas de comercialização dos produtos agrícolas.

É importante assinalar que os pressupostos políticos agrários tomam hoje um el importante, que ao lado da produção agrícola também têm que ser fomentadas ınções sociais e a conservação do ambiente, que a actividade complementar da cultura vai aumentar e por fim que as questões de Exploração e da Gestão dos ursos Hídricos têm que ter a mesma importância como as questões técnicas da ıtrução de grandes obras públicas hidroagrícolas.

iteratura

'IVAL: L' office Regional de Mise en Valeur Agricole du Loukkos XIII. ICID – Kongress, Casablanca, ı. 1987

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ENAECKER, H-CHR: Informação básica sobre Associações de Solos e Águas. Manuscrito 1987

RURAL, 6/1995

G. A disponibilidade de Água para rega e consequências para a agricultura algarvia 4. Congresso do ı. 1995

THE PISTACHIO AS A STUDY CASE FOR CROP TREES IN ARID ZONES

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Introduction

There are two reasons for a scientist to be involved in a search for crop trees suitable for arid zones. One is the interest in development of arid zones for the betterment of life of human beings in these areas. Another reason is the scientific curiosity and the wish to understand the biology and adaptation mechanisms of plants to arid conditions.

I am very glad to have initiated the cooperation between our Portuguese colleagues of the Estação Agronomica Nacional (EAN) and our group at the Blaustein Institute for Desert Research of the Ben-Gurion University of the Negev. In the first two years, since we first met, we have been able to establish the pistachio project and make substantial progress. Here, I want to describe the experience accumulated in the pistachio project as a study case. Carob, cork oak and Leucaenae are now under consideration as potential trees for arid zones as well.

It has been shown by M. Evenari and co-workers that pistachio trees adapt to the climatic conditions of the Negev highlands. The trees develope well and produce fruit on runoff water. Our aim now is to better understand the biology of this crop in order to increase its productivity in arid zones.

Pistachio Fertility

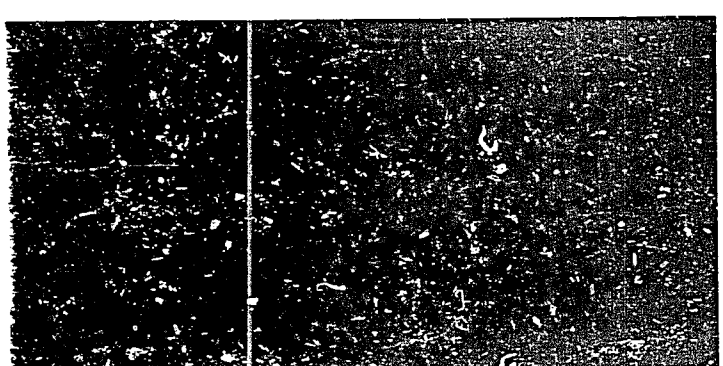
The genus *Pistacia* L. (Anacardiaceae) consists of deciduous trees and shrubs which produce single-seeded drupes. It is well known and reported that *Pistacia* species have a very low seed set. There are reports of trees which have not produce any seed bearing fruits (Grundwag & Fahn, 1969). In a preliminary experiment to estimative biomass production and deposition in the fruit, we found that in one specific tree, out of 4,397 fruits, only three were full. This is of course an extreme case, which, nevertheless, illustrates the problem.

The unique embryology of the pistachio hints at the complexity and potential stages at which development failure could occur. The fruit and seed do not develop

simultaneously. The pericarp reaches its final size and the endocarp becomes sclerified 5 – 6 weeks after pollination (Picture 1).

Picture 1: Pistachio fruits six to eight weeks after pollination. The pericarp is fully developed

During this time the ovule is still small and occupies only a small part of the locule (Picture 2). The fertilized ovule starts to develop rapidly 10-12 weeks after pollination. Full ripening of the fruit and seed occurs about 5 months after pollination.



Picture 2: Pistachio ovule six to eight weeks after pollination. The ovule has not developed yet

From the embryological point of view, the seed production of *P. vera* may be a result of several factores: 1) low pollen fertility and some abnormal pollen development; 2) abnormal female flower development which prevents pollen from reaching the stigmas and/or ovule; and 3) high rate of degeneration of the developing embryo either because of development abnormalities or environmental factores. In this project we focus our experimental efforts on pollen fertility using biochemical and microscopic techniques.

Insoite of the low fruit set, yields of up to 12.0 kg per tree were obtained under desert conditions on runoff water alone (Spiegel-Roy et al., 1977). It indicates that pistachio can be productive and may be economical even before the problem of fertility is solved.

Establishment of Experimental Pistachio Orchard in Algarve

Accompanying the basic research on biology and development of the pistachio, we have started establishing a new experimental pistachio orchard in Algarve. The first site was planted last year (1987) at Lameiras and two new sites were planted last week (on 18 March 1988) at Messines and Senafrim Grande.

The landscape of the site at Lameiras before planting (October1986) is shown in Picture 3. The same area after planting (August 1987) is show in Picture 4 (thanks are due to Prof. Mota for providing me with Picture 4).

Picture 3: The site of the pistachio plot at Lameiras on October 1986 before planting the seedlings.

Picture 4: The site of the pistachio plot at Lameiras on August 1987 with the pistachio seedlings, which were planted in February 1987

Picture 5: A general view of the pistachio nursery at Avdat

Forseeing the need for pistachio seedlings for this project we started a pistachio nursery at Avdat (Picture 5).

The major objective of this nursery method was to develop a normal seedling with a small volume and weight of root suitable for air transportation. This was achieved by air pruning of the root system. The preparation of the seedlings for transportation includes several stages and treatments. The seedling were dug out of the soil and the soil then washed off in water (Picture 6). The seedlings were then disinfected (Picture7) and wrapped in wet paper towels and put in bags for shipment.

Picture 6: The first steps in the preparation of the pisyachio seedling for shipment. Getting the seedlings out of the soil and washing oif the roots in water.

The first shipment of 630 seedlings weighed approximately 80 Kg. This method of preparing the seedling in the nursery for overseas transplanting has been developed by Mr. J. Maurice and is continuously being modified and improved.

Picture 7: The seedlings are disinfected and wrapped in wet towels to keep them wet during transportation.

Summary

The study of pistachio provides us with two avenues of human endevour: 1) basic biological research on infertility in plants and 2) development of techniques and methodologies for growing, transplanting and establishing trees overseas. This project enjoys very enthusiastic cooperation Prof. Miguel Mota and Eng. Abílio Mendes Gaspar of the Estação Agronómica Nacional (EAN) in Portugal and the Plant Adaptation Research Unit of the Blaustein Institute for Desert Research in Israel. This pistachio project can hopefully provide a good study case for other tree crops suitable for arid zones.

PERSPECTIVAS DA CULTURA DE NOVAS FRUTEIRAS EM PORTUGAL

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1. Indrodução

Na CEE há grandes excedentes de alguns produtos agrícolas, o que é devido à melhoria progressiva que se tem verificado na produtividade da agricultura comunitária e ao aumento das importações, feitas a partir de terceiros países, de numerosos produtos que competem vantajosamente com os obtidos no âmbito da Política Agrícola Comum devido aos seus custos inferiores. Porém, a crise resultante desses excedentes não é extensível a Portugal, pois a produção agrícola do nosso País tem-se mantido persistentemente em nível bastante reduzido, em manisfesta oposição ao que se tem verificado nos demais países comunitários. A reduzida dimensão da nossa produção agrícola e que tanto tem afectado o nosso desenvolvimento económico não é de modo algum irremediável,pois deve-se em grande parte ao deficiente aproveitamento das potencialidades existentes.

A diversificação da produção agrícola dentro da CEE contribuirá para a valorização da sua economia e por isso já esta a ser estimulada, nomeadamente através da utilização de culturas produtoras de bens deficitários, como é o caso das proteaginosas, milho, fruteiras tropicais e produtoras de frutos secos, espécies florestais plantas medicinais e aromaticas, flores, etc

A posição de Portugal no que respeita a diversificação cultural é bastante favoravel pois, apesar das suas dimensões territoriais, apresenta grande heterogeneidade de climas e de solos, o que lhe permite utilizar alternativas que a Comunidade pretende desenvolver nos seus países membros. Dentro dessas culturas há a salientar algumas fruteiras pouco utilizadas ou quase desconhecidas no nosso País e que já vêm merecendo a atenção de alguns serviços oficiais e de alguns agricultores

As novas espécies de fruteiras que nos parece deverem ser objecto de estudo mais aprofundado são as seguintes:

a) Tropicais: ananaseiro, bananeira, papaeira e coraseira;

b) Subtropicais: abacateiro, anoneira, maracujá, feijoa, macadâmia, lichia e diospireiro;

c) De folha caduca e próprias das regiões temperadas: actinídia, caria e pistácia.

2. Breve Caracterização Climatica de Portugal

À excepção das regiões com maior altitude, onde há quedas regulares de neve durante o Inverno, os climas de Portugal são do tipo *Cs* de Köppen, isto é, temperados, com estação quente não chuvosa – o que se pode designar por climas mediterrânicos. Tais climas apresentam duas estações bem marcadas (Verão e Inverno) e duas estações intermédias (Primavera e Outono). Porém, a variabilidade climática é bastante pronunciada, o que é devido fundamentalmente ao facto do nosso País se desenvolver sobretudo em latitude, às acentuadas diferenças em altitude e à marcada variação do efeito oceânico.

Nas regiões situadas a Sul do Tejo, com excepção da faixa litoral localizada a ocidente, e na Terra Quente Duriense o clima é do tipo *Csa*, pois o Verão é quente, uma vez que se atingem 22°C de média mensal. Nas restantes regiões, exceptuando as que sofrem quedas regulares de neve durante o Inverno, o clima é do tipo *Csb*, pois a média das temperaturas não alcança 22°C durante o Verão. Nalgumas zonas há excesso de água durante o Inverno, mas durante o Verão a carência hídrica atinge todas as regiões, nalgumas de forma pouco acentuada, enquanto noutras muito marcadamente.

A insolação anual, de 1800 a 3100 horas, e a disponibilidade de energia radiante, de 130 a 170 Kcal por cm² e por ano, variam de forma sensivelmente proporcional ao índice de aridez. Este varia no sentido contrário ao da nebulosidade e da pluviosidade, oscilando esta em média entre 400 e 3000 mm por ano. As necessidades hídricas anuais variam entre 800 e 1200 mm, sendo obviamente maiores nas zonas mais soalhentas, quentes e secas.

Em relação à amenidade do Inverno, há que distinguir entre Invernos «doces» – (D) – e os Invernos «frios» – (F). Nos primeiros as temperaturas médias do mês mais frio são maiores ou pelo menos iguais a 10° C, não havendo geadas ou ocorrendo estas em número reduzido (nunca mais de 5-10 por ano) e com pouca intensidade. Além disso, o número de dias com temperatura média superior a 10°C não será inferior a 300 ou pelo menos a 275.

No que respeita à carência hídrica e de acordo com THORNTHWAITE, distinguem-se três níveis de aridez: «aridez leve ou nula» (s_1), «aridez moderada» (s_2) e «aridez forte» (s_3), consoante o valor desse índice é, respectivamente, menor que 16,7%, está compreendido entre 16,7% e 33,3% ou é superior a este último valor.

Algumas zonas situadas a Sul do País, nomeadamente as zonas costeiras meridionais e ocidentais do Algarve (com climas *CsaD* e *CsbD*, respectivamente) e as zonas costeiras do Alentejo (com climas *CsbD*), têm Invernos muito suaves, pois a

ocorrência anual de geadas é menor que 1 na parte ocidental e menor que 5 na parte meridional. E nalgumas dessas zonas que se iniciaram e vão prosseguir os estudos de adaptação de algumas fruteiras tropicais e subtropicais.

Nas zonas interiores do Algarve e do Alentejo, onde os climas são do subtipo *CsaF*, o Verão é longo, quente e seco, ao passo que o Inverno é frio, tendo geralmente mais de 600 «horas de frio» (temperaturas inferiores a 7°C). Estes climas são favoráveis para as culturas da pistácia e da caria e por isso foram escolhidos para os ensaios com essas fruteiras.

Nas zonas costeiras situadas no Norte e Centro de Portugal os climas são do subtipo *CsbD*, sendo os Estios relativamente frescos e húmidos e os Invernos pouco severos e com geadas limitadas a 2-3 meses. Estas zonas são muito favoráveis para a cultura da actinídia, a qual já despertou o interesse de muitos agricultores.

3. Fruteiras Tropicais

3.1. – Ananaseiro

As principais regiões do Mundo onde se cultiva o ananaseiro (*Ananas comosus* estão situadas entre 25° de Latitude Sul e 25° de latitude Norte. Fora destes limites também se cultiva esta bromeliácea, como sucede por exemplo nalgumas zonas de África do Sul, localizadas entre 33° e 34° de Latitude Sul, e na Ilha de S. Miguel, situada a 38° de Latitude Norte, mas este último caso em estufas de vidro.

Em termos gerais considera-se que o ananaseiro não tolera geadas e por isso a sua cultura encontra-se geralmente confinada a áreas isentas da sua ocorrência. Pode, no entanto, suportar temperaturas ligeiramente inferiores a 0°C desde que tal suceda durante períodos de tempo muito curtos. Temperaturas superiores a 32°C começam a causar prejuízos, que se acentuam a partir de 35°C, especialmente quando a humidade relativa é baixa, pois podem surgir queimaduras nos frutos, dado que as temperaturas internas nestes são nitidamente mais elevadas do que as exteriores. As temperaturas mais favoráveis para o crescimento do ananaseiro são compreendidas entre 21° e 30° C.

Ao proceder à zonagem do ananaseiro em função das temperaturas, Silva (1981) estabeleceu as três faixas seguintes:

– *Faixa A*, que classificou como «*apta*», com temperaturas médias anuais superiores a 19° C;

– *Faixa B*, a que atribui a designação de «restrita», abrangia as zonas com temperaturas médias anuais compreendidadas entre 18° e 19°C;

– *Faixa C*, que classificou como «restrita e inapta», com temperaturas médias anuais inferiores a 18°.

De um modo geral considera-se que o ananaseiro requer 1000 a 1500 mm de chuvas bem distribuídas ao longo do ano, estimando-se as suas necessidades hídricas diárias em 2 a 4 mm.

Tendo o seu sistema radicular grandes necessidades de arejamento, o ananaseiro requer solos com texturas ligeiras ou médias, sendo inadequados os argilosos,

calcários, salgados, compactos ou mal drenados. Os valores do pH mais indicados são os de 5 a 6.

As variedades mais utilizadas nos ensaios realizados em Portugal em abrigos de plásticos têm sido a Cayenne Lise, a Queen e a V. C. Queen. As densidades chegam a atingir 100 000 plantas por hectare, sendo grande a produção e muito boa a qualidade dos frutos.

3.2 Bananeira

As principais zonas produtoras da bananeira estão localizadas entre 30° de Latitude Sul e 30° de Latitude Norte, ultrapassando esses limites algumas zonas produtoras situadas na Austrália, Formosa, Israel, África do Sul e Madeira, ficando todas estas regiões situadas na periferia das isotérmicas que definem aproximadamente os limites que separam as áreas isentas das sujeitas às geadas.

O crescimento da bananeira processa-se entre 15° e 35°C, sendo mais favoráveis os valores compreendidos entre 25° e 30°C. Abaixo dos 12°C sofrem os efeitos da «friagem», distúrbio fisiológico que se pode observar nos frutos verdes e que se manifesta sob a forma de manchas que se vêem sob a forma de traços escuros em corte longitudinal. Temperaturas inferiores a 8° C durante longos períodos podem causar sérios prejuízos e inferiores a 0° matam as plantas.

Ao procederam à zonagem da bananeira, Antunes & Fortes (1980) deram grande importância à «*temperatura média do mês mais frio*», considerando que uma região era «*apta*», «*apta com restrições*», e «*inapta*» consoante esse parâmetro se situava acima dos 18°C, estava compreendido entre 15° e 18°C ou era inferior a 15°C, respectivamente.

Consideram alguns investigadores que a bananeira necessita de mais de 1500 mm de chuvas bem distribuídas ao longo do ano ou de 100 a 150 mm por mês, estimando-se as suas necessidades hídricas diárias em 2 a 5 mm consoante os valores da evapotranspiração.

A bananeira prefere solos profundos, férteis, bem drenados, francos ou franco-argilosos, podendo porém suportar também outros tipos. Tolera valores de pH bastante diferentes, embora se considere que prefere os solos neutros ou ligeiramente alcalinos.

As cultivares utilizadas nos ensaios em curso são Anã, Coccos, William's Hybrid e Grande Anã, mas também estão a ser multiplicadas através das culturas de tecidos a Robusta e a Valery.

3.3. Papaieira

Considera-se que as temperaturas mais favoráveis para o crescimento da papaieira (*Carica papaia*) são as compreendidas entre 22° e 28°C, pois favorecem o crescimento e a obtenção de frutos com elevada qualidade.

A papaieira não tolera geadas e deveria ser cultivada de preferência em regiões onde as temperaturas se mantivessem com reduzidas oscilações ao longo do ano, pois quando descem exageradamente durante o inverno provocam a queda das folhas, grandes decréscimos ou até mesmo paralisias no crescimento, ausência de floração, retardamento na maturação e má qualidade dos frutos. Há diferenças pronunciadas no que respeita ao comportamento das diversas variedades em relação às baixas temperaturas, verificando-se também que as plantas femininas são menos resistentes ao frio do que as masculinas.

A papaieira requer cerca de 1 500 mm de chuvas bem distribuídas ao longo do ano, tornando-se necessário recorrer à rega quando são escassas ou mal distribuídas. Neste caso é habitual o fornecimento de 100 a 150 mm de água por mês.

A papaieira prefere solos profundos e com texturas ligeiras ou médias, boa fertilidade e bem drenados. Os solos argilosos e muito compactos não se devem utilizar, pois dificultam o crescimento das plantas e podem ocasionar a sua morte quando sujeitos ao encharcamento. Os valores de pH mais recomendável são os compreendidos entre 6 e 7.

As variedades de papaieira podem ser hermafroditas ou dióicas. As principais variedades hermafroditas são a Sunrise Solo, Tainung e Tailândia no Brasil e Kapoho Solo, Waimanalo, Higgins e Wilder no Hawaii.

No que respeita às dióicas (nas quais as flores masculinas e femininas estão localizadas em plantas distintas), as principais variedades são a Hortus Gold na África do Sul e Improved Peterson e Bettina na Austrália, estando a ser ensaiadas com bons resultados a Caniflora na Flórida.

Dentro do género *Carica* está a ser ensaiada nalgumas zonas mediterrânicas a *C. pentagona*, espécie subtropical conhecida por babaco, e que é um híbrido entre a *C. pubescens*, papaieira da montanha, e a *C. stipulata*. Os frutos desenvolvem-se por partenocarpia, não havendo, portanto, necessidade de plantas masculinas. Apesar de ser uma espécie subtropical, o babaco apenas é cultivado em estufa, mas os resultados estão bastante longe de corresponder às expectativas, não só devido à fraca qualidade dos frutos mas também aos problemas de natureza fitossanitária que têm surgido.

3.4. Goiabeira

Os crescimentos da goiabeira (*Psidium guajava*) começam em torno dos 15°C, atingindo os valores máximos próximo dos 28°C. Sendo uma fruteira tropical, a goiabeira é sensível ao frio, mas pode suportar geadas ligeiras e sobreviver a temperaturas de – 3° C quando são de curta duração.

Para que a floração e a frutificação se processem em boas condições, a planta necessita de um mínimo de 3,5 a 6 meses com temperaturas médias superiores a 16°C, sendo a qualidade dos frutos bastante prejudicada quando as temperaturas médias durante a maturação se situam abaixo dos 15°C. Também os ventos frios limitam o crescimento vegetativo e prejudicam a floração e o crescimento do fruto. Contrariamente às demais espécies tropicais, a goiabeira poderá ser cultivada ao ar livre em diversas zonas do Algarve, nomeadamente naquelas em que as tempera-

turas medias anuais se aproximam dos 18°C e as temperaturas médias mensais excedem 16°C durante 6 meses e 20°C durante 5 meses.

Embora resista melhor à seca do que a maior parte das outras fruteiras, a goiabeira necessita de mais de 1000 mm de chuva durante o período de crescimento. Pode ser cultivada em vários tipos de solos, desde os mais argilosos até aos arenosos, preferindo, contudo, os que se apresentam férteis, profundos e bem drenados. Pode suportar encharcamentos, revela especial tolerância ao sal e suporta valores de pH compreendidos entre 4,5 e 8,2.

A escolha das variedades depende do fim a que se destinam os frutos – sumos, sobremesa, geleias, sorvetes, goiabadas, doces, etc. – havendo algumas que satisfazem diversos objectivos. Entre as variedades que se podem ensaiar incluem-se a Indonesian Seedless, Allahabad Safeda, Etheridge Selection, Ruby, Miami White, Large White e Supreme.

4. Fruteiras Subtropicais

Contrariamente ao que se passa em relação ao ananaseiro, à bananeira e à papaeira, todas as fruteiras subtropicais devem ser cultivadas ao ar livre.

4.1. – Abacateiro

As flores do abacateiro (*Persea americana*) são hermafroditas, mas há desencontro entre a receptividade do estigma e a libertação do pólen das anteras das flores da mesma planta, fenómeno conhecido por *dicogamia*, que será *protogínica* no caso dessa fruteira pois os pistilos estão funcionais antes das anteras. De uma forma resumida pode dizer-se que há cultivares da *classe A* e cultivares da *classe B*, comportando-se as primeiras como femininas durante as manhãs e como masculinas durante as tardes, ao passo que as segundas se comportam como masculinas durante as manhãs e como femininas durante as tardes. Em face disso é necessário recorrer a cultivares da *classe A* e a cultivares da *classe B* para a constituição dos pomares, pois só assim se garante a obtenção de elevadas produções em consequência das *primeiras polinizarem e serem polinizadas pelas segundas*.

Pelas suas características e exigências climáticas, os abacateiros podem ser classificados em três grupos naturais, havendo ainda a considerar os híbridos resultantes dos cruzamentos feitos entre plantas de grupos naturais diferentes. Os três grupos são os seguintes:

a) *Grupo Antilhano*, que compreende os abacateiros originários das baixas altitudes da América Central e da parte Norte da América do Sul, os quais, não suportando geadas, se revelam desprovidos de interesse para o nosso País.

b) *Grupo Guatemalteco*, que reúne os abacateiros de algumas zonas de média altitude da Guatemala e do Sul do México, os quais podem suportar temperaturas de -2° C. Inclui as cultivares Hass, Queen, Reed, Sharpless, Taylor e Wagner, da *classe A*, e Edranol, Itzamna, Linda, Nabal, Prince e Suardia da *classe B*, amadurecendo os seus frutos durante o Inverno ou durante a Primavera.

c) *Grupo Mexicano*, que agrupa os abacateiros cujos centros de origem se situam nas zonas montanhosas do México e da Guatemala, os quais chegam a suportar temperaturas de -4°C. Engloba as cultivares Duke, Gottfried Zaina, Mexicola, Puebla e Topa Topa, da *classe A*, e Bacon, Winslowson e Zutano, da *classe B*, amadurecendo os seus frutos durante o Verão e durante o Outono.

Além das cultivares acima mencionadas, merecem particular realce os seguintes híbridos resultantes de cruzamentos entre abacateiro pertencentes aos Grupos Mexicano e Guatemalteco:

– Lula e Rincon, da *classe A*;

– Ettinger, Fuerte e Ryan, da *classe B*.

Ao pretender-se fazer a zonagem da cultura do abacateiro em função das temperaturas médias anuais, poderá dizer-se que as regiões com valores superiores a 19°C são favoráveis para todos os grupos e que abaixo dos 17°C começam a surgir grandes restrições, dado que estas últimas são inadequadas para o Grupo Antilhano e marginais para o Grupo Guatemalteco. As regiões em que as temperaturas médias anuais estão compreendidas entre 17°C e 19°C apresentam limitações para o Grupo Antilhano, mas podem ser utilizadas pelos dois restantes Grupos, obviamente com preferência para o Mexicano nas que possuem temperaturas mais baixas. Os abacateiros requerem geralmente solos ácidos, mas quando se utilizam porta-enxertos como G.A.13, o Moaz e o Fuch 20 podem também cultivar-se noutro tipo de solos.

4.2. – Anoneira

A anoneira (*Anona cherimola*) tem exigências climáticas algo parecidas com as do abacateiro, o que não sucede em relação às demais espécies cultivadas do mesmo género, como a fruta-pinha (*A. squamosa*).

Os climas mais favoráveis para a anoneira são os que se apresentam moderadamente frios e isentos de geadas, embora estas possam ser suportadas desde que sejam pouco frequentes e muito ligeiras. As quedas pluviométricas devem atingir os 1200 mm por ano, sendo vantajosa a existência de uma estação seca relativamente longa. Os melhores solos são os de texturas médias, mas também se utilizam os argilosos, como no sul da Califórnia, e os arenosos como na Flórida.

Têm sido ensaiadas algumas cultivares, mostrando boa adaptação a Campas e a Fino de Jete.

4.3. – Maracujá

A espécie mais importante do maracujá é a *Passiflora edulis*, na qual se incluem as formas cultivadas mais importantes e que são conhecidas pelas designações de maracujá roxo (*P. edulis* f. *edulis*) e maracujá amarelo (*P. edulis* f. *flavicarpa*).

O maracujá roxo suporta mal as geadas, pois os lançamentos novos são mortos quando as temperaturas baixam para além de -1° ou -2°C e os próprios tecidos caulinares já lenificados apenas toleram geadas ligeiras de curta duração. O maracujá amarelo ainda é menos tolerante às baixas temperaturas e por isso não é cultivado nas zonas afectadas pelas geadas.

Para ser cultivado em sequeiro, o maracujá requer um mínimo de 900 mm de chuva por ano devendo a sua distribuição verificar-se sobretudo durante o Verão. Quando as quedas pluviométricas são insuficientes ou mal distribuídas torna-se necessário o recurso à rega.

O maracujá prefere solos com texturas médias, férteis, profundos, bem drenados, com valores de pH compreendidos entre 5,5 e 6,5, não suportando encharcamentos.

As flores são hermafroditas, mas também podem ser afectadas pela dicogamia e neste caso, é afectada a autopolinização.

4.4. – Feijoa

A feijoa (*Feijoa sellowiana*) tem poucas exigências climáticas e pedológicas, preferindo, no entanto, climas secos e sem grandes oscilações ao longo do ano. Contrariamente á actinídia, os elevados teores de humidade não lhe são favoráveis.

Muito resistente ao frio, pode tolerar temperaturas que cheguem até -10° C. Como a floração apenas surge em Junho, a planta tolera bem as geadas que possam surgir na Primavera, mas já poderão surgir problemas quando ocorrem no Outono, pois prejudicam os frutos se estes ainda não tiverem sido colhidos.

É muito resistente à seca, adaptando-se a regiões com 750-1000 mm de chuva por ano se estas forem relativamente bem distribuídas. Prefere solos franco-arenosos e ricos em húmus, mas adapta-se bem a solos argilosos e pesados, sendo, no entanto, sensível a elevados teores de calcário. As variedades em estudo são a Mammouth, Sellowiana, Choiceana e Triumph.

4.5. – Macadâmia

Inicialmente cultivava-se a *Macadamia ternifolia*, mas hoje utilizam-se principalmente a *M. integrifolia* e a *M. tetraphylla*, dada as suas produtividades e qualidades das nozes.

Esta fruteira pode ser cultivada em várias zonas tropicais e subtropicais, mesmo em condições de aridez relativamente severas, sendo considerada como adaptável a várias regiões da bacia mediterrânica, como as zonas costeiras do Sul de Espanha e do Algarve, ocupadas pela cultura da amendoeira.

É sensível às geadas e aos ventos fortes, revelando-se a *M. tetraphylla* mais tolerante ao frio. Os solos mais favoráveis são os franco-argilosos, medianamente férteis, bem drenados e com pH compreendido entre 4,5 e 6,5.

4.6. – Lichia

Enquanto novas, as lichias (*Litchi chinensis*) não toleram temperaturas negativas, mas depois de adultas podem suportar geadas ligeiras, embora as suas folhas sejam muito afectadas. Comportam-se melhor em climas com 3-4 meses secos e relativamente frios durante o Inverno, florescendo no final desta estação. Durante o crescimento do fruto, as temperaturas deverão ser relativamente quentes, havendo necessidade de recorrer à rega quando as chuvas forem insuficientes.

Depois das colheitas deverão manter-se elevadas as temperaturas e as disponibilidades hídricas e nutritivas para garantir a recuperação das reservas das árvores e o crescimento dos lançamentos onde se irá verificar a produção do ano seguinte. Porém, nas zonas susceptíveis de ocorrência de geadas, dever-se-ão evitar os crescimentos durante o Outono, pois os ramos novos são bastante mais sensíveis às baixas temperaturas.

Desenvolve-se bem em vários tipos de solos, desde os arenosos até aos fortemente argilosos, podendo mesmo suportar os que possuem fraca drenagem. Prefere solos ligeiramente ácidos, devendo aplicar-se calcário quando o pH for inferior a 5.

4.6. – Diospireiro

A maior parte das plantas cultivadas do género *Diospyros pertence* à espécie *D. Kaki*, vulgarmente conhecida pela designação de diospireiro japonês, diospireiro oriental ou simplesmente diospireiro.

O diospireiro já é cultivado em várias zonas de Portugal, mas apenas através das variedades adstringentes ou taninosas e que só podem consumir-se quando os frutos se apresentam bastante maduros.

Nos anos mais recentes e devido principalmente aos trabalhos de melhoradores japoneses já há um número apreciável de variedades que produzem frutos doces ou não adstringentes, os quais podem ser consumidos com a polpa ainda firme e como se fosse uma maçã, ou com a polpa relativamente mole. Estes frutos apresentam ainda a vantagem de possuirem um período de consumo maior do que os taninosos.

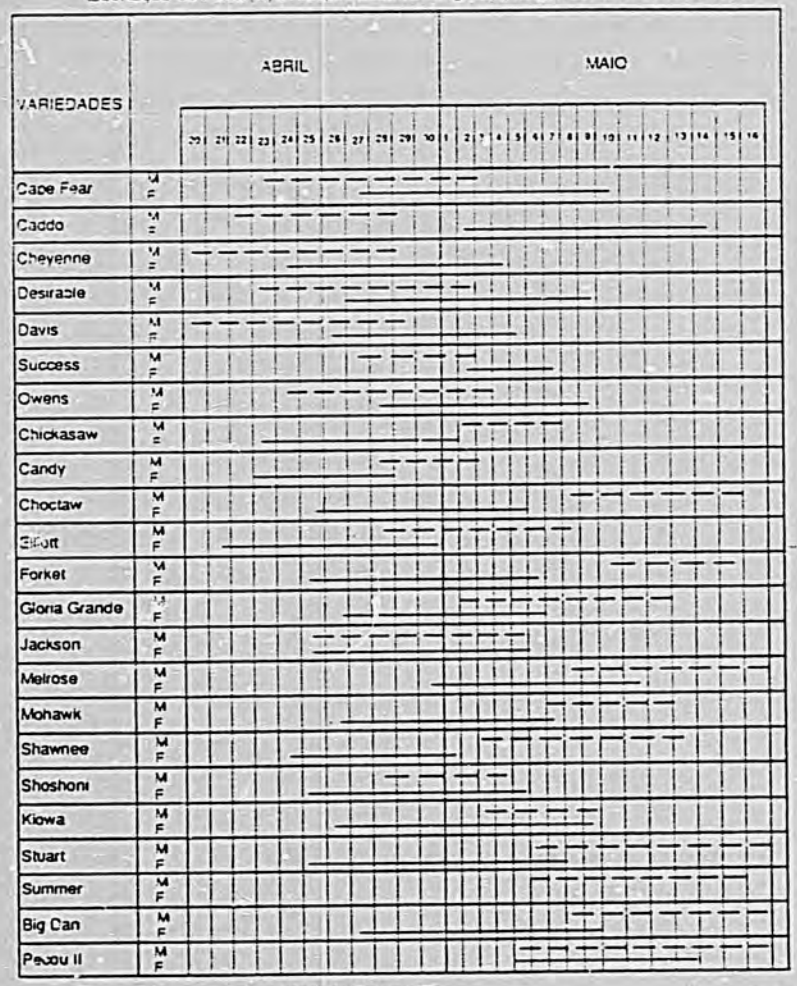
O diospireiro é uma das poucas fruteiras subtropicais que têm folha caduca e que entram em dormência durante o período invernal. Muitas das suas variedades revelam poucas exigências em frio para quebrarem a dormência e assegurarem desabrochamentos e rebentações uniformes – 200 a 400 horas com temperaturas inferiores a 7°C.

Para completarem a maturação dos frutos, os diospireiros necessitam de uma estação quente relativamente longa, requerendo as variedades produtoras de frutos doces de climas mais quentes do que as produtoras de frutos adstringentes. Para as primeiras a melhor qualidade do fruto é assegurada quando a insolação que decorre desde o vingamento do fruto até á maturação excede as 1400 horas e as temperaturas médias durante o período de maturação oscilam entre 16° e 22°C.

O diospireiro pode ser cultivado em vários tipos de solos, preferindo, porém, os franco-arenosos, bem drenados e com pH compreendido entre 5,5 e 6,5.

CUADRO I

Libertação do Pólen (M) e receptividade do estigma (F) nalgumas variedades de caria.



As temperaturas demasiado altas também são desfavoráveis, pois aumentam a evapotranspiração, podendo provocar queimaduras nas folhas e nos frutos. Com uma área folhear muito abundante e uma grande transpiração, necessita que a humidade relativa da atmosfera se mantenha elevada durante a maior parte do período de vegetação.

A actinidia requer solos ligeiros ou de texturas médias, férteis, profundos e com teores de água relativamente elevados, mas não tolera encharcamentos, suportando mal os solos argilosos e pesados. O pH deverá ser ligeiramente ácido, não suportando essa fruteira os solos calcários ou salgados.

A actinidia é uma planta dióica, estando, portanto, os dois sexos situados em plantas diferentes. As principais variedades femininas em culturas são a Hayward, Abbott, Bruno e Monty, sendo as masculinas mais utilizadas a Matua, Tomuri e M-3.

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PISTACHIO NUT PRODUCTION IN THE NEGEV

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Abstract Pistachio trees have adapted well to the climatic conditions of the Negev highlands.[2] Our present aim is to study the biological mechanisms of plant development, fruit setting and quality, and agrotechnical practices determining pistachio productivity under desert conditions. Composition of nuts was studied in two consecutive seasons. It was shown that the Kerman variety, which is preferred in western markets, has a higher percentage of protein (32.2%) compared with Sfaks (25.4%), Avdat (20.7%) and Larnaka (24.2%). Irrigation of trees with brackish water resulted in larger Kerman fruits and nuts and increased dry weight per seed as compared to nuts from trees irrigated with non-saline water. The proteins, lipid and fatty acid composition of seeds was similar when trees were irrigated with brackish, tap or flood water. Addition of either tap or brackish water resulted in more indehiscent nuts, 50% and 67%, respectively.

INTRODUCTION

The genus *Pistachia* consists of dioecious trees and shrubs, the flowers of which are wind-pollinated.

There are four native species of *Pistachia* in Israel: *P. atlantica*, *P. palaestina*, *P. lentiscus* and *P. saportae*. The commercially important species *P. vera* was brought to Israel from Iran. The cultivation of pistachio is widespread in Mediterranean and Middle Eastern countries. The major pistachio nut production at present is in Turkey and Iran.[1]

There are several experimental pistachio plantations in Israel: in the Galilee, Judea and central Negev. The results presented in this paper were obtained from fruits of trees at the experimental orchard of the Avdat Farm located in the Negev highlands, a desert area with an average yearly precipitation of 50-100 mm, cold winters and hot, dry summers. Evenari and co-workers have shown that pistachio can survive and produce nuts under harsh desert conditions even when runoff water only is used.[2] However, increasing pistachio productivity to its full potential in the desert requires more than erratic floods.

The major research problems confronting us are:

1. Physiological and agrotechnical - selection of root-stocks and scions; response of trees to irrigation regimes and water quality; response of trees to mineral and nitrogen nutrition.

2. Pest management - identification of pathogens and insects causing damage and development of appropriate treatments. Epicarp lesion is one severe disease which sometimes causes losses of greater than 30% of the crop.[3] It is not yet certain whether the cause for this disease is an insect, a genetic or physiological disorder, or a manifestation of scion-rootstock incompatibility.

3. Low seed setting - a common feature of the female inflorescence is that many of its flowers do not produce seeds.

4. Indehiscence - dehiscence of the endocarp (shell) of the pistachio nut is an important factor determining its economical value. Unfortunately, sometimes more than 40% of the fruits are indehiscent.[4]

5. Propagation - P. vera cuttings do not produce roots, therefore it is propagated by grafting. The recent development of tissue culture techniques opens a new perspective for more efficient pistachio propagation.

6. Fruit quality - there are not enough studies on the biochemical composition of the fruit and the contribution of its proteins, lipids and other compounds to the nutritive value and acceptability by the consumer.

In this paper, we report some preliminary data on fruit composition of major pistachio varieties grown at the Avdat runoff farm with different water qualities: 1. flood water; 2. tap water; and 3. brackish water.

MATERIALS AND METHODS

Pistachio fruits of mature trees of the following varieties: Sfaks, Avdat, Kerman, and Larnaka, grown at Avdat runoff farm and maintained on flood water were harvested in the 1984 season. Fruit parts were separated and the fresh weight of each part determined. The seeds were oven-dried at 80°C to a constant weight and analyzed for Kjeldhal-N. Lipids were extracted by soxhlet using chloroform methanol 2:1 (v:v) and ash was determined by burning the dry matter in a furnace at 550°C for 4½ hrs. Lipids were transmethylated with methanol-acetyl chloride[5] and the resulting fatty acid methyl esters (FAME) were analyzed by gas chromatography on an SP-2330 capillary column. FAME were identified by cochromatography with known standards.

The effect of water quality on seed composition was determined in the 1985 season. Fruits of mature Kerman trees (Atlantica rootstocks) were analyzed as described above. The water treatments began in the 1984 season and were as follows: 1. flood water; 2. tap water containing either 600 mg/L TSS, total soluble solids, (850 liters/tree) or 4000 mg/L TSS (850 liters/per tree).

RESULTS AND DISCUSSION

FRUIT COMPOSITION OF PISTACHIO VARIETIES

Fresh weight distribution in fruit parts of four major pistachio varieties is shown in Table 1. The exo-, meso- and endocarp, which are non-edible, constitute the major part of the fruit. Kerman and Larnaka produced the biggest seeds (0.65 and 0.60 gr./seed, respectively).

Chemical analysis of seeds is shown in Table 2. Kerman seeds had substantially higher concentrations of proteins (32.2%) and ash (8.9%) as compared to the other varieties analyzed. Protein and lipids were inversely related in Avdat and Larnaka. Avdat had relatively less protein (20.7%) and more lipids (53.6%) while Larnaka had relatively more proteins (24.2%) and less lipids (48.0%). The relationship between seed proteins and lipids and the nutritive value and consumer preference for certain varieties has not yet been tested scientifically. However, it is known that the western consumer prefers the Kerman nut because of its larger size (Table 1). In addition, the data presented here may indicate that higher proteins and ash content of the seed is commercially preferable.

Avdat and Larnaka appear similar except for slightly higher concentrations of oleic acid (C18:1) in Larnaka (71.3%) than in Avdat (66.9%) and higher concentrations of linoleic acid (C18:2) in Avdat (18.9%) than in Larnaka (14.0%) (Table 3).

We have studied the effect of water quality (flood, tap and brackish water) on the weights and indehiscence of Kerman fruits (Table 4). Brackish water irrigation produced the biggest fruits and nuts on a fresh and dry weight basis. Both tap water and brackish water produced significantly bigger fruits than floodwater, probably due to the fact that the trees irrigated with floodwater received a substantially lower amount of water (1985 total precipitation was 73.8 mm). Water quality also had a significant effect on the extent

TABLE 1. Comparative of fresh weights of pistachio fruit parts of major varieties grown at Avdat.

Fruit part	Whole fruit	Exo- & Mesocarp	Endocarp	Seed coat	Seeds
Variety	Fresh weight* (g)				
Sfaks	1.78 ± 0.09	0.62 ± 0.06	0.61 ± 0.05	0.12 ± 0.01	0.42 ± 0.06
Avdat	2.06 ± 0.17	0.77 ± 0.08	0.59 ± 0.05	0.15 ± 0.02	0.55 ± 0.04
Kerman	2.98 ± 0.28	1.06 ± 0.11	0.99 ± 0.12	0.27 ± 0.04	0.65 ± 0.09
Larnaka	2.35 ± 0.15	0.91 ± 0.08	0.64 ± 0.05	0.02 ± 0.04	0.60 ± 0.06

* Ten full fruits of each variety were analyzed.

TABLE 2: Chemical composition of seeds of major pistachio varieties grown at Avdat

Component	Protein (%N x 6.25)	Lipids	Carbohydrates* + Fiber	Ash
Variety	Percentage of dry matter			
Sfaks	25.4	-	-	4.6
Avdat	20.7	35.6	20.4	5.3
Kerman	32.2	-	-	8.9
Larnaka	24.2	48.0	23.3	4.4

* Obtained by difference

\- Not determined

TABLE 3. Fatty acids composition of pistachio nuts grown at Avdat

Fatty acid	16:0	16:1	18:0	18:0	18:2
Variety	Percent of total fatty acids				
Avdat	11.8	1.0	1.4	66.9	18.9
Larnaka	12.1	1.1	1.5	71.3	14.0

Effect of water quality on seed composition

TABLE 4. Effect of water quality on fresh and dry weights and dehiscence of Kerman fruits*

Matter	Fresh matter						Dry matter			Number of fruits
Fruit Part	Fruit (whole)	Nut dehiscence	Nut indehiscence	Seed dehiscence	Seed indehiscence	Seed dehiscence	Seed indehiscence	Seed dehiscence		dehiscence
Treatment				gram/unit			%			%
Floodwater	2.60	1.44	1.59	0.77	0.66	96.6	86.3	71		
Tap water	3.01	1.50	1.52	0.74	0.63	81.9	93.8	50		
Brackish water	3.09	1.80	1.87	0.97	0.69	66.6	91.0	33		

* Several hundred fruits were analyzed in each treatment.

of dehiscence. Trees irrigated with brackish water produced considerably less dehiscent nuts (33%) than with floodwater (71%). This may be due either to strengthening of the endocarp (shell), especially along the abscission zone, in nuts of trees irrigated with brackish water, or that nuts in this treatment did not develop enough to produce the pressure required to rupture the endocarp. A third possibility is that the fruits of trees irrigated with brackish water did not develop to the dehiscence stage by harvest time, while the nuts of flood water treatments had already reached this stage. This last possibility might be related to the larger quantity of water in the brackish water treatment (850 l/tree) than in the flood water treatment (see above). This possibility is supported by the high percent of indehiscence (50%) also in the tap water treatment (850 l/tree).

The effect of water quality on proteins and lipids in Kerman seeds was determined (Table 5). The indehiscent seeds contained a higher percentage of lipids in all treatments compared to the dehiscent seeds. Protein content of dehiscent and indehiscent seeds was similar. Brackish water produced nuts with the highest protein content (27.7%) and lowest lipids content (39.7%). There were only small differences in the fatty acids composition of seeds of the different water treatments and between dehiscent and indehiscent nuts. (Table 6).

Irrigation of trees with brackish water at a level of 4000 mg/liter TSS did not have an adverse effect on pistachio nut composition as compared with flood water (Tables 5 and 6). Irrigation had a positive effect on fresh and dry weights of pistachio nuts. Nut filling under these conditions requires, presumably, a longer time to allow dehiscence.

Our experimental work in the near future will concentrate on the combined use of brackish and run-off water. Brackish water will supplement the water requirements of the trees for maximal yields during years of drought or insufficient

TABLE 5. Effect of water quality on protein and lipid content of Kerman nuts

Treatment	Component: Protein (%N x 6.25)		Lipid	
Nut State	dehiscent	indehiscent	dehiscent	indehiscent
	Percent of dry matter			
Floodwater	24.1	24.0	42.5	53.7
Tap water	25.5	22.3	50.5	34.9
Brackish water	27.7	27.8	39.7	54.4

TABLE 6. Effect of water quality on fatty acids composition of Kerman nuts

Treatment	Nut state	Fatty acids* 16:0	16:1	18:0	18:1	18:2
Floodwater	Dehiscent	12.2	1.3	1.1	57.8	27.3
	Indehiscent	12.3	1.3	1.2	56.8	28.4
Tap water	Dehiscent	13.1	1.5	1.3	56.8	27.0
	Indehiscent	12.7	1.5	0.9	54.6	30.9
Brackish water	Dehiscent	12.2	1.5	1.3	56.5	28.0
	Indehiscent	11.6	1.2	0.9	56.9	28.8

* Percent of total fatty acids.

precipitation. Run-off, beside providing water for the trees, will also wash out salts from the soil profile during years of abundant precipitation. The physiological and agrotechnical consequences of this practice, as well as its effect on soil structure, will be studied on a large scale between 1987 and 1990.

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Germination of *Pistacia vera* L. pollen in liquid medium

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Summary. The osmotic effect of Polyethylene glycol (PEG) has been shown to be sufficient to induce the germination of *Pistacia vera* L. pollen in liquid medium. The prehydration of the pollen in a saturated atmosphere for approximately 10 h was necessary to obtain maximum in vitro germination. Imbibition of the pollen in water resulted in the rapid leakage of solutes into the medium. These solutes consisted of approximately 50% carbohydrates, of which sucrose (0.65 µmol/mg), glucose (0.77 µmol/mg) and fructose (0.78 µmol/mg) were the major sugars; the remaining 50% comprised proteins with the following major molecular weights 63 kDa, 60 kDa, 59 kDa, 40 kDa, 36 kDa, 35.5 kDa, 31 kDa, other organic matter and minerals.

Key words: *Pistacia vera* L. – In vitro germination – Pollen – Osmotic effect

Introduction

Pollen grains of *Pistacia vera* L. have been considered to be difficult to germinate in vitro (Crane et al. 1974; Crane and Iwakiri 1981; Vithanage and Alexander 1985). This is in spite of the bicellular nature of its pollen (Copeland 1955; A. Golan-Goldhirsh unpublished data), which usually ensures ready germination (Knox et al. 1986). Recently it has been shown that controlled hydration of these pollen prior to incubation in germination medium increased percent germination (Polito and Luza 1988). The germination of pollen in vitro is greatly influenced by the relative humidity at which the pollen is pretreated (Gilissen 1977). The assessment of pollen quality by its in vitro germination is a useful method for determining the acceptability of pollen for artificial pollination (Stanley and Linskens 1974) as well as in biochemical studies on pollen viability. However, negative results must be interpreted cautiously as a lack of pollen germination may reflect either inviability or a deficiency in some requirement for pollen germination (i.e. hydration state, medium composition, temperature, etc.). Pollen of some species can germinate in water (Duffield 1954), while other species require more complex media for germination (Stanley and Linskens 1974). The importance of water and the effect of the osmotic potential of the medium on pollen germination has been reported extensively (Heslop-Harrison 1979; Dumas et al. 1988; Hoekstra 1986, and others). Nevertheless, there is still a lack of understanding of the early events in pollen hydration and germination (Crowe et al. 1989). In the present article we report the effect of water and medium osmotic potential on the germination and structure of *Pistacia vera* L. pollen in liquid medium.

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Materials and methods

Pollen of *Pistacia vera* L. cv 'Chico' were collected during the season of 1988 and sieved through a 90-µm sieve net. The pollen were kept at 4° C in containers plugged with cotton wool. The water content of the pollen was approximately 5% throughout the storage period.

Pollen germination in vitro

Pollen germination in vitro was done after hydration in a water-saturated atmosphere in closed containers or by direct imbibition in distilled water at room temperature. The liquid medium for germination contained 1.3 mmol/l calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), 0.81 mmol/l magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 1 mmol/l potassium nitrate (KNO_3), 1.6 mmol/l boric acid (H_3BO_3) and 0.5 mmol/l sucrose (Harris et al. 1987). Sucrose or polyethylene glycol (PEG) were added to this medium as indicated. Pollen grains (40 mg) were added to 5 ml medium in a 100-ml Erlenmeyer flask and allowed to germinate for 12 h at 26° C on a rotatory shaker at 100 rpm. An aliquot (20 µl) of the suspension was taken for determining pollen germination using an automatic pipette fitted with a 200-µl capacity tip, the distal 2.5 mm of which had been cut off. This prevented breakage of the pollen tubes (Harris et al. 1987). Pollen tube length was measured on a Image Processing System (Kontron M15) attached to a Zeiss IM microscope via a video camera (Sit 66). Pollen grains were stained with Alexander stain for light microscopy (Alexander 1969). Scanning electron mi-

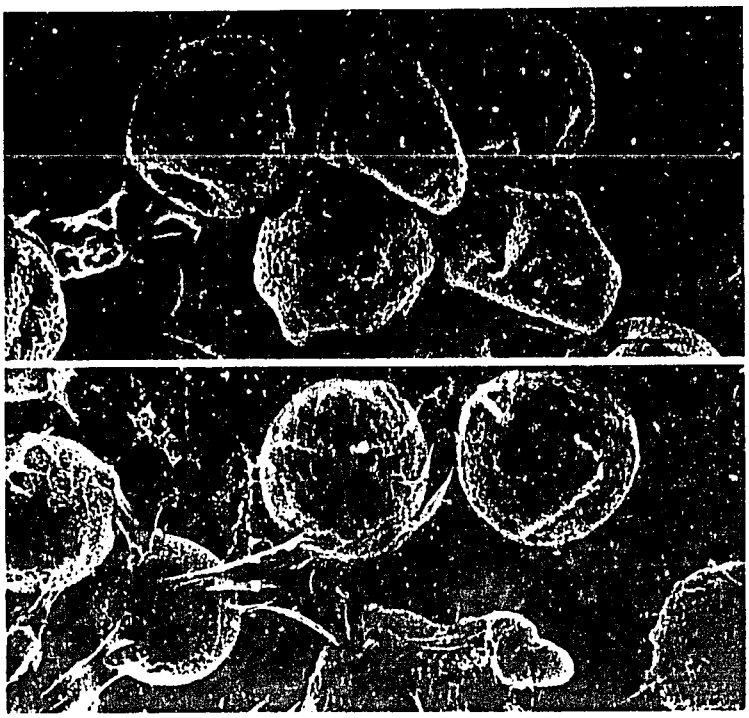

Fig. 1 A, B. Scanning electron micrograph of *P. vera* L. pollen. A Dry pollen, B water-imbibed pollen. *Bar* = 10 μm

crographs of gold-coated specimens after freeze drying were taken on a Hitachi S 900 microscope.

SDS-Polyacrylamide gel electrophoresis

SDS-PAGE was carried out on 10% polyacrylamide gel according to Laemmli (1970) and stained with coomassie brilliant blue.

Analytical procedures

Total C, N and S were determined with a Carlo Erba nitrogen analyser model 1500 (Carlo Erba Strumentazione, Milan, Italy). K, Ca, Mg, P and B were determined with an inductively coupled plasma emission spectrometer (Perkin Elmer, model ICP/5500, Ueberlingen, Germany). Nitrate was measured with a nitrate electrode (Orion, model 93-07) and sulfate was determined, semi-quantitatively with a reagent kit for photometric analysis (Merck, Microquant 14789). Vitamin C was determined fluorometrically using a centrifugal analyser equipped with fluorescence immunoassay attachment (Vuilleumier and Keck 1989). Sugars were determined by HPLC with a BIO-RAD HPX-87C column (Richmond, Calif.). The osmolalities of the media were determined by a vapour pressure osmometer (Wescor, 1500 B). Electrical conductivity was measured with a conductometer (E 518 Metrohm, Herisau); this was converted to osmolality assuming that 1 mS·cm^{-1} = 14.2 mOs·kg^{-1}.

Results

Pollen hydration

A scanning electron micrograph of water-imbibed *P. vera* L. pollen is shown in Fig. 1 A, B. In the dry state the pollen appeared shrunken with a convoluted surface (Fig. 1 a). After being subjected to water for approximately 10 min, the pollen swelled and became spherical (Fig. 1 b). No wall rupture was seen, but there was a leakage of solutes to the medium (Fig. 2). It appeared as if the water-extractable matter came out the wall matrix. The vesicles (v, Fig. 2) on the wall surface resisted the water wash. During the three washes of the pollen with water (ca. 10 min each time) there was a rapid leakage of solutes, with approximately 32% of the pollen dry matter leaking out in the first wash (Fig. 3). In the following two washes only small amounts of dry matter came out of the pollen (Fig. 3). The leakage of solutes from pollen suspended in germination medium was difficult to measure because of the high concentration of solutes in the medium itself. Preliminary data (not shown) indicated that in germination medium both the rate of leakage and the quantity of solutes leaked were lower than in distilled water. The composition of the water-washable solutes from the pollen in distilled water is shown in Table 1. Approximately 50% of these solutes were carbohydrates, of which sucrose (0.65 μmol/mg), glucose (0.77 μmol/mg) and fructose (0.78 μmol/mg) were the major sugars. The nitrogenous portion of the dry matter contained a protein fraction that was composed of a large number of proteins (Fig. 4). The major polypeptides had molecular weights of 63 kDa, 60 kDa, 59 kDa, 40 kDa, 38 kDa, 36 kDa, 35.5 kDa and 31 kDa (Fig. 4). Nitrate could not be detected with the nitrate electrode, and sulfur was mainly in the form of sulfates forming approximately 1% of the dry matter (Table 1).

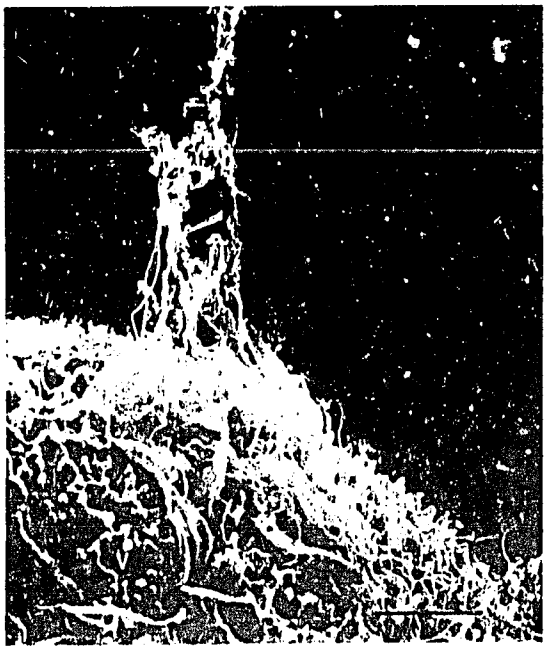

Fig. 2. Scanning electron micrograph of *P. vera* L. pollen surface after washing with distilled water. r-vesicles. *Bar* = 2 μm

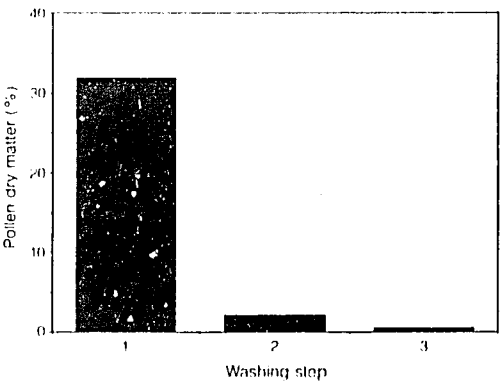

Fig. 3. Water washing of *P. vera* L. pollen, in three steps of approximately 10 min each

Osmotic effect

The *P. vera* L. pollen germinated poorly after imbibition in distilled water. However, after prehydration in a water-saturated atmosphere there was an increase in germination in both liquid nutrient media (Fig. 5). When the water content of the pollen was approximately 20% water, germination was highest in the sucrose-containing medium (Fig. 5).

The low osmotic potential required for pistachio pollen germination led to the hypothesis that an osmotic component is strongly involved in the initiation of pollen germination. The replacement of sucrose with PEG at the same osmolality (1000 mosmol/kg) resulted in about 10% pollen germination (Fig. 5). While germination in medium containing PEG was lower than in medium containing sucrose, it did indicate that an osmotic signal might be involved in pistachio pollen germination. PEG does not enter the cells; therefore, the low osmotic po-

Table 1. The composition of water-washable solutes from *Pistacia vera* L. pollen

Component	Dry weight (%)
Nitrogen	3.82 ± 0.03
Carbon	39.76 ± 0.4
Sulphur[a]	0.31 ± 0.01
Ash:	6.94 ± 0.04
Potassium	3.85
Calcium	0.11
Magnesium	0.11
Phosphorus	0.92
Boron	0.002
Vitamin C	0.021
Sugars:	50.3 ± 3.0
Sucrose (μmol/mg)	0.65 ± 0.06
Glucose (μmol/mg)	0.77 ± 0.08
Fructose (μmol/mg)	0.78 ± 0.06

[a] As 1% sulphate

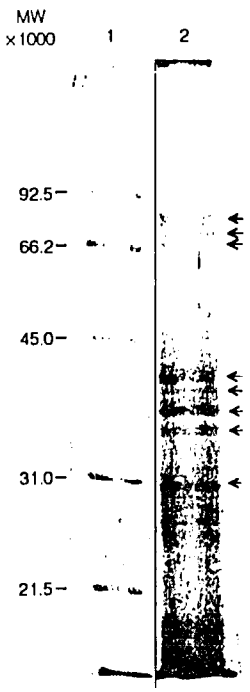

Fig. 4. SDS-PAGE of pistachio pollen water-washable fraction (*2*) and standard proteins (*1*). The *arrows* point to the major polypeptide bands

ential of the medium led to shrinkage of the germinated pollen (Fig. 6a), in contrast to well swollen pollen in sucrose-containing medium (Fig. 6b). Pollen tube length in both media is shown in histograms (Fig. 7). The pattern of pollen tube length distribution was similar in both media. There was a large variability in tube length, 250 ± 142 μm and 263 ± 172 μm in sucrose and PEG, respectively. This indicated that pollen viability and maturity at dehiscence and dispersal were quite variable.

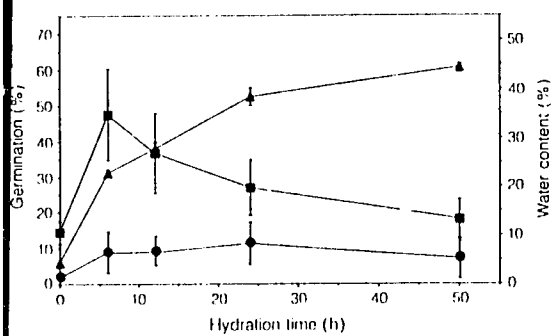

Fig. 5. Germination of pistachio pollen in media containing sucrose (■ ■) or PEG (● ●). The osmolality of each medium was approximately 1000 mosmol/kg. ▲ ▲ the water content of prehydrated pollen

Discussion

The test most commonly used to assess pollen viability is in vitro germination. The known difficulties in germinating pistachio pollen can be partially overcome by prehydration, as has been shown by Polito and Luza (1988) and in this work (Figs. 5 and 6). While artificial medium cannot fully simulate the complex pollen-stigma interaction in vivo, pollen tube development in vitro appeared to be normal (Fig. 6b) under the appropriate conditions. Furthermore, in a number of taxa the percentage of in vitro germination of stored pollen can be correlated with its ability to set fruits and seeds following in vivo pollination (Janssen and Hermsen 1980; Visser 1955).

In the experiments reported here, we have examined the early stages of pollen hydration and germination and shown that rapid hydration of the dry pollen did not result in much cell breakage, nor in germination. The membranes of these pollen grains were apparently not effective in preventing the movement of solutes out of the pollen (Fig. 3). Pollen germination in the sugar-containing medium was substantially increased by prehydration of the pollen in a humid atmosphere (Fig. 5). These results may be explained by the model proposed by Crowe et al. (1989): the membrane phospholipids in the dry organism (pollen) are in the gel phase, and upon a sudden exposure to water a fast phase transition

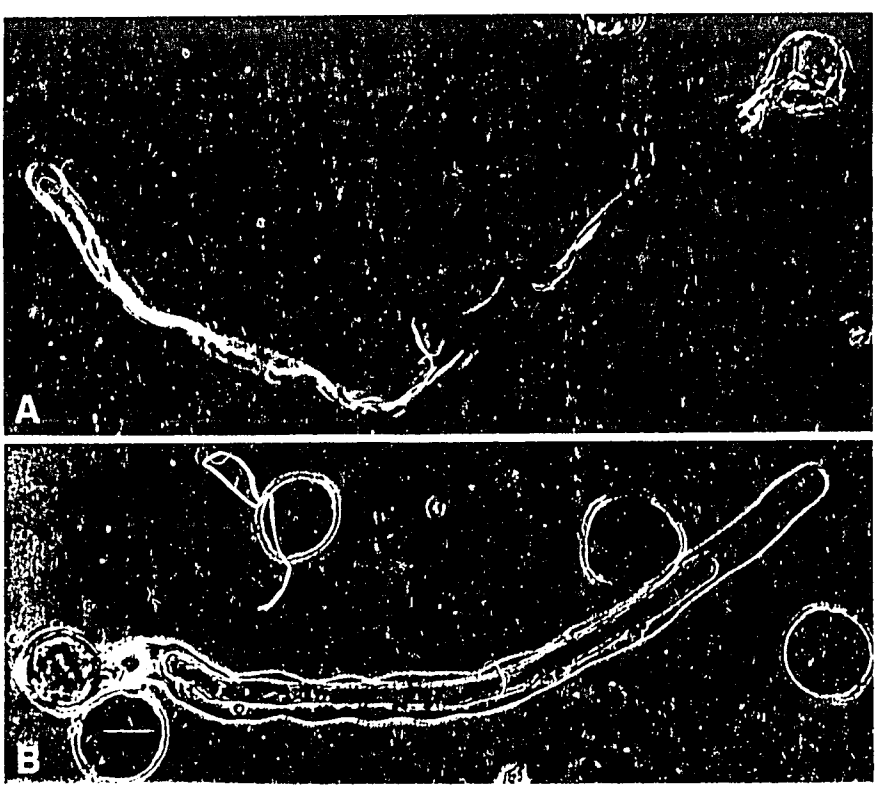

Fig. 6A, B. Light microscopy micrographs of *P. vera* L. pollen germinated in media containing PEG (A) or sucrose (B) (1000 mosmol/kg). ×250

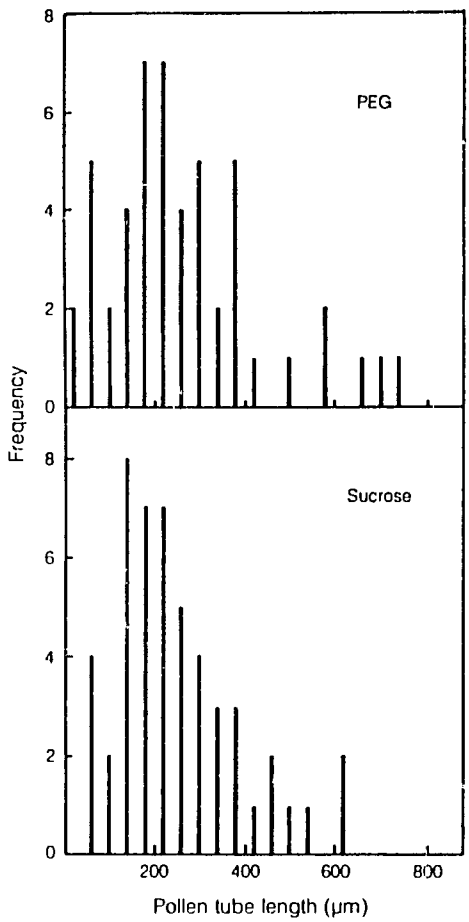

Fig. 7. Histograms of pistachio pollen tube length after 10 h of germination in media containing sucrose or PEG

occurs to a liquid crystalline phase that results in leakage. When the pollen were exposed to partial prehydration in a humid atmosphere, up to 50% of them germinated in the sugar solution (Fig. 5). According to Crowe's model (Crowe et al. 1989), these pollen grains did not undergo phase transition and therefore did not leak. One can not eliminate another possibility that the soluble fraction might have come from the wall layers partly derived from the haploid gametophyte and partly from its diploid parent. Evidence for this has come from the localization by immunofluorescence of the origins of the antigens present in the leachates from intact pollen (Heslop-Harrison 1975). Protein release during germination (Fig. 4) has been reported extensively (Heslop-Harrison 1975; Kamboj et al. 1984; Jackson 1989; and others). It has been suggested that these proteins may play a role in the interaction between pollen and stigma (Heslop-Harrison 1975). The SEM picture (Fig. 2) of water-washed pollen showing the removal of materials from the wall layers may support the possibility that at least part of the leachate originated in the pollen-wall layers and not in the cytoplasm. Furthermore, the presence of substantial amounts of solutes in the cell-wall layers was supported by the observation (data not shown) that large amounts of solutes were also quickly released from prehydrated pollen. At this hydration state it could be assumed (Crowe et al. 1989) that the membrane integrity was reestablished, which would have prevented the leakage of solutes from the cytoplasm.

In dry pollen only a minor amount of potentially osmotic-active substances is in a soluble form. When the water content of the pollen is 5%, the maximum solubility of glucose is three times lower than the amount present in the pollen. One-third of the sugars analysed consisted of glucose, which contributed approximately 38% to the osmotic concentration. The water content of the pollen was close to 50% after 44 h of hydration. An osmotic potential of −9.7 MPa could then be generated by the osmotica contained in the pollen. It seems unlikely that a major part of the solutes was found in the cytoplasm, because osmotic potentials much lower than −10 MPa could result during hydration of the dry pollen. Osmotic potentials of halophyte cells are −5 to −8 MPa, and osmotic potentials of most plant species lie between −0.4 and −2 MPa (Salisbury and Ross 1978).

The low water content of pistachio pollen and low osmotic potential of the medium required for germination may be related to their phytogeographical origin in the dry Irano-Turanian region. In species where high water content in the pollen is required for germination (e.g. *Zea mays*: 60%), the medium osmolality is also lower, probably in simulation of a wetter stigma. It has been speculated that in a wind-pollinated species like pistachio, high osmotic pressure is needed on the stigma to allow for a controlled hydration and release of solutes from the pollen and secretion from the stigma into the grain. This osmotic compatibility is critically important in the early interactions between the pollen grain and stigma (Heslop-Harrison 1979).

The importance of osmotic balance between the concentration of osmoticum in the medium and that contributed by the solutes contained in the pollen has been recognized since the earliest attempts to germinate pollen in artificial medium. The successful germination of *Pistacia vera* L. pollen required a high osmotic concentration in the medium. Low water potentials in the pollen provided the pollen with a great capacity to hold water vapour. This was certainly required for pollen hydration under low relative humidity conditions, conditions which this plant species is frequently exposed to. Maintenance of a high turgor pressure, a prerequisite for pollen tube elongation in stigma and style, will be promoted by a high internal solute concentration.

Sucrose could be substituted for by PEG for induction of germination (Fig. 5) (Roberts et al. 1983). Indeed, pollen germinated in PEG appeared shrunken (Fig. 6a), and a lower percent germination was obtained than in sucrose-containing medium (Fig. 5). We propose that the net osmotic effect is important in initiating pollen germination. The large variability in pollen tube size probably reflects the fact that pistachio is wind pollinated and

that the pollen grains leave the anther at different stages of maturity (Wagner et al. 1989), which leads to different rates of water uptake upon rehydration and different rates of germination.

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Microscopic aspects and protein composition during microsporogenesis in *Pistacia vera* L.

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Abstract

The development of pistachio (*Pistacia vera* L.) male inflorescence bud was examined microscopically and by electrophoretic analysis of the protein composition at the same developmental stages during microsporogenesis. An Inflorescence Bud Polypeptide, 32 KDa (Ibp32), wasidentified, it was not found in vegetative buds. It concentrated in the reproductive zones of the bud and disappeared between the pollen mother cell (PMC) stage and the tetrad stage in pollen grain development.

Key words: *Pistacia vera* L., inflorescence bud, microsporogenesis, pollen, protein.

The *Pistacia vera* L. (common name pistachio) is a species of the genus *Pistacia* (Anacardiaceae), which is exploited commercially. The latest monograph study by Zohary (1952) recognizes eleven species of *Pistacia*. This genus is unique in that its species are dioecious, staminate and pistillate inflorescences borne on different individuals of a population, and are wind pollinated. Only a small part (approx. 4%) of all plants on earth are dioecious, which makes the pistachio quite attractive for studies on molecular aspects of sexual reproduction in plants. A common feature of the female inflorescence of pistachio is that many of its flowers do not produce seeds. The appearance of seedless fruitsin *Pistacia vera* L. has been recorded by several authors (Savastano, 1929, Whitehouse & Stone, 1941 and others). The staminate and pistillate inflorescences are panicles that may consist of one to several hundred individual flowers. Developmental aspects related to pistillate inflorescence were reported by Grundwag & Fahn, (1969) and Takeda, Crane & Lin, (1979). However, no description of the development of pistachio staminate inflorescence was found in the literature.
Here, we describe microscopic changes during microsporogenesis of pistachio staminate inflorescence, and the accompanying changes in bud protein composition. It is the first report of identification of a 32 KDa polypeptide which disappears from the reproductive male bud during bud dormancy break and microsporogenesis.

Materials and Methods

Axillary male inflorescence buds of *Pistacia vera* L. Chico cv. trees were collected during development, every season between 1987 to 1990. The trees are growing in the experimental orchard at the Avdat Run-Off Farm in the Negev Desert of Israel. The buds were prefixed in a mixture of 2.5% glutaraldehyde, 7.5% paraformaldehyde in 30 millimolar sodium phosphate buffer pH 7 (Rowley & Walles, 1985). For light microscopy bud segments were embedded either in paraffin or in araldite. Specimens in araldite were cut into sections 2μm thick and in paraffin into 10μm thick. The paraffin sections were stained with Alcian green Saffranin, and the araldite sections stained with both Methylene blue azur ii and Basic Fuchsin.
Buds for electrophoresis were collected directly into liquid nitrogen, and stored at -70°C until extracted for analysis. Extraction of bud protein and preparation for electrophoresis were done as described before (Golan-Goldhirsh et al., 1990). SDS-

Polyacrylamide gel electrophoresis (SDS-PAGE) was carried-out on 12.5% polyacrylamide gels according to Laemmli (1970).

Results

Overall sequence of development

Figure 1 shows the overall sequence of male inflorescence bud development in pistachio and five specific stages in microsporogenesis, during which microscopic and protein analyses of the bud were carried-out. There are two major bursts of growth during differentiation of a vegetative bud to a flowering bud (Figure 1). A leaf at each node on the shoot subtends a single axillary bud, most of these differentiate into inflorescence buds during April and grow to their ultimate size by late June (First burst of growth, Figure 1). Generally, one or two axillary buds located distally on the new growth are vegetative. They are considerably smaller than inflorescence buds. Male Inflorescence buds begin expansion in the last week of March of the following year and anthesis occurs generally during the first half of April (Second burst of growth, Figure 1).

Microscopic changes during Microsporogenesis

After reaching its ultimate size following the first burst of growth (Figure 1) in late June, a young anther consists of a mass of homogeneous meristematic cells (archesporial) surrounded by epidermis (Fig. 2). This situation persists until bud expansion begins toward the end of March of the following season. As the anther develops the archesporial cells divide to form a primary parietal layer on the outside and a primary sporogeneous layer on the inside (Fig. 3). Eventually, the former gives rise to the wall layers by repeated periclinal and anticlinal divisions. The sporogeneous layer develops into the microspore pollen mother cells (Meiocytes). At the tetrad stage the microspore mother cells become enclosed within a special callosic wall, undergo meiosis and give rise to tetrads of microspores (Fig. 4). After the microspores are released from the tetrads, the tapetum layer degrades (Fig. 5), each microspore enlarges and undergoes an asymetric division producing the vegetative and the generative cells of a mature pollen grain (Fig. 6).

Electrophoretic protein pattern during microsporogenesis

The electrophoretic pattern of male bud proteins during development (Fig. 7) corresponds to the developmental stages analyzed microscopically (Figs. 2 to 6). In addition, lane F figure 7 shows the pattern of an extract of buds collected during early May, before the floral induction period was complete. Although a few slight changes in the electrophoretic pattern during development can be seen, the most notable change was in a 32 KDa polypeptide. It accumulates during bud formation, towards the end of the first burst of growth (Figure 1), in the transition from a vegetative to a reproductive bud and stayed apparently unchanged during dormancy, until the beginning of the second burst of growth (Figure 1; A, figure 7). This polypeptide disappeared between the pollen mother cell (PMC) stage and the tetrad stage (B to C, Fig. 7). A comparison of protein extracts of the rachis and flower primordia (reproductive) zones of the bud (Fig. 7) showed that the 32 KDa is concentrated in the reproductive zone of the bud rather than in the vegetative zone.

Discussion

A flower bud polypeptide 32 KDa (fbp32) was identified, whose appearance and disappearance from the inflorescence bud is closely correlated with the transition from vegetative to reproductive bud and with pollen formation (microsporogenesis), respectively. It may be significant that Ibp32 is synthesized during early development of the bud and remains apparently unchanged during bud dormancy, disappearing during inflorescence opening (second burst of growth, Figure 1). Could it be involved in dormancy regulation? For the moment Ibp32 may provide an important molecular marker for male flower development in pistachio and potentially in other trees.

The microscopic pattern of pistachio bud development shown in figures 2 to 5 is quite similar in other plants (Shivanna & Johri, 1985). However, time of anthesis and the duration of flowering is of extreme importance in dioecious species. Under natural conditions the bloom period of male trees often precedes that of female trees. Therefore, artificial pollination is practiced in pistachio orchards, and the pollen vitality is essential (Polito & Luza, 1988; Golan-Goldhirsh et al., 1991). Spraying winter oil has been used to overlap male and female flowering by induced early dormancy break (Procopiou 1973; Spiegel-Roy, Asaf & Garmi, 1972). The Ibp32 as a molecular marker, should enable early evaluation of the effect of such treatments at the molecular level.

Some of the developmental problems of the *Pistacia vera* L. includes abscission of flowers and young fruits, a large number of the nuts (drupes) that set and remain on the tree to maturity contain none, or underdeveloped seeds. The Ibp32 can serve as a marker in studies of developmental processes in pistachio leading ultimately to higher productivity of the pistachio. Preliminary experiments indicated specificity of Ibp32 to reproductive buds (unpublished data). This is indicative of its potential functional specificity to reproduction in pistachio.

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Legend to figures

Figure 1. Approximate timing of *Pistacia vera* male inflorescence bud development.

Figure 2. Longitudinal section in a stamen at the archesporial (Ar) cells stage (A), as it is during bud dormancy, also showing a short filament (F) and sepals.

Figure 3. Cross-section in a differentiating anther at the pollen mother cell (PMC) stage (B), showing anther epidermis (E), wall cells (W), tapetum (T) and sporogenous tissue (Sp).

Figure 4. Cross section in an anther at the tetrad stage (C) showing microspores, tapetum (T), callose (Ca), nucleus (N), nucleolus (Nu), plastids (Pl) and small vacuoles (V).

Figure 5. Cross section in an anther after microspores (Mi) separation from the tetrad stage (D).

Figure 6. Cross section in a mature pollen grain, showing starch granules (S) and wall layers, exine (Ex) and intine (I), stage (E).

Figure 7. Electrophoretic analysis of *Pistacia vera* male inflorescence bud at several developmental stages. The lanes are marked by letters corresponding to the developmental stages indicated on figure 1. Lane F, represents archesporial cells at an early stage of floral induction, and St is a lane of a standard proteins mixture with the molecular weights indicated on the side lane.

Figure 8. Electrophoretic analysis of reproductive (1) and vegetative (2) zones of *Pistacia vera* male inflorescence bud.

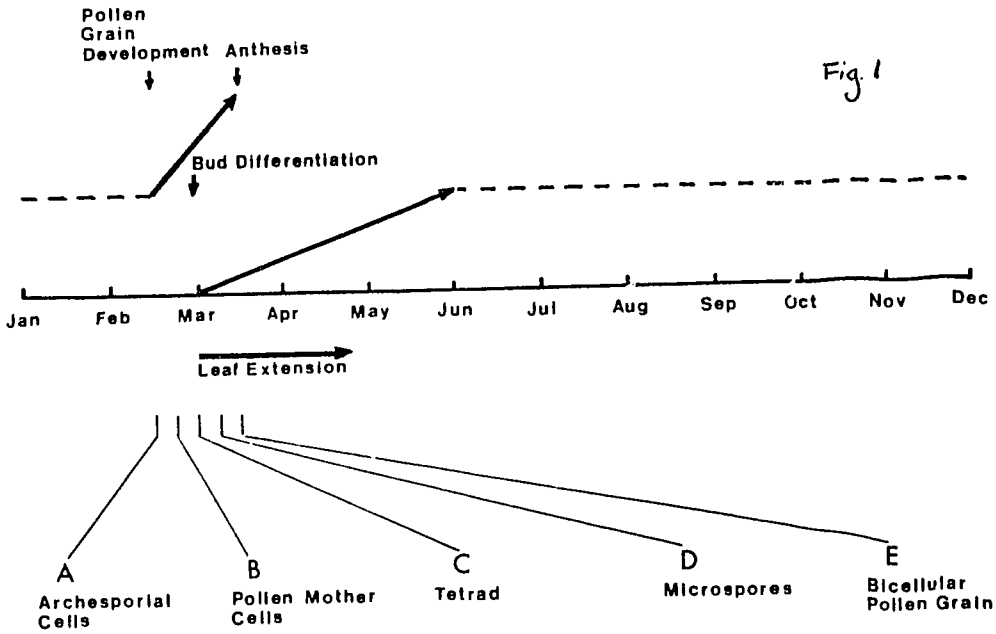

Pollen
Grain
Development
Anthesis
Bud Differentiation
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Leaf Extension
A
Archesporial Cells
B
Pollen Mother Cells
C
Tetrad
D
Microspores
E
Bicellular Pollen Grain

Fig. 1

Fig. 2

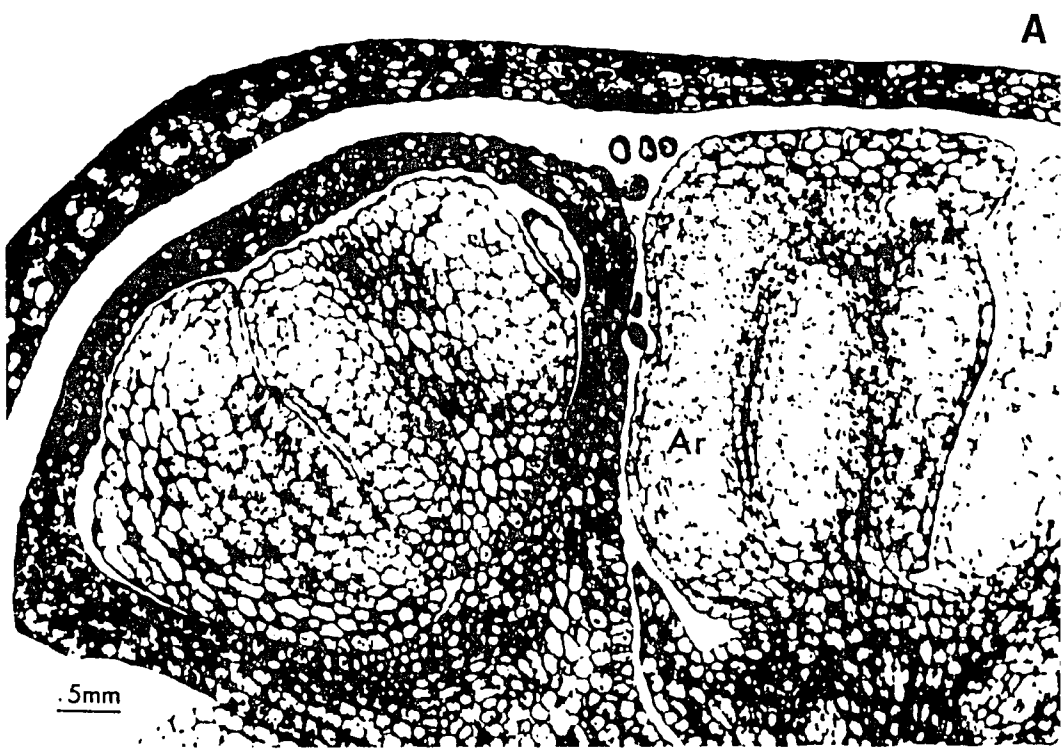

A
Ar
.5mm

Fig 3

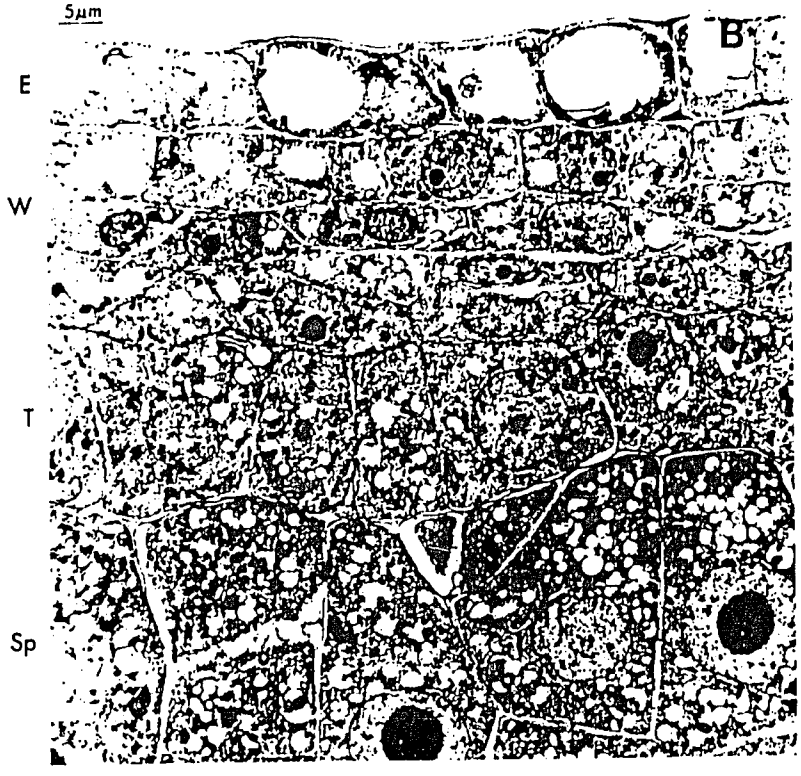


Fig V

Fig 5

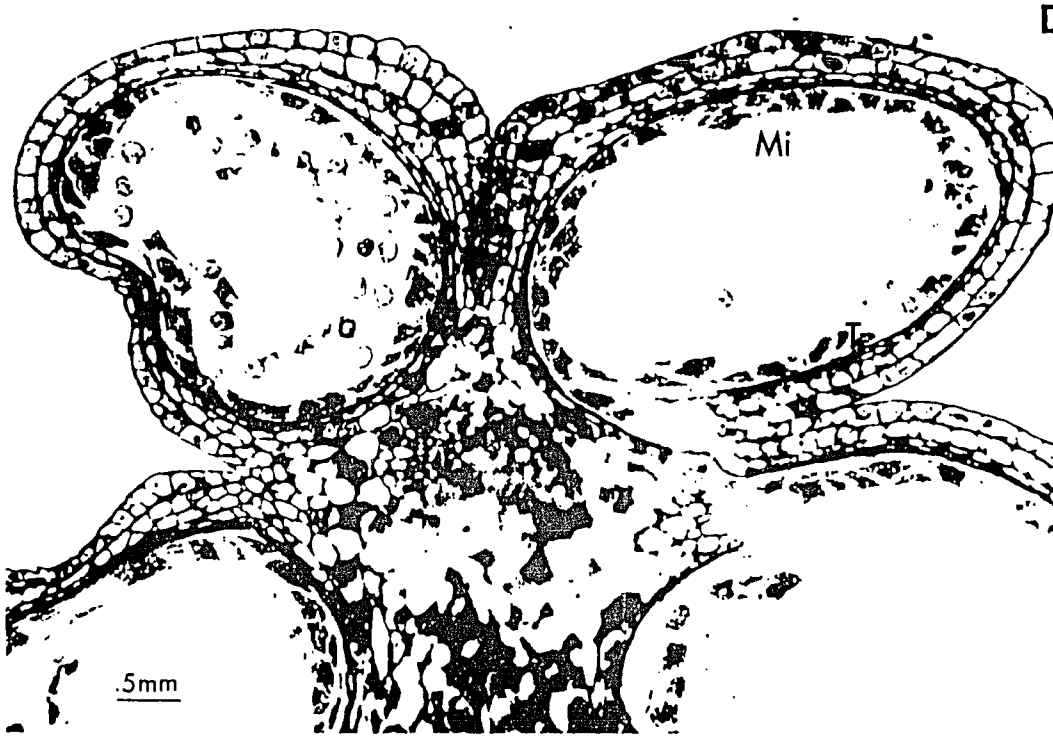


Fig 6

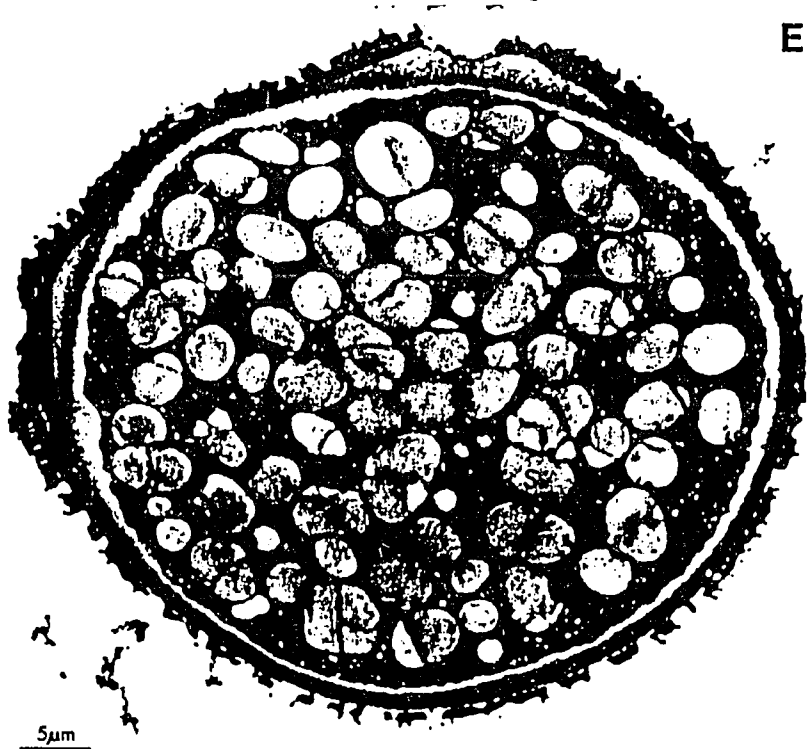


Fig. 7

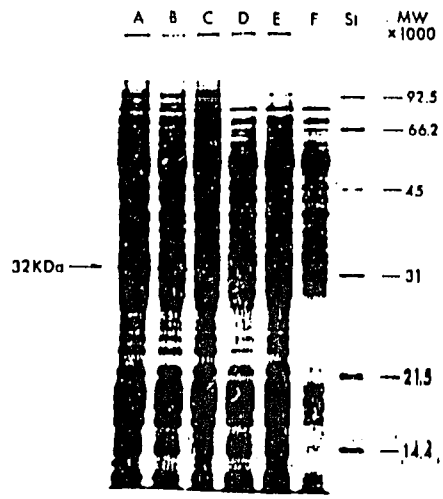
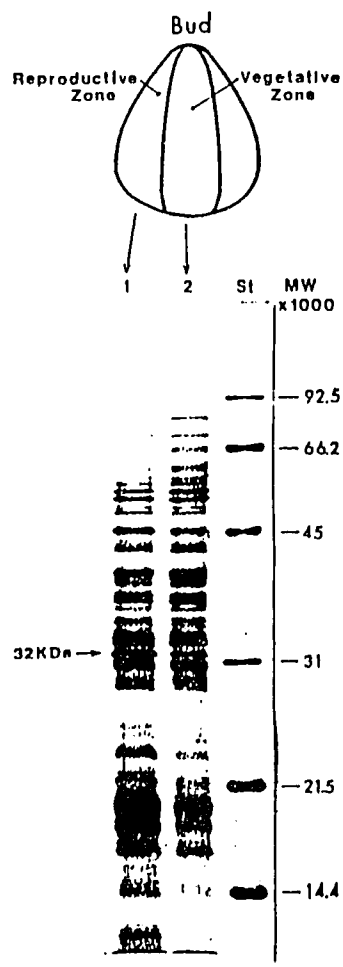

A
B
C
D
E
F
St
MW
x1000
92.5
66.2
45
31
21.5
14.4
32KDa

Fig 8


Bud
Reproductive Zone
Vegetative Zone
1
2
St
MW
x1000
92.5
66.2
45
31
21.5
14.4
32KDa

Polypeptides associated with sexual development of *Pistacia vera* L. inflorescence bud

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Dioecious plant species, where staminate and pistillate inflorescences are borne on different individuals of a population, offer a unique advantage over hermaphrodite species for the study of sexual reproduction in plants. Some important crop plants are dioecious e.g. *Cannabis sativa, Vitis vinifera, Asparagus officinalis, Humulus lupulus, Carcia papaya , Simmondsia chinensis* and *Pistacia vera* . The study of genetic control of sex in these plants has contributed greatly to our understanding of evolutionary aspects of sex determination and to the improvement of crop production [1]. The *Pistacia vera* L. (common name pistachio) is a unique dioecious and deciduous tree species, which is productive under harsh desert climate [2,3]. Flowering in *Pistacia vera* seedlings is usually delayed 5 to 8 years, therefore early sex determination in young seedlings would be of great practical importance.

We have identified and purified an Inflorescence Bud Polypeptide of 32 KDa (Ibp32) from pistachio. There was a close correlation between its accumulation and floral induction and its disappearance and flowering. Making use of poly- and monoclonal antibodies (mAb) against this polypeptide, we have identified a new 27 KDa polypeptide (Ibp27), which appears to be specific tofemale inflorescence buds. This allows us to examine the sex relatedness of the Ibp27 peptide and may allow early gender diagnosis in pistachio by a fast and simple procedure.

Flower formation in higher plants is a complex process controlled by genetic as well as environmental factors [4]. Although, it is an integrated process, three major phases can be recognized in deciduous trees, floral induction (evocation), bud dormancy and flowering (Figure 1). In recent years intensive research is done on flower development using homeotic genes, mutations which specify a wrong organ at the wrong place[5,6]. The model plants of these studies are *Arabidopsis thaliana* and *Antirrhinum majus*., which offer significant experimental advantages as model organisms. However, the pistachio as a deciduous and dioecuous fruit tree offers unique developmental traits, i.e. flower bud dormancy and chilling requirements for flowering and distinct separation of the sexes. Physiological aspects of sexual development in plants have been described in great detail[1,7]. The potential of modern molecular biology in elucidation of sex determination in plants has only recently started to emerge [8].

Phenological and microscopic aspects related to pistachio pistillate and staminate inflorescence have been described[9,10,11]. Much of the information on pistachio inflorescence bud morphology and development is based on studies by Crane and Iwakiri [12]. There are two major bursts of growth during bud development (Figure 1). A leaf at each node on the shoot subtends a single axillary bud. In the Negev plateau of Israel, floral induction (evocation) when most of these differentiate

into inflorescence buds occurs during April (Spring) and grow to their ultimate size by late June (First burst of growth, Figure 1). This will vary slightly with environment, amount of winter chilling and cultivar. Male inflorescence buds undergo microsporogenesis and flowering in the last week of March (Beginning of Spring) of the following year and anthesis occurs generally during the first half of April (Second burst of growth, Figure 1). The general macroscopic pattern of female buds development is simillar to that of male buds, however, after the initial inflorescence differentiation to rachis, lateral branches and sepals between April to June, there is a quiescent period of about three months, then pistil growth initiates in early October and continues until March, when individual flowers open[13].

Time of anthesis and the duration of flowering is of extreme importance in dioecious species. The bloom period of male seedlings often precedes that of female seedlings. Therefore, in the wind pollinated pistachio, staminate and pistillate cultivars having overlapping bloom periods must be planted in an orchard to obtain effective pollination [13].

We have shown recently that during the early stages of microsporogenesis a 32 KDa polypeptide (Ibp32) disappears from the maleinflorescence bud[11]. This polypeptide was purified to electrophoretic homogeneity (Figure 2).

The timing of accumulation of the Ibp32 is shown in figure 3. Using a set of specific poly- and monoclonal antibodies raised against this polypeptide, proteins of buds collected during the first burst of growth (Figure 1) were subjected to immunoblotting analysis (Figure 3). Female (lanes 1,2 &3, figure 3) and male (lanes 4,5 & 6, figure 2) bud proteins are compared at the period of floral induction. Ibp32 accumulates during this process to the highest concentration toward the end of June, the beginning of the bud dormant period. The Ibp32 is expressed throughout the quiescent period and disappears quickly between the pollen mother cell (PMC) stage and the tetrad stage at the beginning of the second burst of growth (Figure 1), before the tetrad stage of microsporogenesis[11]. Although the antibodies were raised against the 32 KDa peptide (Ibp32), they recognized a female bud-specific 27 KDa polypeptide (Ibp27) in addition to the 32 KDa (Figures 3 and 4). A comparison of bud proteins of female and male trees, harvested during bud dormancy shows the sexual relatedness of the 27 KDa polypeptide (figure 4). None of the male trees tested (8 trees) expressed the Ibp27 polypeptide, but 26 of 37 female trees did. These polypeptides were detected in reproductive buds only, neither in vegetative bud nor in leaf (data not shown). The genetic female sex linkage of Ibp27 is now under investigation in a large number of trees. The findings so far indicate a potential functional and/or structural specificity to reproduction in pistachio. The close correlation of Ibp27

with female buds is intriguing and points to a diagnostic potential. The fact that these peptides accumulate in large quantities makes it less likely that they have a regulatory role, nonetheless, they represent a different developmental pattern in male and female trees and can serve as molecular markers. It would be important to examine if Ibp27 is an independent gene product, an alternative splicing product[14] of Ibp32 or its post-translational processing product through proteolysis. If the first possibilty turns-out true, it would be of great interest to characterize each one of the genes and their products in relation to their regulation and function. If the Ibp27 is a separate gene product, it may be possible to detect the gene in juvenile pistachio by Southern blot and PCR and develop a diagnostic procedure for early gender determination in pistachio. If Ibp27 is a result of alternative splicing, it should give insight on regulation of sex expression in plants in analogy to what was recently reported in *Drosophila* [15]. If it is a product of proteolysis, it may be interesting to search for the enzymatic activity which leads to the specific modification of the female bud protein. Even if the Ibp27 is transitory in females, as part of a different pathway of turnover of Ibp32, it is of biochemical interest to determine its cellular location and examine its unique function in female buds. The finding that the Ibp32 is synthesized during early development of the bud, remains unchanged during the dormancy period and disappears during flowering constitutes an intriguing pattern. In preliminary experiments we found cross-reactivity between pistachio Ibp32 and immunologically related bud proteins of other deciduous trees (unpublished data), using the anti-Ibp32 antibodies. Could it be involved in maintenance of dormancy or, possibly, in sensing an environmental signal for dormancy break? In any case, this protein should provide an important molecular marker for inflorescence bud development in pistachio and potentially in other trees.

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Legend to figures

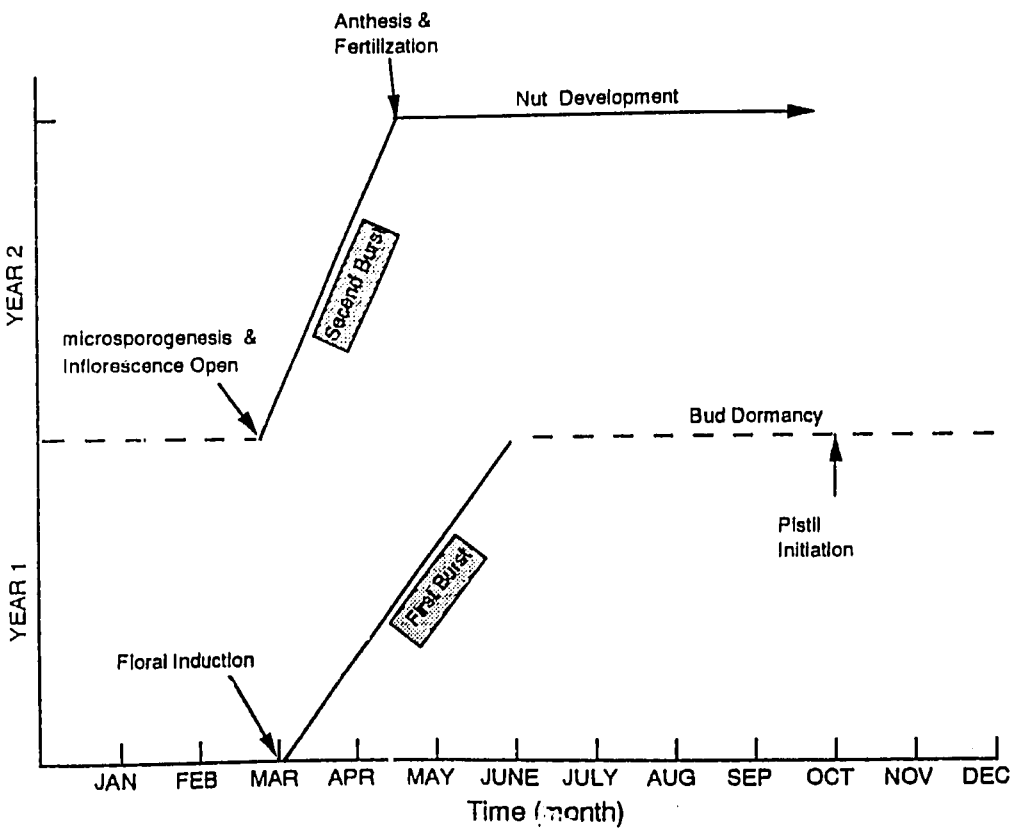
Figure 1. Approximate timing of *Pistacia vera* L. male and female inflorescence bud development at the Negev plateau of Israel.

Figure 2. SDS-polyacrylamide gel electrophoresis of female inflorescence bud extract (lane 1), showing the 32 KDa and 27 KDa polypeptides, and the electrophoretically purified 32 KDa polypeptide (lane 2). 10% SDS-polyacrylamide gel under reducing conditions was done according to Laemmli [16]. Lanes 1 and 2 contained 50 μg and 5 μg proteins, respectively, determined according to the method of Bradford [17].

Figure 3. Immunoblot analysis [18] of female (lanes 1,2 & 3) and male (lanes 4,5 &6) inflorescence buds of *Pistacia vera* L. A 50 μg Protein sample [17] was applied to each lane. Anti-serum from BALB/C mice containing polyclonal antibodies produced against electrophoretically pure male 32KDa (diluted 1/100), was used for the wesern blot. The second antibody was a Peroxidase conjugatedaffinity pure Goat Anti-Mouse IgG, F(ab') 2 Fragment (Jackson Immunoresearch Laboratories, Inc.), used at a dilution of 1/1500. Lanes 1 and 4 - buds of May 4 (early floral induction); Lanes 2 and 5 - buds of May 28 (advanced floral induction); Lanes 3 and 6 - buds of November 10 (dormant bud); Bl - blank buffer.

Figure 4. Immunoblot analysis [18] of male (lanes 1,2,3 & 4) and female (lanes 5,6,7 & 8) inflorescence bud extracts, showing the 32 KDa and 27 KDa polypeptides. The procedure is as described in figure 2, each lane contained 16 μg protein [17] and represents a different tree. Tree's identification numbers at the Avdat Run-off Farm, according to lane are as follows: lane 1 - 529B; lane 2 - 516B; lane 3 - 515B; lane 4 - 513; lane 5 - 540; lane 6 - 517; lane 7 - 514; lane 8 - 512; Bl - blank buffer.

Fig. 1


Anthesis &
Fertilization
Nut Development
YEAR 2
Second Burst
microsporogenesis &
Inflorescence Open
Bud Dormancy
Pistil
Initiation
YEAR 1
First Burst
Floral Induction
JAN
FEB
MAR
APR
MAY
JUNE
JULY
AUG
SEP
OCT
NOV
DEC
Time (month)

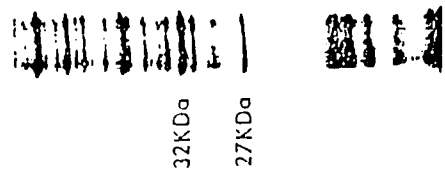


Fig 2

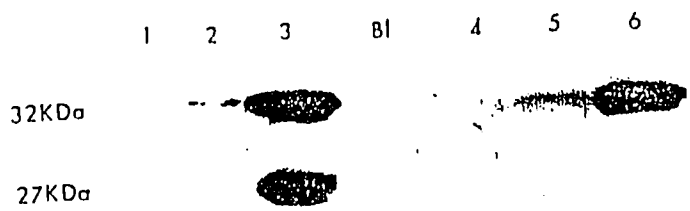


Fig 3

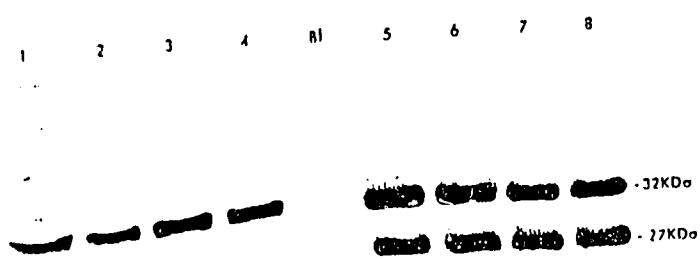


Fig 4