Adoption of biointensive IPM to enhance the development of organic date palm cultivation in the Arab countries

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ABSTRACT

Recently, there has been an increased demand on organic date in the developed as well as developing countries including the Arab countries. However, there are certain challenges for developing organic date palm cultivation in the Arab countries which are the main global producers of dates. Since the pest management practices should be applied in accordance with the valid standards of organic date production, the insufficient management of pests is one of the major reasons of such constraints. In fact, knowledge of organic-agriculture standards and available required information of the all components of IPM strategy is very important to develop adequate programs to manage the pest problems in organic farming; available data indicate that the basic components include: biological and ecological aspects of the target pest, field monitoring and scouting, threshold /action levels and natural controls, whereas, the methods and tactics which are commonly used as main and potential components to implement the IPM program include: regulation and legislative interventions, agricultural methods, attractants and pheromone traps and biological control. When the organic management practices alone cannot prevent or control pests, a biological or botanical substance may be applied through biointensive integrated pest management (Bio-IPM) programs. Consequently, bio-IPM is not just about management of pests alone, it is a sustainable crop production system based on sound eco-system analysis. However, there are certain constraints on its wide-range implementation in the Arab region. This paper highlights the current situation of IPM levels in Arab region and the need to overcome constraints and encourage the implementation of bio-IPM programs in organic date palm farms.

Key words: Organic date, Pests, Adoption and implementation, Biointensive IPM

INTRODUCTION

Organic agriculture includes all agricultural practices that promote the environmentally, socially and economically sound production of food. Crop production and pest control methods in organic agriculture are governed by strict standards and rules imposed by the International Federation of Organic Agriculture Movement (IFOAM) and national regulations. These standards applies to the unprocessed and processed products that carry or are intended to carry descriptive labelling referencing organic production methods. On the other hand, organic production is generally associated with different challenges; the major challenges include low quality palm cultivars, poor farm management, pest and disease control (and inadequate IPM: integrated pest management), harvesting, processing and marketing, shortages of national qualified and trained staff and labour, and insufficient research and development (Mahmoudi, *et al.,* 2008). Regarding organic date palm cultivation, dates shall refer to organic production only if they come from a farm system employing management practices that seek to nurture ecosystems in order to achieve sustainable productivity; and that provide weed, pest and disease control through a diverse mix of mutually dependent life forms, recycling of plant and animal residues, crop selection and rotation, water management, tillage and cultivation (United Nations, 2003; Azadi *et al.,* 2006; El-Zemaity, 2007b; Safwat, 2007).

Some Arab producers have diversified into organic production of dates. For example, Tunisia export certified organic dates to the European countries, the main market is Germany. Tunisia exported 678 tones of organic dates (The official production Figure was 107 000 tones for all varieties) in 2000-2001, up 60 percent from 425 tones in the previous season (Fruitrop, 2001).The recent data indicated that in 2011, 6,000 tons of organic dates were harvested in Tunisia, of which 4,000 tons (67%) was exported; 68% of this went to Germany, 11% to the United States and 7% to Morocco (Source: Freshplaza.com). Although Tunisia accounts for only 2 percent of world date production, its share of global exports in value is 21 percent. It represents 55 percent of EU imports in value. Tunisia exports about the same quantity of processed and natural dates. Algeria came the second with a market share of 20 percent of EU imports in value. The official production Figure in 2000 was 365 000 tones for all varieties. Algeria exports more natural dates than processed dates, as there is a lack of processing capacity. The quasi-totality of Algerian dates is destined for France (Fruitrop, 2001). Among organic fruits, date palms are of major importance in other Arab counties such as Egypt, UAE, Palestine and Saudi Arabia. Organic date production of these countries is locally distributed (Hartmann, *et al.,* 2012).

As conventional date palm the organic date palm cultivation and its fruits could be subject to attacks by several pests that are, in most cases, well adapted to the oasis environment. The main causes of date palm damage include insect pests, rodents and diseases (Naturland, 2002; Blumberg, 2008; Mahmoudi, *et al.,* 2008). The damage caused by such pests is considerable and leads to heavy economic losses. Most of pest control operations employing pesticides are either restricted or not permitted not only in organic date but also at all in organic products. The principles of pest control in organic farming are based on: (i) prevention of infestation, (ii) avoiding the contamination of organic foods by any form of infestation, (iii) avoiding any contamination of organic foods with plant protection products, and (iv) the use of substances which not adversely affect the environment. Generally, IPM is a set of management activities that farmers implement to maintain the intensity of potential pests at levels below which they become pests, without endangering the productivity and profitability of the farming system as a whole, the health of the farm family and its livestock, and the quality of the adjacent and downstream environments. Consequently, IPM is not just about management of pests alone, it is a sustainable crop production based on sound eco-system analysis. However there are certain challenges that constrain its wide range implementation (Guan Soon, 1996; Dhaliwal and Heinrichs, 1998).

Considering all mentioned previously, it is highly expected that the Arab countries which are the main producers of dates will face some of constraints in developing of organic date cultivation. So, the present paper highlights the current situation of IPM levels in Arab region and the need to overcome constraints and encourage the implementation of bio-IPM programs in organic date palm farms.

DISCUSSION

Possible causes of date palm damage in organic cultivation

All parts of offshoots and mature date palm tree could be exposed to the infestation by different abiotic as well as biotic disorders includes pests and diseases. The main pests which include insects; mites; plant pathogens; weeds; rodents and birds are similar in most of the Arab countries (Fig. 1). Some of these pests are considered serious pests in certain countries, whereas considered moderate or minor pests in the others. Among of these pests the two main serious pest-threats in date palm plantations now are the Red Palm Weevil (*Rhyncophorus ferrugineus*) and the fungal disease Bayoud (*Fusarium oxysporium*) (Calcat, 1959; Carpenter and Elmer,1978; Al-Azawi, 1986; Howard *et al.,* 2001; Zaid *et al.,* 2002). Other date palm disorders such as environmental, physiological and propagation factors could be causes of considerable damage in each country. The occurrence of date palm pests and/or injury symptoms is depending on the development-stage and the environmental factors. Naturland, 2002 reported that most of the problems concerning disease and pests have different causes, i.e. (a) monoculture cultivation and use of non-resistant and/or of few varieties; (b) insufficient distance between species that grow to the same height, failure to trim agro forestry systems; (c) unfavorable soil conditions like degenerated or poor soil, soil not deep enough for roots, lack of organic material, high salinity etc and (d) unsuitable site conditions (deep water table, insufficient irrigation, drought, temperature, high rainfall level etc.). It is worth mentioning that the absence of adequate management of such disorders could cause considerable damage and lead to heavy economic losses.

Requirements of pest control in organic date palm

Pest control (including insect pests, diseases and weeds) shall be centered on organic management practices aimed at enhancing crop health and minimizing losses caused by such pests. When the organic management practices alone cannot prevent or control possible pests, a biological or botanical substance or other substances may be applied (British Pest Control Association, 2002). However, the conditions for using the substance shall be documented in the organic plan. Pest management plan should be based on essential considerations: (1) appropriate practices should be adopted to prevent pests and avoiding the contamination of organic food by any form of infestation from microorganisms, insects or other pests; (2) control measures should be achieved mainly by means of scrupulous cleaning procedures and hygiene controls adopted within and around warehouse and storage areas, food preparation areas and for all contact surfaces, within particular emphasis given to the frequent and regular cleaning of inaccessible areas; (3) the permitted pest control substances which does not adversely affect the environment may be used if these practices are ineffective and must be used without any risk of contamination; and (4) the use of chemical means of pest control should be kept to minimum, and restricted substances should lead to the organic products losing their organic status. These emphasize that the pest management practices should first involve the removal of pest habitat and food; second, the prevention of access and environmental management (light, temperature and atmosphere) to prevent pest intrusion and reproduction; and third, mechanical and physical methods (traps), permitted lures and repellents. On the other hand the operator shall, however, ensure that any pest control substance used does not come in contact with the organic raw materials or product, and shall record the use and disposition of all such substances. These requirements could be implemented through the bio-IPM strategy which emphasizes on proactive measures to redesign the agricultural ecosystem to the disadvantage of a pest and to the advantage of its parasite and predator complex (UIUC, 1997).

Biointensive integrated pest management (Bio-IPM) system and planning the suitable program

Biointensive integrated pest management (bio-IPM) is a system approach to pest management that is based on an understanding of pest ecology. It begins with steps to accurately diagnose the nature and source of pest problem, and then relies on a range of preventive tactics and biological measures to keep pest populations within acceptable limits (Leslie and Cuperus, 1993; Steiner, 1994; Altieri, 1994). Reduced risk pesticides are used if other tactics have not been adequately effective, as a last resort and with care to minimize risks. Generally, bio-IPM has many of the same components as conventional IPM, including monitoring, use of economic thresholds, record keeping, and planning (El-Zemaity, 2006). On the other hand, bio-IPM system is affected by several factors such as: economic costs and benefits of individual components; emergence of new pests, resistance or unusual weather problems; the skill and competence of field personnel conducting scouting, designing tactics and assessing effectiveness of given strategies; the impact or importance of preventive practices; availability, or lack thereof of effective alternative pest management products; and the complexity of interactions among pests, beneficials, cropping practices and control measures. Moreover, all IPM programs, regardless of the situation, share the components of monitoring the pest population and other relevant factors; accurate identification of the pest; determining injury levels and threshold that trigger treatment; timing treatments to the best advantage; spot-treating for the pest; selecting the least – disruptive tactics; evaluating the effectiveness of treatment to fine –tune future actions and educating all people involved with the pest problem (El-Zemaity, 2007a).

Good planning must precede implementation of any IPM program, but is particularly important in a biointensive program. Planning should be done before fruiting season because many pest strategies require steps or inputs, such as beneficial organism habitat management that must be considered well in advance. Attempting to jump-start an IPM program in the beginning or middle of a season generally does not work.

The current situation of IPM levels in the Arab region

Measuring the success and improving the efficiency of IPM actions by adopting better application practices require accurate evaluation of the current management programs. The success of the IPM program can be measured by the ability to maintain infestation levels below threshold level or a given % in a target area. In fact, information on the degree of adoption and evaluation of IPM practices in the Arab countries is very lacking (El-Zemaity, 2006). Regarding the actual implementation of IPM along the Arab region, it could be classified to 3 categories of adoption (low, medium and high – level IPM), with the exception of chemical control level which no practices of IPM (or no IPM) are employing and the system is essentially dependent routinely on insecticides (El-Zemaity, 2013). The adopted practices of the three categories may include: (1) low – level IPM, employing at least the most basic IPM practices-scouting and applications in accordance with economic threshold/ area–wide management; (2) medium – level IPM, some preventive measures, coupled with efforts to cut back on broad spectrum of insecticide use and (3) high – level IPM, integration of multiple preventive practices to control the insect without relying on insecticides such as in organic farming. The high – level IPM is the most advanced IPM and termed as the bio- intensive IPM. The actual percentages of current IPM adoptions levels in each Arab country are not well known. This may require encouraging researches on the evaluation of the adopted IPM programs under the local condition of each country. Such researches have become necessary to improve our understanding of the success and true impacts that can be expected from the commonly used IPM practices.

Successful implementation of Bio-IPM approach

1. **Pest identification (pest diagnosis)**

A crucial step in any IPM program is to identify the pest. The effectiveness of both proactive and reactive pest management measures depend on correct identification. Misidentification is actually harmful and costs time and money. Help with positive identification of pests may be obtained from university personnel, private consultants, the cooperative extension service, books and websites. After a pest is identified, appropriate and effective management depends on knowing answers to a number of questions related to the pest life cycle and the role of agricultural practices in enhancement its natural control. So, monitoring (field scouting) and economic injury and action levels are used to help answer such questions as well as to make adequate analysis of the overall situation of a date plantation through agro-ecosystem analysis.

2. **Agro–ecosystem analysis (AESA)**

The objective of AESA is to build awareness of the relationship that exists between organisms in the environment and to make good management decisions. The AESA should be done weekly to monitor conditions of crop, weather, soil, pests (including diseases and weeds) and beneficial organisms (predators and parasites). To conduct proper AESA, it is highly recommended to spend some time discussing the needed information, observations and recording results. This discussion should lead to the correct way to observe date palm plantation and chosen observed trees. In-field observation of represented sample tree should carefully be observed for the presence of any pests, beneficials, injury symptoms and signs on the different tree parts (growing point, inflorescences, leaves, fruits, trunk/stem, off shoots, bulb, roots, whole plant). Soil surface also observed for any ground – dwelling pests or beneficials. The results of observed pests and associated organisms, as well as different leaf spot disease symptoms should be recorded on AESA chart (Fig.1A&B) or presented in inspection table or illustrate AESA chart.

3. **Proactive tactics of bio-IPM system**

Cultural control and pest- resistant cultivars - All agricultural methods should be utilized to create a non-suitable environment for the multiplication of the pest and offer suitable habitat for beneficial organisms. On the other hand cultivars should be resistant to major pest(s), appropriate for the area, commercially available, should have appropriate mode of resistance and must have a market.

Mechanical and physical controls - Methods included in this category utilize some physical components of the environment, such as temperature, humidity, or light, to suppress the pest. Common examples are covering the fruit bunches with plastic nets, flaming, soil solarization, and plastic mulches to kill weeds or to prevent weed seed germination.

Biological control - Biological control is the use of living organisms - parasites, predators, or pathogens - to maintain pest populations below economically-damaging levels, and may be either natural or applied. The first step in setting up a biointensive IPM program is to assess the populations of beneficials and their interactions within the local ecosystem. This will help to determine the potential role of natural enemies in the managed agricultural ecosystem. It should be noted that some groups of beneficials (e.g.,spiders, ground beetles, bats) may be absent or scarce on some farms because of the lack of habitat. These organisms might make significant contributions to pest management if provided with adequate habitat. Possible natural enemies of the main date palm pests are listed in Table (1).

Reactive pest management options

Since, IPM requires continuous assessment of a situation (UIUC, 1997), there are certain key questions that must be answered before implementing any management strategy such as: Is treatment necessary? What are the alternatives to prohibited substances that can inhibit pests? What are commercial sources for these alternatives? Where should the treatment talk place? When should action be taken? and Which tactics should be used?. The answer of these questions required to emphasize that the mere presence of a pest doesn't necessarily warrant treatment. Some times a fairly large population of pests can be tolerated while other times the presence of a single pest is intolerable. In addition, the determination in treatment will vary among individuals. Also, pest managers must look to the whole system to determine the best place and timing to solve the problem. A successful IPM program is based on taking "a whole system" or eco-system approach to solve a pest problem (Leslie and Cuperus, 1993). We must think of both the living and non-living components when determining which approach to take, and each component has impact on every other component (Altieri, 1994).

Choosing practices/ tactics

Organic control practices for the main pests (i.e. insects, diseases and weeds) are based on non-chemical sanitation, physical, mechanical, cultural, and biological means as well as organically-permitted products including approved chemicals. Since no single practice is effective for all possible pests that threaten the crop, a combination of such practices is necessary. Proactive and reactive practices or tactics should be chosen to achieve the organic control measures (Fig. 2). Steiner, 1994 reported that the proper selection of control techniques is among the bases of successful management of insect pests. During the growing season there are numbers of practices to maintain healthy plants including adequate fertilizing, irrigation and mulch. Preventive devices, sticky colored yellow, black light and pheromone traps are excellent trapping techniques and can be used as survey tools, and may offer protection to plants. These practices could make fields unattractive to pest species. However, sometimes this may be not enough when the levels of pest populations or damage are not acceptable. The use of bio-pesticides including microbial products, botanicals and biochemical substances in these cases are necessary practice. Permitted and restricted pest management tools in organic farming are listed in Table 2.

CONCLUSION

Adoption and overcome of bio- IPM constrains to improve the effectiveness of current programs used in organic date cultivation is needed. Furthermore, new management programs for organic agriculture need to be designed, where the crop environment discourages pest development. Also, the role of training of organic farmers and farm groups should be emphasized as a key feature of successful programs in learning and implementing new practices. Meanwhile the IPM continuum could be achieved according to the following action plan:

1. Define an appropriate IPM continuum for the country or the region.
2. Establish at what stage we are now.
3. Establish realistic objectives in consultation with all stakeholders.
4. Recommended action to industry and to government.
5. Establish new positions of crop management specialists.
6. Recruit a professional with research and extension expertise in the area of bio - intensive IPM.

Literature Cited

Al-Azawi, A.F. 1986. A survey of insect pests of date palms in Qatar. Date Palm J. 4:247-266.

Altieri, Miguel A. 1994. Biodiversity and pest management in agro-ecosystems. The Haworth press. Binghamton. NY. 185p.

Azadi, H.; Hosseininia, G.H. and Azadi, M. 2006. ‘North-South GM crops transfers and organic farming: The question of food security in South countries’. Paper presented at the International Conference of Economics of Poverty, Environment and Natural Resource Use, 17-19 May 2006, Wageningen, The Netherlands.

Blumberg, D. 2008. Review: Date Palm Arthropod Pests and Their

Management in Israel. Phytoparasitica 36(5):411-448

British Pest Control Association, BPCA. 2002. Pest Control Requirments for Use in Organic Farming & Food Production. Gleneagles House, Vernon Gate, Derby DE11UP.

Calcat, A. 1959. Diseases and pests of date palm in the Sahara and North Africa. Bull. Entomol. Res.11:287-303.

Carpenter, J.B. and Elmer, H.S. 1978. Pests and diseases of the date palm. U.S. Dep. Agric. Agric. Handbk.527:1-42.

Dembilio, Ó. and Jacas, J.A. 2013. Biological Control of Rhynchophorus ferrugineus. AFPP- Palm Pest Mediterranean Conference, NICE- 16-18 January, 2013.

Dhaliwal, G.S. and Heinrichs, E.A. 1998. Critical issues in insect pest management. Commonwealth Publishers, New Delhi, India. 287 pp.

El-Zemaity, M.S. 2006. IPM and organic farming. Arab J. Pl. Prot. Vol.24, No.2:174-176.

El-Zemaity, M.S. 2007a. Developing and using expert systems for management of date palm pests in Arab region- What kinds of information may be needed?. Fourth Symposium on Date Palm in Saudi Arabia Proceedings, King Faisal University, pp:1167-1178.

El-Zemaity, M.S. 2007b. Organic date and bio-intensive IPM . Egypt. J. Agric. Res., 85(1):119-130.

El-Zemaity, M.S. 2013. Future and Challenges of Integration Biotechnology Products in RPW Management Programs. Paper Presented in Fifth Symposium on Date Palm in Saudi Arabia, King Faisal University, Alahsa, 3-5 November 2013.

FAO. 1995. Report of the Expert Consultation on Date Palm - Pest Problems and their control in the Near East. (22-26 April, 1995); Al-Ain, United Arab Emirates.

Freshplaza .Tunisia changing to organic: great demand for dates and fruit. Avaliable in www.freshplaza.com

Fruitrop. 2001. Tunisia: date exports increasing, p.6, n.76, January 2001, Montpellier, France

Guan Soon, L. 1996. Integrated pest management in developing countries. In: Biotechnology and integrated pest management. CAB International, p. 61-75.

Hartmann,M; Khalil, S.; Bernet, T.; Ruhland, F. and Al Ghamdi A. 2012. Organic Agriculture in Saudi Arabia. Available online: www.sofa.org

Hassan, B.H.; Al- Rubeai, H.F.; Al- Jboory, I.J. and Viggiani, G. 2003. Pseudoligosita babylonica n.sp. (Hymenoptera: Trichogrammatidae), egg parasitoid of

Ommatissus lybicus Bergevin (Homoptera: Tropiduchidae) in Iraq. Boll. Lab. Ent. Agr. Filippo Silvestri 59: 75-78.

Howard, F.W.; Moore, D.; Giblin-Davis, R.M. and Abad, R.G. 2001. Insects on Palms. CAB International, Wallingford, UK.

Hubaishan, M.A. and Bagwaigo, A.A. 2010. The effect of the egg parasitoid Pseudoligosita babylonica on dobas infestation in Doaan, Shichawi and Asid (Hudramout, Yemen). Proc. 4th Int. Date Palm Conference, Eds.: A. Zaid and G.A.Alhadrami, Acta Hort. 882, ISHS 2010,pp.451-455.

Leslie, Anne R. and Gerritt Cuperus. 1993. Successful implementation of integrated pest management for agricultural crops. CRC Press. Boca Raton, FL. 193p.

Mahmoudi, H; Hosseininia, G.; Azadi, H. and Fatemi, M. 2008. Enhancing Date Palm Processing, Marketing and Pest Control Through Organic Culture. Journal of Organic Systems, Vol.3, No.(2): 29-39.

Naturland e.V. 2002. Organic Farming in the Tropics and Subtropics- Date Palm. – 1st edition, www.naturland.de

Steiner, P.W. 1994. IPM: what it is, what it isn't., IPMnet NEWS. October.

Safwat, M. S. A. 2007. Organic Farming of Date Palm and Recycling of Their Wastes. African Crop Science Conference Proceedings Vol. 8. pp. 2109-2111

United Nations. 2003. Organic Fruit and Vegetables from the Tropics. United Nations Conference on Trade & Development. New York and Geneva.

University of Illinois at Urbana- Champaign, UIUC. 1997. IPM adoption: How far along the continuum are we? http://ipm.uiuc.edu/bulletin/ pastpest/ articles/v 9713f.html

Zaid, A.; de Wet, P. F.; Djerbi, M. and Oihabi, A. 2002. Chapter XII: Diseases and Pests of Date Palm. In: A. Zaid (ed.) Date Palm Cultivation. FAO Plant Production and Protection Paper 156. Rev.1. Rome, 2002.

Tables

Table.1. Possible natural enemies of the main date palm pests

English/ Scientific name	Possible Natural Enemis	Source
White scale/ *Parlatoria blanchardii* Targ	Hemisarcoptes malus, Chrysoperla vulgaris, Cardiastethus nazarenus, Coccinellidae (29 species), Nitidulidae (5 species), *Mycetaeidae* (1 species), *Aphytis mytilaspidis*, *Cybocephalus nigriceps, Cybocephalus rufi frones, Chilocorus bipustulatus var. iraniensis* and *Chilocorus sp*.	FAO, 1995.
Red scale/ Phoenicococcus marlatti. cockerell,	General predators, such as *Pharoscymnus anchorago* (Fairmaire), are considered as active predators.	Zaid et al., 2002
Red palm weevil (RPW)/ Rhynchophorus ferrugineus Oliv.	Entomopathogenic nematodes (*Heterorgabditis species or Steinernema sp.*) - Entomopathogenic fungi (*Beauveria bassiana, Metarhizium anisopliae*) - Entomopathogenic bacterium (*Bacillus thuringiensis*)	Dembilio and Jacas, 2013.
The dubas bug/ *Ommatissus binotatus var. Lybicus* (De Bergevin)	The egg parasitoid *Pseudoligosita babylonica* (*Hymenoptera: Trichogrammatidae*).	Hassan et al., 2003; Hubaishan& Bagwaigo, 2010.

Table.2. Permitted and restricted pest management tools in organic farming.

Permitted	
- Carbon dioxide, nitrogen, freezing, heating and vacuum treatment. - Mechanical, sound or light barriers. - Electric flying insect control units. - Tamper resistant bait stations. - Pheromone traps & sticky boards. - Diatomaceous earth & amorphous silica. - Particle film barriers (processed kaolin clay). - Sugar esters - Compost teas.	- Botanical products. - Microbial products. - Organically approved chemicals (Bordeaux mixture, sulfur and copper) Restricted (Substances used only in case of immediate threat to organic foods becoming unfit for consumption due to infestation) - Pyrethrum derived only from a natural source. - Synthetic pyrethroids for the treatment of sealed units.

Figures

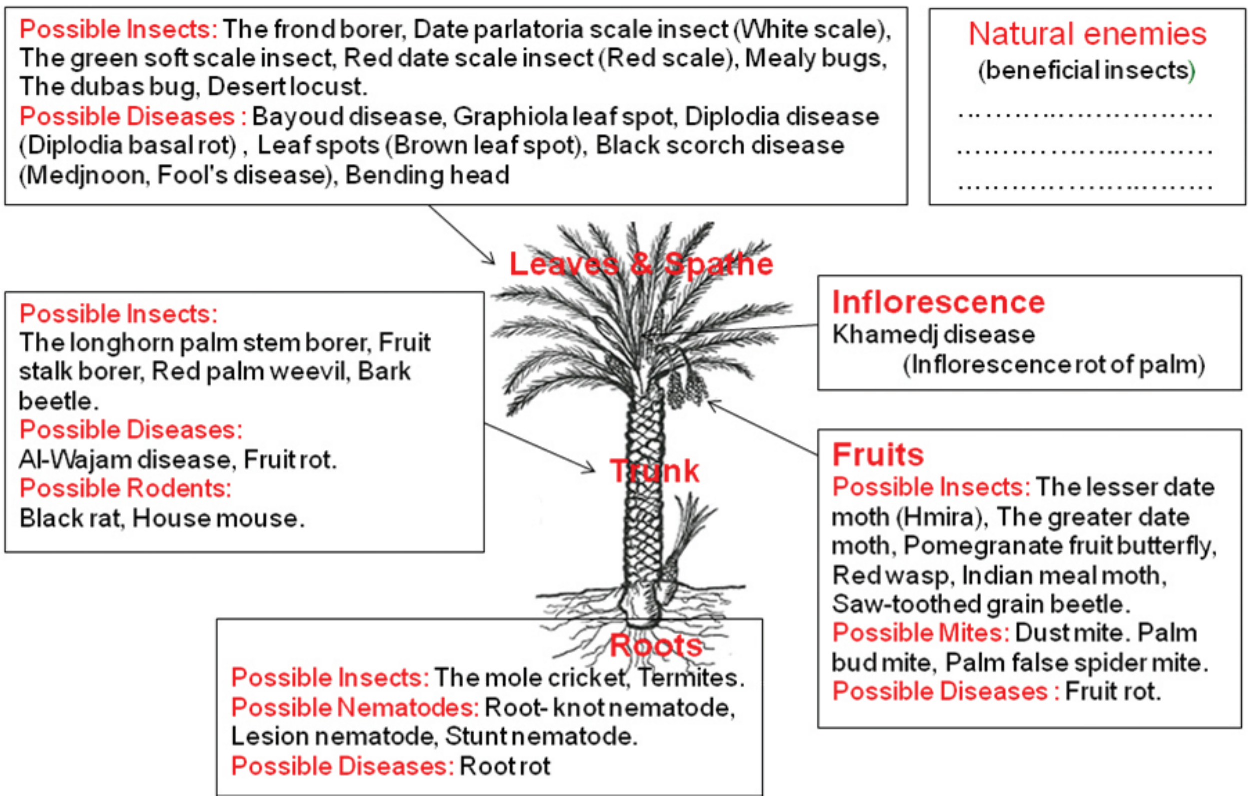

Fig.1A. Agro-ecosystem Analysis Chart for Date Palm

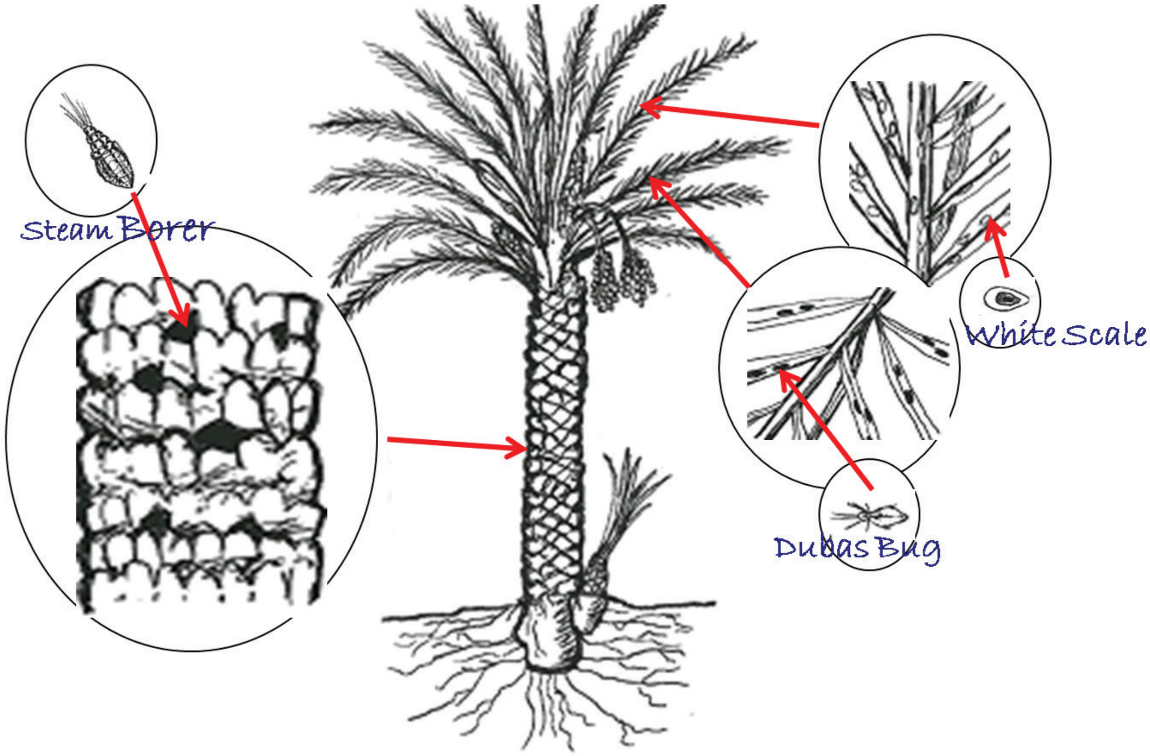

Fig. 1B. Agro-ecosystem Analysis Chart for Date Palm

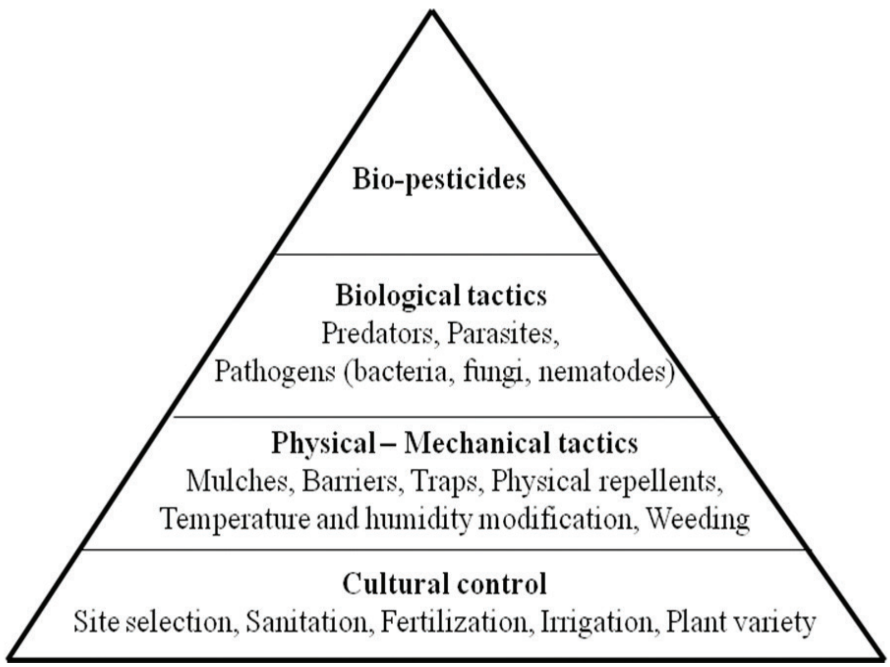

Fig.2. Pyramid of bio-IPM tactics (proactive and reactive options).

Relationship between Iranian male and female date palm elite cultivars by codominant marker (microsatellite)

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ABSTRACT

High and excellent Date production is related to pollen use from elite male stocks, which is called Metaxenia phenomena. Using 8 pairs of SSR primer investigated genetic diversity and relationship between 7 male and 19 female Date palm cultivars collected from throughout Iran. DNA extracted via Sambrock CTABbr2 procedure (1998) with little modification. All the primers could produce bands. Collectively 56 alleles produced with average 7 alleles per locus. No more than 2 alleles per locus found that means Date palm is diploid plant. Mean excepted heterozygosity was 0.723±0.045, mean observed heterozygosity 0.753±0.029 and polymorphism information content (PIC) 0.670±0.049. Inbreeding coefficient (F) mean was -0.346±0.219 showed these cultivars did not obtain by near crossing, and need wide crosses to new elite female or male Date palm cultivar improvement. Based on Nei's distance indices and Ward clustering obtained dendogram separated the studied cultivars into 3 groups that follow the geographical distribution. Distance between males and females were not more than distance within males or females. Therefore could not select proper male stock for specific female cultivars based on their distance. But observed heterozygosity was high for elite male stokes, so can conclude that may select it on its higher heterosigosity. Heterosis of male stock beside establishment of high pollen production potential, increased general combining ability between pollen mass and ovules of spots because its more diverse produced pollens, result to improve fruit quantity and quality that result to Xenia and Metaxenia.

Key words: Date palm, SSR, Iran, Male and Female Cultivars, GCA, Genetic Distance, Xenia and Metaxenia

INTRODUCTION

Date palm tree is perennial monocot, heterozygous, and belongs to the Arecaceae family and is one of the most important horticultural crops in arid and semi-arid countries. Researchers have proved that the type of pollen used for pollination is effective on quantitative and qualitative characteristics of fruits (seeds and edible parts) (1). Most scholars believe that Date palm originated from Mesopotamia (Middle East) or Africa. Therefore, Iranian male stocks probably have lot of diversity. Hamwieh et al (2010) considered distribution palm as function of environmental factors and diverse ecosystems in arid climates throughout many countries. To date many studies had taken on diversity of Date palm cultivars in the world; also, some investigations have been made to determine best male pollinator for famous female cultivars. Billotte et al. (2004) designed 16 primers using Billotte et al. (1999) protocol and were able to show palm polymorphisms across the genome. Mirbabaee et al. (2011) have used a slightly modified Fiasco method to design new 9 primers for SSR positions of Date palm. Arabnezhad et al (2012) using the new AAG and AG-rich repeats SSR markers and adaptation them with the cloned DNA sequence of Date palm were able to design 25 primer pairs, and studied genetic diversity among 16 genotypes from different geographical areas, but only 22 primers were able to demonstrate polymorphism between cultivars. Akkak et al. (2009) identified 41 binary rich repetitive sequence microsatellites from palm gene library, and after screening 17 microsatellite primers, studied 31 cultivars of collected palm trees from California and Algeria. Al-Ruqaishi et al (2007) were used microsatellite markers for screening and analysis of genetic diversity in Date palm genotypes derived from somatic embryos in Oman. Ahmed et al. (2009) were used microsatellite markers for analysis of genetic diversity and relationships among 15 varieties of female palm trees of Qatar. From 16 primers, that have ability to raise transparent bands, 10 primers demonstrated more clearly single bands, but six other primers did not show clear bands. However Hamwieh et al (2010) stated although the typical features such as codominance and high polymorphism, microsatellite markers are less used in Date palm trees.

The first step to improve of Date palm is determination of suitable male for pollination the popular cultivars. Therefore, some efforts have been made around the world, including Iran (Tallaie A. R., Panahi B. 1997; MirShekari A., Hassan-Pour A. 2001; Jahan-Tigh A., Panahi B. 2009). However, these researches even fail to cover important commercial cultivars of key Date producer areas. In addition to hardness of working in the key Date production areas, because too apparent similarity between palm cultivars is difficult to distinguish them based on morphological properties. According to classical breeding theory, the distance between the male and female may results in more production due to more consistent heterosis, that proposed a hypothesis to determine the proper males. But, this hypothesis is not checked base on genetic relationship between known compatible male and female yet. In other hand, a proper male must to have characteristics such as earliness and high pollen production. Also between molecular methods only codominant ones that are able to identify the exact nature of diversity and relationships between different genotypes. In this order, SSR markers were used in this study to investigate the relationship between Iranian male pollinator and popular Date palm female cultivars beside their variation.

MATERIALS AND METHODS

Plant materiel: In this study, seven known pollinator male and 19 cultivars of commercial females has been collected from the biggest Dates producer provinces of the country, including Khuzestan, Kerman and Sistan and Baluchestan (Fig 1).

Total genomic DNA extraction: The young leaves (which are white or yellow) washed with sterile water to remove the wax. About 200-300 mg of leaves, washed and chopped fine grinding in a mortar with liquid nitrogen, then DNA was extracted follow Sambroke et al. (2004) protocol with some modification. The extracted DNA was dissolved in sterile water and stored at refrigerator temperature for 18 hours. Quantity and quality of DNA were determined using a Nanodrop spectrophotometer and 1% Agarose gel.

PCR and SSR amplification: Amplification reaction was done by Bio-Rad thermal cycler in a volume of 25 μL containing 80-60 ng genomic DNA, 2.5 ml 10xPCR buffer, 0.7 ng MgCl2, 1 mM dNTP, 1 unit Taq enzyme and 1 nM primer. Thermal cycles composed a cycle initial denature 95˚C, 35 round include 30 seconds 95˚C denature, 30 seconds annealing temperature for each primers (Table 1), 45 seconds 72˚C extension temperature, and finally one cycle of 10 min at 72°C extension temperature. Then produced segments separated on polyacrilamid gel 0.8%.

Data analysis: After determining the bands length, data scored based on the presence (1) or absence (0) and saved in Excel. The distance matrix computed on Nie genetic similarity coefficient, diversity indices (Table 2) calculated by GenAlEx6.2 software and phylogenetic tree plotting by SAS based on Ward procedure.

RESULTS AND DISCUSSION

All eight pairs showed high levels of polymorphism with total 57 alleles. Billotte et al (2004) with 16 primers estimated polymorphism rate of about 76% and the number of alleles at each locus around 14. Akkak et al (2009) with 17 primers on 31 cultivars obtained average number of alleles 6.4 per locus, and observed polymorphism 63%. Arbnejad et al (2012) with 22 primers in 16 cultivars estimated 106 alleles overall, with an average of 4.8 alleles per locus, and polymorphism 67%.

Based on the Nie similarity coefficient, no resemblance was seen between Astaamran and Halavy, or Zahdi and Rabbi, which all four cultivars belong to the Khouzestan province and adjacent areas. Most similarity (0.671) was between Ashgar from Khouzestan and Golgoly belong to Sistan and Baluchistan. Cluster analysis (Figure 2) divided the clones into 3 groups. Male Verdi, Astaamran, male Samesmavy, male Ghannami, male Jarvis, Barhi and Zahedi were in first group; most cultivars such as male Sabzparak, Almehtery, male Jalogh1, Halili, male Fenouch, Ashgar, Golgoly, Hemravy, Shkar, Majoul, Mazafati and Rabbi in second group; and cultivars like Barim, Jouzi, Helavy, Gantar, Khadzravy, Dairi and Deglet-Nour in third group. Apart from important cultivars like Deglet-Nour with core from southern Algeria and Majoul from Morocco, according dendrogram could conclude that its distribution corresponded roughly with geographic dispersion. However, the cultivars of Khousestan and adjacent areas divided into two quite distinct groups, and Kerman and Sistan va Baluchestan cultivars laid in a group between this tow. Because the same soil and climate, farmers of this two adjacent province have been swapping the same genetic palms. Arbnejad et al (2012) based on Nie genetic distance analysis clustered 16 cultivars into three major categories distinguished Africans, Iranians and Iraqians. Kheirallah et al (2013) divided 30 Iraqi cultivars into two major categories by bootstrap method, one of which was divided into 3 subgroups.

Analysis of molecular variance (AMOVA) showed a difference of about 5 percent between male and female palms, while internal variance was close to 95% in both groups (Table 3). Comparison of genetic variances (Table 4) also revealed there is no significant difference between male and female cultivars in terms of genetic background, and as Pournabi et al (2011) showed there is only one DNA band difference between male and female Date palms. Also in dandogram, Zahedi and Astaamran females, Verdi, Smsmavy, Ghannami and Jarvis males were with each other in one cluster also, but Rabbi and Halavy females were in opposite cluster. While studying Khierallah et al (2013) was put two versions green and yellow Ghannami in two distinct clusters on both sides and apart from females cluster. These issues show that superior males like Ghannami may have no far distance with particular females like Astaamran, or near to some of them like Halavi. Therefore, farness or closeness of the genetic distance does not cause performance of a male for a specific female. So, the more distance between males and females to choose the superior male hypotheses seems incorrect.

Mean of observed heterozygosity was 0.724 and excepted was 0.759 (Table 5). Akkak et al (2009) estimated expected heterozygosity 66%, observed 50%, Arbnejad et al (2012) showed average expected heterozygosity 72%. As observed, heterozygosity rates estimated in this study was over than previous researches that was not less than 50%. This level of heterozygosity in Date palm is unique among plants, which is caused by its dioecious nature and have maintained via somatic proliferation.

Comparison of observed heterozygosity (Table 5) showed there is no significant difference between male cultivars with 0.661±0.111 and female with 0.787±0.041. Observed heterozygosity levels were in most favorite male (Ghannami) 0.875, in Vardi and Fanouch 0.750, in Jarvis and Sabzparak 0.625, in Samesmavi and almost unknown cultivar Jalough 0.500. Heterosis superiority of the male cultivar in addition to its ability to produce more pollen, with more diverse pollen grain production allowing it to increase general combining ability (GCA) between the pollen and ovum, cause improvement in quantity and quality of the fruits. Because, Because, more heterosis in male cause greater variation in pollen, create a competitive situation that increases the possibility that from the 3 ovules ready to insemination, pollen fertilize which have a greater genetic distance. Thus, Xenia and Metaxenia phenomena in the stone and fruit level will cause increase to quality and production. Therefore, when choosing the male stock must select clones with heterozygosity rate more than 0.75.

Observed heterozygosity levels in the number of preferential trading cultivars such as Mazafati, Braim, Majool and Ashgar was 1, in Astaamran, Barhi, Khadzravy, Dglet-Nour, Dairee and Gantar was 0.875, in Shakar, Halili and Golgoly was 0.750, in Halavy, Hamravi and Zahedi 0.625 and in Almahtary, Jouzie and the Rabbi was 0.750 (Table 6). As seen, the quality and adaptability level of female cultivars will be reduced, when there is decrease in amount of heterozygosity. Also, It seems females that have more heterosis, addition to better growth and production, with more diverse ovule production, create better competitive conditions for diverse pollen grain reception. Therefore, when selecting the female cultivars also, must choose one that has greater heterosis.

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References

Ehdaie B., 1976. Plant Breeding, 1st ed., Jondi Shahpour University Publication, Ahwaz, Iran, page 271-272. [in Persian]

Ahmed A., Asmaa A., Al-Qaradwi V., 2009. Molecular phylogeny of Qatari date palm genotypes using simple sequence repeats marker. Biotechnology, 8(1): 126-131.

Akkak A., Scariot V., Torello Marinoni D., Boccacci P., Beltramo C., Botta R., 2009. Development and evaluation of microsatellite markers in *Phoenix dactylifera* L. and their transferability to other Phoenix species. Biologia Plantarum, 53(1): 164–166.

Al-Ruqaishi I.A., Davey M., Alderson P., Mayes. S., 2007. Genetic relationships and genotype tracing in date palms (*Phoenix dactylifera* L.) in Oman based on microsatellite markers. Plant Genetic Resources,6(1): 70–72.

Arabnezhad H., Bahar M., Mohammadi H.R., Latifian M., 2011. Development, characterization and use of microsatellite markers for germplasm analysis in date palm (*Phoenix dactylifera* L.). Scientia Horticulturae, 134:150–156.

Billotte N., Lagoda P.J.L., Risterucci A.M., Baurens F.C., 1999. Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. Fruits, 54(4): 277–288.

Billotte, N., Marseillac N., Brottier P., Noyer J.L., Jacquemoud-Collet J.P., Moreau C., Couvreur T., Chevallier M.H., Pintaud J.C., Risterucci A.M., 2004. Nuclear microsatellite markers for the date palm (*Phoenix dactylifera*L.): characterization and utility across the genus Phoenix and in other palm genera. Molecular Ecology Notes, 4: 256-258.

Elmeer K.H., Sarwath J., Malek J., Baum M. Hamwieh A., 2011. New microsatellite markers for assessment of genetic diversity in date palm (*Phoenix dactylifera*L.). 3 Biotech, 1:91–97.

Hamwieh A., Farah J., Moussally S., Al-Sham K., Almer K., Khierallah H., Udupa S., Lababidi S., Malek J.A., Aaouine M., Baum M., 2010. Development of 1000 microsatellite markers across the date palm (*Phoenix dactylifera* L.) genome. Acta Horticulturae, 882: 269-277.

Jahan-Tigh A., Panahi B., 2009. Study effects of five male date cultivars pollen on chemical properties of Madhafti of Souran date. Proceeding of 6th Iranian Congers of Horticulture Sciences, Abstract. [in Persian]

Khierallah H.S.M., Bader S. M., Baum M., Hamwieh A., 2013. Genetic diversity of Iraqi date palms revealed by microsatellite polymorphism. J. Amer. Soc. Hort. Sci. 136(4):282–287. 2011.

Mirbabaee S.A., Mardi M., Mahmoodi P., Pirseyedi S.M., Abbasi A., Farsi M., Soleimani H., Bakhshikhaniki X., Mohajeri-Naraghi S., Zeinolabedini M., KHayam-Nekouei S.M., 2011. Development of now microsaellit marker from an enriched genomic library of date palm (*Phoenix dactylifera* L.). Horticultural Science and Biotechnology, 86(5):539-54.

MirShekari A., Hassan-Pour A., 2001. Study effects of pollen sources on physical and chemical properties of Shahani of Jahrom date. Journal Iranian Agriculture Sciences, 24(2): 119-127. [in Persian]

Sambrook J., Russel D. W. 2001. Chapter 6, Protocol 7; Rapid Isolation of Yeast DNA, in: Molecular Cloning: A Laboratory Manual, 3rd edit. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp: 559-562.

Tallaie A. R., Panahi B., 1997. Effect of pollen on physical properties of Madhafti of Jiroft date. Journal Iranian Agriculture Sciences, 28(1): 11-16. [in Persian]

Tables

Table 1. Primers characteristics

Locus	Length (bp)	Repeat motif	Sequence 5′→ 3′	Ta (˚C)	Refr.
PDAG1006	199-250	(CT)22	F:GCCACAGGAAGCACATTTAG R:CCACACCTTAATCACAAACTCC	51	4
PDAG1005	377-450	(AG)10	F:GTATGTTCCATGCCGTTCTAC R:AGCCACATCACTTGGTTCA	51	4
PDAAG1023	213-240	(AAG)10	F:AGACGCTCACCTTGGAACTT R:ACCCCGCTCATGAATTAGG	54	4
PDAAG1025	214-250	(AAG)15-A6-(AAG)3	F:CTTCTCCACTGGCATCTTCC R:CACCCGTTGGGCATCTTA	53	4
DP169	180-220	(AAT)12	F:GCATGGACTTAATGCTGGGTA R:GGTTTTCCTGCCAACAACAT	54	7
DP172	199-240	(AGG)11	F:GGTGTTTGGGCCTATTTCCT R:GTCCCTCCTCCTCTGTCC	56	7
PDCAT14	140-160	(TC)19(TC)16	F:TGCTGCAAATCTAGGTCACGAG R:TTTACCCCTCGGCCAAATGTAA	57	2
mPdCIR044	280-332	(GA)19	F:ATGCGGACTACACTATTCTAC R:GGTGATTGACTTTCTTTGAG	47	5
F: forward and R: reveres primer					

Table 2. Diversity indices formulas and description

Na = No. of Different Alleles
Ne = No. of Effective Alleles = 1 / (Σ pi2)
I = Shannon's Information Index = -1 x Σ (pi x Ln (pi))
Ho = Observed Heterozygosity = No. of Hets / N
He = Expected Heterozygosity = 1 - Σ pi2
UHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) x He
F = Fixation Index = (He - Ho) / He = 1 - (Ho / He)
Fis = (Mean He - Mean Ho) / Mean He
Fit = (Ht - Mean Ho) / Ht
Fst = (Ht - Mean He) / Ht
Nm = [(1 / Fst) - 1] / 4
Where pi is frequency of the ith allele.

Table 3. AMNOVA of SSR marker for Iranian male and female clones

Source	df	SS	MS	Est. Var.	%
Among Sexes	1	10.776	10.776	0.369	5%
Within Sexes	24	167.955	6.998	6.998	95%
Total	25	178.731		7.367	100%

Table 4. Compression of SSR diversity between Iranian male and female clones

Sex	df	SSWP	MSWP	F	Pr > F
Male	6	44.42857	7.404762	1.079007	0.414087
Female	18	123.5263	6.862573		

Table 5. Mean and SE over Loci for each Sex

Pop	Male		Female		Total	
	Mean	SE	Mean	SE	Mean	SE
Na	5	0.327327	6.75	0.559017	5.875	0.385951
Ne	3.83969	0.31284	5.033649	0.451091	4.436669	0.306716
I	1.437645	0.072816	1.70643	0.094511	1.572038	0.067272
Ho	0.660714	0.111109	0.786915	0.040844	0.723815	0.059458
He	0.727041	0.022797	0.787219	0.022953	0.75713	0.017451
UHe	0.782967	0.02455	0.808761	0.023412	0.795864	0.016722
F	0.099964	0.142488	-0.00782	0.06443	0.046073	0.076808

Table 6. Observed hetrozygosity of different Date palm clones

Male			Female					
No.	Clone	Ho	No.	Clone	Ho	No.	Female Clone	Ho
1	Vardi	0.750	8	Istaamran	0.875	18	Dayri	0.875
2	Ghanami	0.875	9	Ashgar	1.000	19	Zahidi	0.625
3	Smesmavi	0.500	10	Almehtary	0.375	20	Shakar	0.750
4	Sabze parak	0.625	11	Barhi	0.875	21	Ganthar	0.875
5	Jarvis	0.625	12	Berim	1.000	22	Medjool	1.000
6	Jealagh1	0.500	13	Jozi	0.375	23	Helaly	0.750
7	Fonoch	0.750	14	Khathrawy	0.875	24	Methafaty	1.000
			15	Helawi	0.625	25	Golgoly	0.750
			16	Hamrawy	0.625	26	Rabey	0.375
			17	Deglet-Nour	0.875			

Figures

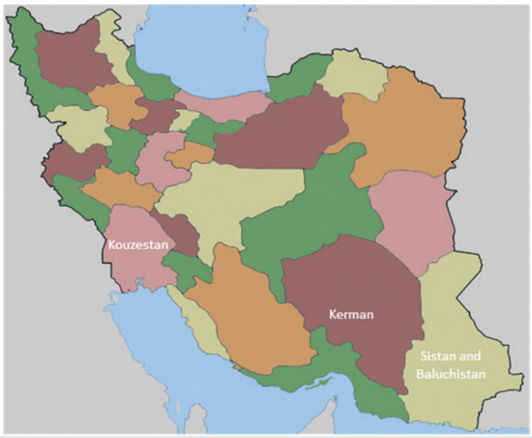

Fig 1. Map of Iranian provinces

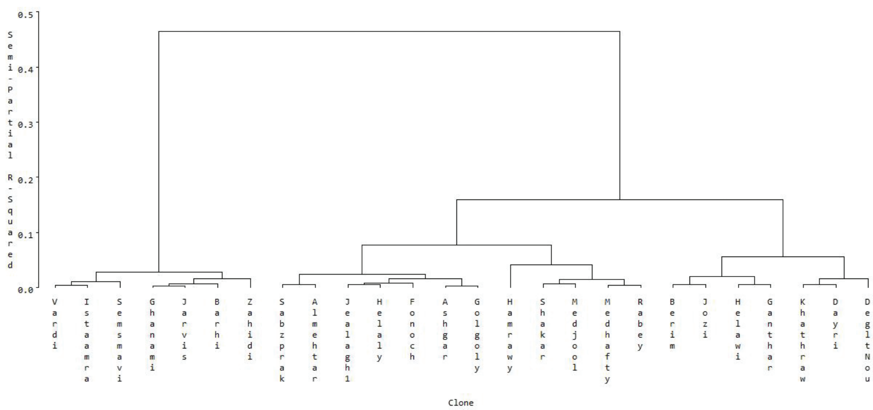

Fig 2. Dendogram of 7 male and 19 female clones of Iranian Date palm