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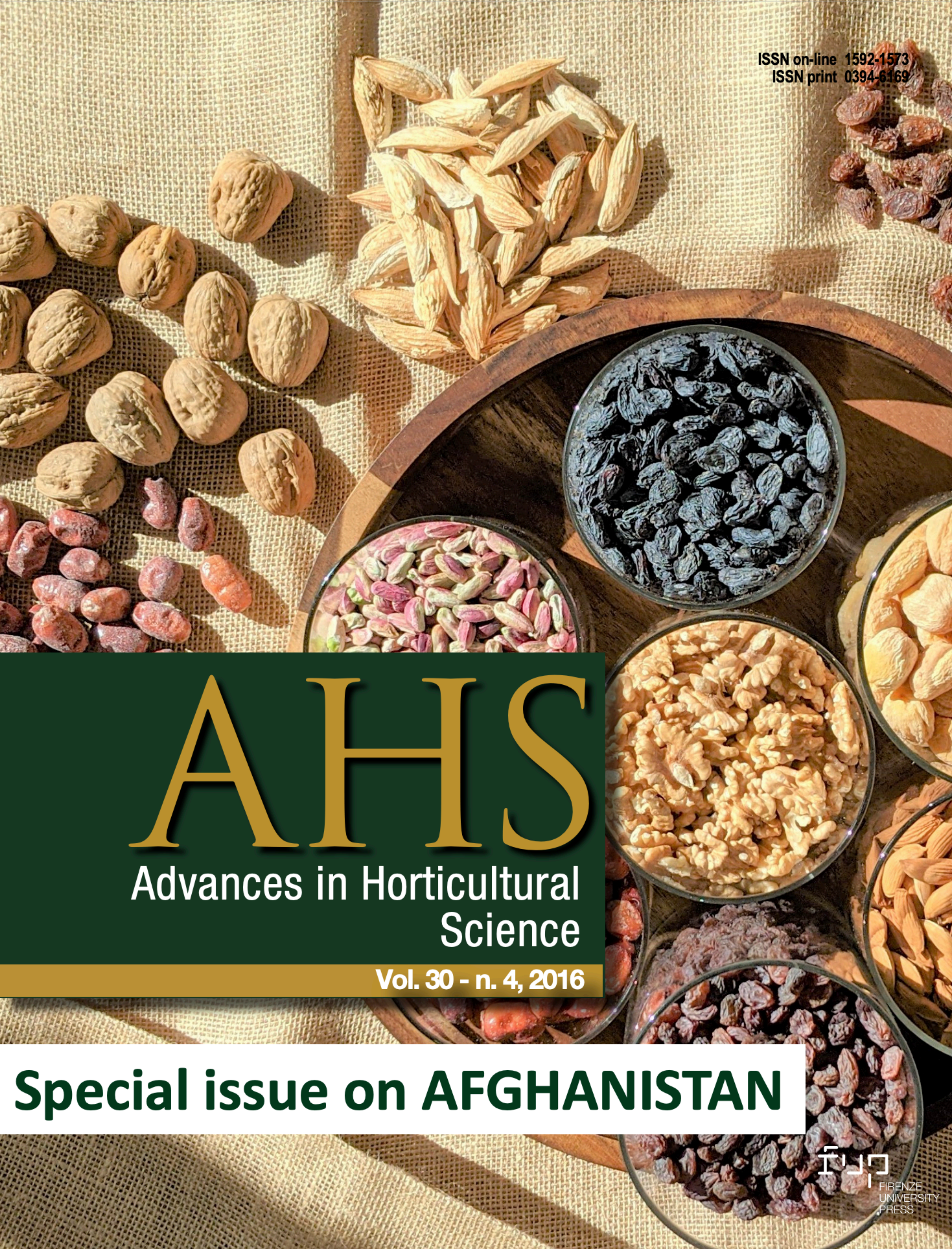

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Foreword

When I was asked to prepare a brief preface to this special issue of the journal Advances in Horticultural Sciences, I gladly accepted because I thought that the topic of the recovery and enhancement of indigenous germplasm of a country so far away and so problematic as Afghanistan was fascinating and full of great potential in various fields of agricultural science and beyond.

While I was reading the articles included in this issue, I heard news of a further attack by Isis on the architectural masterpieces of the city of Palmyra, Syria, on 20 January. The facade of the Roman theater and the spectacular Tetrapylon were destroyed, irretrievably. Although there is no clear link between the two events (the recovery of the fruit germplasm of Afghanistan and the destruction of historical monuments) it set me thinking that these two events are actually part of a single negative picture of modern times that man, consciously or not, is perpetrating against the biological, cultural and artistic gifts that our ancestors have been handing down to us for centuries.

The systematic deletion of the vestiges of the past carried out by Isis is not so different from what decades of uninterrupted war have caused to a country like Afghanistan, which for historical and cultural reasons, is one of the most important points of contact and exchange between cultures and the biodiversity of the East, the Middle East, Africa and Europe. A country which through commercial and military exchanges along the Silk Road, has witnessed a merging of genotypes of many species of agricultural interest, as well, of course, of traditions and cultures belonging to people who had never met before.

It is easy to imagine what kind of genetic variability has been able to generate through these exchanges which allowed the spread, throughout the rest of the globe, of fruit species which today are some of the most cultivated worldwide.

In the temperate climates where these crops have since been established, the strong selection imposed by man in favour of genetic or phenotypes traits of production and commercial interest has meant that, over the centuries, the gene pool of these species has been increasingly shrinking. This became particularly acute when specialized vegetative propagation began to be used through grafting techniques in order to obtain almost total uniformity in the reproduced trees and the fixing of those positive characters that are essential from an economic point of view.

However in an area like Afghanistan, these species have survived, as witnessed by the Authors of the articles in this special issue, in an environment in which the technological evolution of agricultural practices has remained almost stationary since the beginning of the last century. In this environment selective processes have taken place that are much closer to the natural processes than to those imposed by man for cultivation purposes.

Given that Afghanistan is a country rich not only in contradictions, but also in geomorphological variability, soil and climate, the strategic importance of the conservation of its biodiversity is clear. This country, which is considered by many as 'the land of dust', with extreme climatic conditions, both in terms of drought and flooding, high and low temperatures, and a topography with considerable altimetric changes, has led to an evolutionary process of adaptation to these conditions on the part of both cultivated and non-cultivated species.

These conditions thus suggest the presence of genes that can resist extreme climatic conditions, which may be a key to the future adaptation of fruit tree species grown in temperate climates to the progressive and unstoppable changes that climate changes are causing.

Unfortunately, geneticists from around the world have established that the fruit tree species grown in temperate areas no longer have, and maybe have never even had, a gene pool that would lead to improvements in the resistance abiotic stress that has become increasingly frequent.

Hence the vital importance of participatory research programs such as those described in this special issue.

And here again lies the analogy between the destruction of the archaeological sites and the gradual loss of irreplaceable biodiversity that has been handed down for generations and generations: the loss of this heritage permanently eliminates the future possibility of re-establishing a balance between us and our environment and achieving harmony between the people, nature and history of a country.

It is difficult, however, to assume that the preservation of this heritage can be obtained through protective measures aimed merely at conservation, without any organized and responsible participation by the local populations. Just as the statues of the Bamiyan Buddhas and ancient books and statues of the Mosul Museum were not saved from the devastating fury of the Taliban and Isis, so too the fragile Afghan germplasm of the fruit tree species would have little chance of survival if its conservation was confined to collection orchards designed like an open air museum but subjected to some nefarious purpose in a country where the emergency of war, combined with the food and economic emergency, leaves little room for investments in an unproductive genetic heritage that cannot actually be exploited by the local people.

The papers in this special issue thus represent an effective model of approach to the problem which, in my opinion, is the only way to reach such a high objective - the protection of biodiversity threatened by genetic erosion. A fundamental part of this approach is also the participation of local people who can then gain a possible means of livelihood and income, providing that they are properly trained and supported both technically and culturally.

This is an enormously difficult process, most importantly due to the need to recover all that knowledge that enabled the older generation to pass on, until a few decades ago, the cultivation and planting techniques of suitable plants for the particular geographical area in question. The gradual abandoning of agricultural practices for food in order to concentrate on the almost universal production of opium poppies has led to the disappearance of several generations of farmers and the almost irretrievable loss of know-how in agriculture. Introducing new and effective cultivation and propagation techniques for fruit species can play a vital role in the reconstruction of an agricultural micro-entrepreneurship, which is both self-sufficient and motivated to succeed.

The challenge for researchers who are attempting this enterprise is very great. We can only hope that their efforts will be rewarded by the expected results so that they can guarantee for tomorrow the survival of our agricultural production if we can keep that genetic heritage that will represent the basis for future breeding programs.

For now we can only wish the greatest success to the researchers in this volume who, with great personal sacrifice, have started working on the challenge with so much enthusiasm and so much hard work.

Good luck and thank you!

Rossano Massai

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From traditional orchards to advanced fruitculture: establishing the bases of commercial horticulture in Afghanistan

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Key words: fruit, germplasm, plant nursery.

Abstract: The Afghan economy is based essentially on the primary sector and, namely, on fruit production. A cross road of trade along the "Silk route", local traditional fruitculture based on the *bagh* (home garden) is widely variegated in terms of species and varieties. Afghan fresh and dried fruits (namely raisins) and nuts, sweet and rich in flavors, are demanded by domestic and foreign consumers. The lack of traceability and quality of the propagation material has been considered one of the basic bottle necks hindering development of an advanced horticulture. During the period 2006-2015 the Perennial Horticulture Development Project (PHDP), funded by the EC-EuropeAid Program, has been supporting the Ministry of Agriculture, Irrigation and Livestock of Afghanistan through a process of collection and selection of local fruit varieties in order to improve the private nursery system, as well as perennial horticulture. About 850 accessions of different fruit tree species have been collected and entered into the National Collections at six PHD Centres, where they undergo a standardised characterisation and identification procedure in order to be formally registered in a national list, hence contributing to their safeguard and protection. The best accessions were used as source for a traced propagation system after phytosanitary indexing and also in numerous adaptive experimentations. The PHDP contributed in capacity building at technical and institutional level, fostering the establishment of nursery sector associations, providing expertise and developing the Afghanistan National Nursery Growers Organization (ANNGO) and the apex Afghanistan National Horticulture Development Organization (ANHDO), mainly concerned with value chains.

1. Introduction

Historical, cultural and institutional aspects

Afghanistan, a variegated and multiethnic country, is the result of 5000 years of continuous interaction between different cultures and civilizations, and conflicts with neighbouring powers. More recently the social, educational, and economic situation of this country has been shaped by a conflict dating back more than 30 years. The effects of war have had an overwhelming negative impact on governmental structures (e.g. Ministry of Agriculture and Universities), causing an almost complete absence of guidance and regulations for promoting agriculture, the leading sector of the Afghan economy. Notwithstanding the destruction generated by the conflict, the rural cultural heritage and social structure has coped with these events better than urban areas where human resources were deeply hit, hence increasing the country's isolation from the international community. At present, material rehabilitation and human capacity building are still a priority in Afghanistan.

Afghan universities in particular were greatly affected by the conflicts to the point that their level of education and research is still below adequate standards. This is mainly due to a lack of financial support (even for physical re-building and the payment of salaries) and a generalized very poor human capacity (with a few outstanding exceptions). Similarly, public institutions such as the Ministry of Agriculture need international support in order to re-assume their expected role in society.

Over 75% of the Afghan people live in rural areas

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and the agriculture sector contributes about half of the GDP of the country. Afghan agriculture needs to grow at least 5% per year over the next decade (World Bank, 2014). This is a big challenge as technology, communication and transport, irrigation, and education are substantially deteriorated due to the conflicts and lack of maintenance.

Both public and private institutions lack physical infrastructure, necessary regulatory frameworks, and skilled staff to build a modern and competitive agricultural sector.

Environment

Two-thirds of total surface of the country (652230 km^2) is made up of mountainous terrain and dry plains with little or no vegetation. Only 12% of the land is ever cultivated due to high declivity and low rainfall, and out of approximately 8 million ha of cultivated land, only about 2 million can be irrigated. Although there are areas rich in high quality groundwater (from melting snow), the irrigation capacity decreases during the summer. A limited number of dams and irrigation schemes were built during the 1950s and the 1960s and efforts are currently on going to rehabilitate water resource infrastructures and extend the irrigated areas.

Water resources are the main limiting factor for agriculture in Afghanistan. Precipitation is limited, ranging between 200 and 500 mm/year between December and March in agricultural areas. Rain-fed agriculture does not exist, except in limited areas where "spring wheat" cultivation is possible.

Though arid and often a semi-desert, the Afghan landscape also forms a gigantic watershed close to the Himalayan and central Asian region, with five major river basins and tremendous underground water resources yet to be fully studied and developed.

Fruit cultivation and crops in general are possible only on irrigated land, hence the typical Afghan landscape of dry, deserted mountains and greenery on the foothills, around aquifers and canals. In the irrigated areas, there is high potential for species diversification.

The climate is harsh in central and northern Afghanistan, with very cold winters and very hot, dry and, in many areas, windy summers. Nevertheless, the most common temperate fruit species are grown from 250 m up to 2-3,000 m above sea level. Citrus and similar evergreen species can be grown in parts of eastern Afghanistan; southern areas have generally mild winters. High mountain chains divide the country into hundreds of deep, narrow valleys with specific microclimates, each one with different typical fruit varieties, which are essentially determined by the interaction between altitude and orography. A good indicator of this is the yearly mean number of frost-free days which ranges from 137 in the Salang area (elevation of 3172 m), 188 in Gazni (2183 m) to 315 in Jalalabad (566 m) (College of Agricultural and Environmental Sciences, 2003).

The land tenure

The land tenure in Afghanistan is extremely diversified, but characterized by fragmentation and a prevalence of smallholdings and subsistence farming (see table below). Almost 30% of the irrigated land is owned by 70% of the farms with less than 5 ha. These include 11% of the land and 45% of the owners with less than 2 ha. However, farm owners of more than 10 ha own 70% of the irrigated land (Table 1) (Maletta and Favre, 2003). The generally small size of the farms is making it problematic to achieve an economy of scale. Nevertheless, as we have seen in many parts of Europe, it could be possible to mitigate this constraint by promoting producers' associations. That is, whenever safety and the social and economic conditions make it possible as the path towards farmers' associations is extremely difficult in the present context.

Table 1 - Distribution of irrigated land in Afghanistan

Farm size (ha)	Farms (%)	Land (%)
>50 Ha	1	15
20-49.9	5	19
10-19.9	9	18
5-9.99	14	18
2-4.99	26	19
<2 Ha	45	11

Commercial horticulture is currently taking place, prevalently in small size orchards often with mixed production, which is a handicap as the market requires high quality and standardization of productions. However, the conditions exist for even small, commercially oriented farmers to plant orchards of commercial size (i.e. 1 ha or more) with the right commercial varieties on suitable rootstocks, now provided by the Afghanistan National Nursery Growers Organization (ANNGO) certified nurseries. These factors, along with the promotion of producers' associations could render Afghan fruit growers much stronger and competitive in the domestic and international market.

The development of horticulture in the general agricultural context of Afghanistan

For many centuries, the livelihood of the Afghan people has been associated with the cultivation of fruits and nuts and this has become an intimate part of their life, food, culture, and identity. What is today's Afghanistan developed at a cross road of the trade along the "Silk route", having a comparative climatic advantage over its neighboring countries in hot and humid South Asia and very cold Central Asia. These became - and still are today - the natural outlets for Afghan fruits and nuts.

Afghanistan's strategic position explains the broad diversification with many temperate fruit species which originated in the Middle East, and central and eastern Asia, as confirmed by old records showing a strong traditional linkage between the Afghan people and fruits (fresh and dried fruits and nuts). For instance, Qasem Ebne Yousof Abunasre Herawi, in his "Guide to agriculture" of the XIV Century, mentioned over 100 types of grapes for the area of Herat. *Bagh* is the word meaning "home garden" - an area usually protected by clay walls, forming a special micro-climate - where a wide range of species and varieties are grown (often from seed) and from which most local varieties originate. *Bagh* also mean specialized mono-specific orchards that supply most of the fruits. Scattered trees disseminated in community areas also consistently contribute to fruit production (e.g. nuts like pistachios and seldom "wild" pomegranates).

The main perennial fruit species cultivated in the country are grapes (fresh and raisin), almond, pomegranate, apricot, plum, pistachio, citrus (potential "niche"), olive (potential "niche"). The main annuals (vegetables, excluding pulses and cereals) cultivated are onions, potatoes, cumin, and melon, and other cultivated vegetables (including tomato, cauliflower, broccoli, spinach, lettuce, chilli, pepper, okra, cucumber, watermelon, etc.) (College of Agricultural and Environmental Sciences, 2003).

Afghan consumers are eager for very sweet, extremely low acidic and tasty fruits; the sunny, dry weather contributes to the necessary requirements and facilitates fruit drying and conservation. Fruits represent a valuable product both for subsistence and profit. Fruit and nut production in 2014 reached 1567000 t (Fig. 1), representing more than 16% of the entire primary production of this country, which confirms Afghanistan's vocation for fruitculture, nevertheless yields are very low if compared to those of countries with advanced fruit-growing systems (even if hardly comparable because of inconsistency of statistical data). For instance, Afghan yield is about 38% of that of Italy for peaches and nectarines. These figures point to problems in terms of fertility, production systems and cultural practices. The adoption of innovative strategies to increase yield and to reduce looses during post-harvest management and marketing, two issues far for being exhaustively afforded and solved, is a must in order to face the high growth rate of the population (average of 3.2%, against 1.2% of the world in the last ten years).

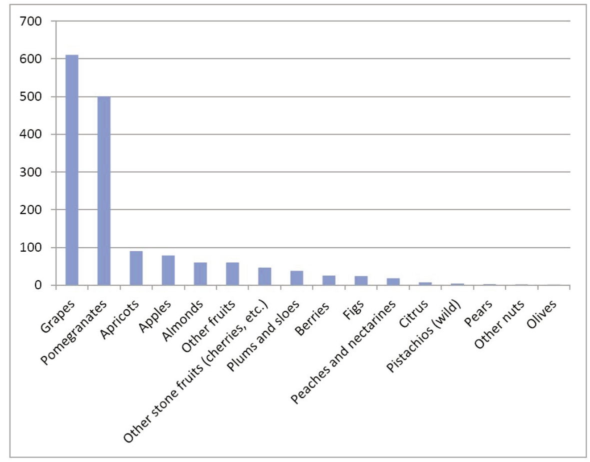

Fig. 1 - Fruit production of Afghanistan in 2014 (1,000 metric tons). FAO, 2016; other sources.

In the 1970s the production of fruit and nuts was estimated as 34% of the total export of Afghanistan and agriculture as 51% of the total GDP (Guimbert, 2004), while exports were only 5.2% of the total GDP (underlining that most of the agricultural production was consumed in the country, thus the importance of the internal market).

Over the last 35 years, following the Russian invasion and internal conflicts with foreign interventions, the production and export of fruits and nuts has continued, but Afghan exports lost ground and the important segment of the international market (i.e. EU and USA) was taken by competing production from Iran and Turkey, which attained higher quality standards.

In the last ten years, efforts to revive horticulture have been ongoing with the support of various donors including the EU, WB and USAID. Between 2006 and 2015, Afghan trade regained some of the lost ground and Afghanistan became the seventh largest exporter of raisins (US$ 150 million) and the eleventh largest exporter of almonds (US$ 110 million) (World Bank, 2011). Fruits and nuts constitute 35% of Afghanistan's total exports. This sector still has great potential for further growth and increased income for rural households.

Today, the estimated area invested in horticulture is 340,000 ha, representing 14% of the total irrigated land. Around 2 million people are involved in horticulture to varying degrees and the contribution to the GDP is estimated at 1.4 billion USD (34% of the agriculture GDP contribution and 6.7% of total) (Pain and Jensen, 2014; World Bank, 2014).

Brief description and observations regarding the present cropping pattern

Although Afghanistan is in the midst of a major shift towards commercial agriculture, the general cropping pattern is still wheat-based. For a long time, government and international donors have subsidized wheat seed, based on concerns for food security. The very idea of investing part of the land in fodder production is not even an option for most of the rural families, which own or manage a small farm and are mostly focused on subsistence needs. The needs for animal feed are fulfilled through grazing, crop residuals or agro-processing by products (such as cotton and flax), kitchen waste or imported industrial feed (for stall fed animals). Wheat straw is largely used as a fodder (often mixed with Urea or seed cakes) and it has a market of its own. Most of the limited areas invested in fodder crops are intercropped with fruit trees. Because of the importance of livestock in the rural economy, the demand for fodder is extremely high and its production can be an income generating activity, as we can see in various parts of the country were fodder like fresh alfaalfa is sold in bundles in bazaars.

There is an important economic consideration that concerns the commercial farms. Afghanistan has entered into the global market and could import wheat from regions that produce at a lower cost and have a surplus (namely the Pakistani Punjab, one of the largest grain producing areas in the world). The economic return of an orchard in Afghanistan can be four to five times higher than one of the same area invested in wheat production. Moreover, the cost of wheat production in Afghanistan is much higher than in Punjab or other traditional wheat exporting areas. Therefore, the investment in temperate fruit production is a natural choice for commercial farms in Afghanistan, rather than trying to compete in areas where they are at a disadvantage. It is interesting to note that this is a very fortunate circumstance in which economic, climatic and environmental considerations join hands.

As cropping patterns are the result of economic and social factors that have operated for centuries, changes are not easy. However, a shift towards commercial agriculture is in progress and in the near future we can expect that economic factors will drive the most progressive farms towards a greater investment in horticulture, with change and rationalization of the cropping pattern. Yet the process may be very slow or limited to a few farms or the private sector may adopt a short-term vision and neglect the important environmental factors.

Here lies the important role of the public sector to accelerate and incentivize the process. The Ministry of Agriculture and various donor agencies and development projects could consider introducing in development packages promotion and incentives for changes that rationalize the cropping pattern in order to fully exploit the commercial potential of Afghanistan's agriculture and at the same time mitigate, halt, and reverse ongoing soil fertility degradation.

Opportunities and constraints of Afghanistan fruitculture

The main opportunities and positive factors for development are: comparative climatic advantages in the region (dry and hot spring/summer), long tradition of horticulture and reputation of Afghan varieties, opportunity to replace growing imports in domestic market (World Bank, 2014); opportunity to increase yield and quality through adoption of suitable rootstocks and varieties, intensive cropping systems and suitable orchard management practices; high demand for quality fruit saplings; rich genetic diversity. Furthermore the development of horticulture is part of the national development strategy and supported by international donors, few private agribusinesses are starting to invest in quality control, and more farmers associations are under formation. Notwithstanding security issues, the presence of consistent international donors developing projects in the primary sector guarantees an emergent agriculture.

The main constraints of the Afghan fruit-culture can be summarized as follows: small land holdings, small commercial orchards, lack of standardized product, poor orchard management, low yield, lack of infrastructure and substandard storage, sorting, packaging, marketing, transport facilities, high cost of quality inputs, low quality standards, inadequate public services (extension, applied research, metereological services), growing imports to fill higher standards demand in main cities, poor range of varieties in vegetables and lack of applied research to extend marketing opportunities and marketing season, define cropping calendars, etc.

2. The Perennial Horticulture Development Project (PHDP I and II)

Origin and objectives of PHDP

During 2002-2003, the Italian Cooperation funded FAO through a grant for the restoration of the Afghan germplasm. Shortly thereafter, the FAO staff approached the European Commission for financial support and in 2004 the European Commission Delegation organised a feasibility study focused on the development of the Ministry of Agriculture, restoration of the old mother stock nursery systems, and some germplasm collection. Actually the subjects involved were: a) germplasm collection and nursery development; b) clean germplasm systems; c) provision of facilities for the development and testing of new varieties and new growing systems of horticultural crops; d) training programmes for implementing agencies; training for senior extensionists/MAIL staff/farmers/traders; linkages between MAIL and universities; and e) development of an integrated horticultural research and technology transfer system.

The objectives of the Perennial Horticulture Development Project were defined in the overall scheme of a number of horticultural development projects taking into account the Agriculture Master Plan (7), following a design aimed at emphasizing the involvement of the Ministry of Agriculture, Irrigation and Livestock (MAIL). The included objectives were the maintenance of reference collections, research and extension production methods and regulation of the industry, backed up by appropriate analytical laboratories and an inspection and quarantine system, and minimizing MAIL involvement in the production of fruit trees and in the development of mother nurseries. As stated in the project web pages "The specific objective of the project is to develop a demand oriented and export led perennial horticulture industry". A major purpose of the project was the development of the nursery sector to strengthen and to qualify fruit production, by establishing a traced nursery system based on the propagation of true-to-type local varieties.

Funding and partnerships

In 2006 the European Commission-EuropeAid Program funded, through a public/private consortium formed by IAK Agrar Consulting GmbH, AHT GmbH and the University of Florence-Italy, the Perennial Horticulture Development Project (PHDP) (www.afghanhorticulture.org) in support of MAIL. A second phase was supported by the EC for the period 2010-2015 to a wider consortium (Agriconsulting SpA, Department of Agri-food and Environmental Science - University of Florence, Department of Agricultural Sciences - University of Bologna, Centro Attività Vivaistiche, Landell Mills UK) with the contract "Technical assistance to MAIL to strengthen the planting material and horticulture industry in Afghanistan (EuropeAid/129-320/C/SER/AF/2)".

Activities related to the germplasm collection

The framework comprised the following steps: a) individuation of superior trees in productive orchards; b) cataloguing and definition of the *in situ* National Collection; c) propagation from the *in situ* original mother plants; d) establishment of the *ex situ* National Collection; e) characterisation and evaluation; f) foundation of traced and clean mother stock nurseries (MSN) (Fig. 2). The concept underly-

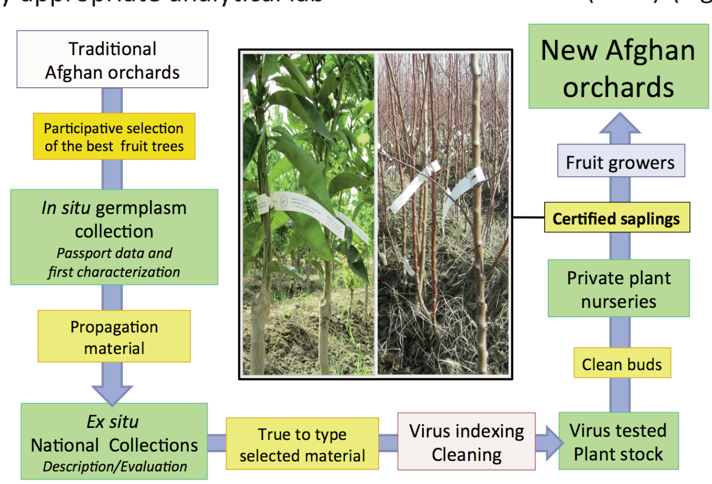

Fig. 2 - Scheme of PHDP germplasm activity: from the collection in the traditional mixed bag to the establishment of new specialized orchards. Source: PHDP.

ing all the activities can be summarized with the call for a meeting with local nurseries and fruit-growers: *It is the intention of the PHDP to undertake various initiatives that create and develop a system that ensures the availability of the best possible planting material for the perennial horticulture industry in Afghanistan, and to ensure the long term sustainability of such a system owned by the private sector.*

Support activities, capacity building and sustainability

The workplan included the establishment of six centres (the main one located at Badam Bagh - Kabul, the others in Herat, Jalalabad, Kandahar, Kunduz and Mazar-e-Sharif) and a Laboratory of Biotechnology for plant indexing and micropropagation. Long term sustainability involved essentially handing over the system built up by PHDP to the local staff of MAIL and of two organizations, the "Afghan National Horticulture Development Organization" (ANHDO) and the "Afghan National Nursery Growers Organization" (ANNGO)" (Giordani *et al*., 2014). Apart from the described objectives, PHDP activities were related also to public and private sector institutional and regulatory reform and training and extension.

PHDP main results

A previous publication described the main goals achieved by PHDP up until 2013 (Giordani *et al*., 2014). In the present paper, the authors summarise the achievements of the project once it was officially completed (December 31, 2015). More detailed information is available on the web (afghanhorticulture.org).

Table 2 - Number of accessions belonging to the *ex situ* National Collection of Fruits and Nuts of Afghanistan located in the six PHDP Centers

Species	Location of PHDP Centers	Number of accessions
Almond	Mazar and Kunduz	106
Apricot	Kabul and Mazar	128
Apple	Kabul and Kunduz	81
Pear	Kabul and Kunduz	53
Sweet and Sour Cherry	Kabul and Herat	28
Japanese and European Plum, Myrobalan	Herat and Kandahar	77
Peach	Herat and Kandahar	116
Grape	Herat and Kandahar	139
Pomegranate	Kandahar and Jalalabad	79
Fig	Kandahar and Jalalabad	16
Citrus	Jalalabad	66
Date palm	Jalalabad	6
Loquat	Jalalabad and Kandahar	12
Persimmon	Jalalabad	24
Olive	Jalalabad	10
Total		935

Source: PHDPII Report - Agriconsulting.

The national collection of fruits and nuts of Afghanistan

After the surveys of 2006-2008 in the most important areas of fruit production when the best and most "profitable" varieties were chosen together with fruit growers (who were recognized as "custodial" of the *in situ* selected accessions), an *in situ* collection formed of over 850 accessions was defined. *Ex situ* collection orchards were established in the six PHDP Centers in plots with six to ten replicates; they constituted the National Collection (NC) (Table 2). Among fruit species, only citrus, loquat and persimmon accessions, donated by CRA-Centro di ricerca per l'agrumicoltura e le colture mediterranee - Acireale (Italy) and IVIA-Valencia (Spain) have been almost completely imported and placed in Nangarhar valley (Jalalabad Province) because of its warm climatic conditions. Some accessions of cherry, peach, plum, apple, and pear were recovered from plants derived from previously imported propagation material. On the other hand, apricot, almond, grape, and pomegranate varieties were mostly collected locally. The standardised procedures of characterisation (e.g. adoption of internationally recognized specific descriptor lists for the different species) started in 2009 and up to now around 80% of all collectable data have been inserted in a database (Table 3).

Table 3 - Status of characterisation of the accessions collected in the National Collection of Fruits and Nuts of Afghanistan

Species	Progress in description and characterization
Almond	56 varieties fully described. Register printed and distributed
Apricot	72 varieties fully described. Register printed and distribute
Pomegranate	78 varieties fully described. Register under translation
Plums	50 varieties fully described. Register in preparation
Peach	82 varieties (description almost completed)
Cherries	24 varieties fully described. Register under translation
Grape	60% of description completed
Apple	50% of description done; started description of fruits
Pear	50% of description done; started description of fruits
Citrus	Fruit description in process (only for first evaluation purposes)
Fig	in progress
Loquat	in progress
Persimmon	in progress
Date	in progress
Pistachio	Not started yet, accessions recently introduced
Olive	Not started yet, accessions recently introduced

Source: PHDPII Report - Agriconsulting.

After completion of the description and individuation of true-to-type and unique identified local varieties for each species, the accessions, as previously done for almond and apricot (Ministry of Agriculture, Irrigation and Livestock, 2014, 2015), will be registered by the Ministry of Agriculture, Irrigation and Livestock of Afghanistan. Actually, the NC represents both a repository of local germplasm and the officially guaranteed source of material to establish the Mother Stock Nurseries (MSNs) in different parts of the country.

Capacity building

An operative Laboratory of Plant Biotechnology (LBP) has been established in Kabul through scientific, educational and technical collaboration of the Department of Agri-Food and Environmental Science of the University of Florence, the Department of Agriculture of the University of Bologna, and the company Centro Attività Vivaistiche (CAV) of Tebano (Ra) - Italy. The LBP verifies the health status of national and exotic germplasm of fruit trees, applying international standards (e.g. European Plant Protection Office protocols) and, being equipped with the facilities for plant tissue culture, it is in charge of micropropagation of the most valuable rootstocks for peach and cherry.

Another result focused on sustainability and based on local human resources was the establishment of two organizations. The first, the Afghan National Nursery Growers Association (ANNGO), represents 30 Nursery Growers Associations spread throughout the country. All the involved private nurseries (about 1000) have accepted the ANNGO regulatory system for the production of true-to-type and traced saplings, deriving from MSNs with clean material from the NC, to be sold to fruit-growers. ANNGO also provides services related to business improvement and marketing promotion, sanitary controls, monitoring of the quality of planting material, improvement of nursery techniques and production of clonal rootstocks, certification and inspection services including labeling of certified saplings, technical training and dissemination of innovations, and publication of the catalogue. The production of certified saplings has increased from 340000 in 2012, to 1500000 in the last selling season (2015). The second organization, the Afghanistan National Horticulture Development Organization (ANHDO), is actually an NGO devoted to the development of a modern horticulture in partnership with MAIL and the private sector, and it is promoted and tutored by PHDP. Its aim is to provide continuity to PHDP main activities and to enable horticulture stakeholders to improve the industry by providing technical services, upgrading capacities, and promoting private-public coordination.

Adaptive research

Following a bottom-up procedure, adaptive research has been integrated within the PHDP framework from the very beginning (Fig. 3). There were 25 scheduled trials in 2015, most of them related to the topics considered to be at the base of improvement of fruit growing productivity and quality, such as self- and inter-compatibility in almond, plum and apricot varieties; grafting compatibility with vigour reducing

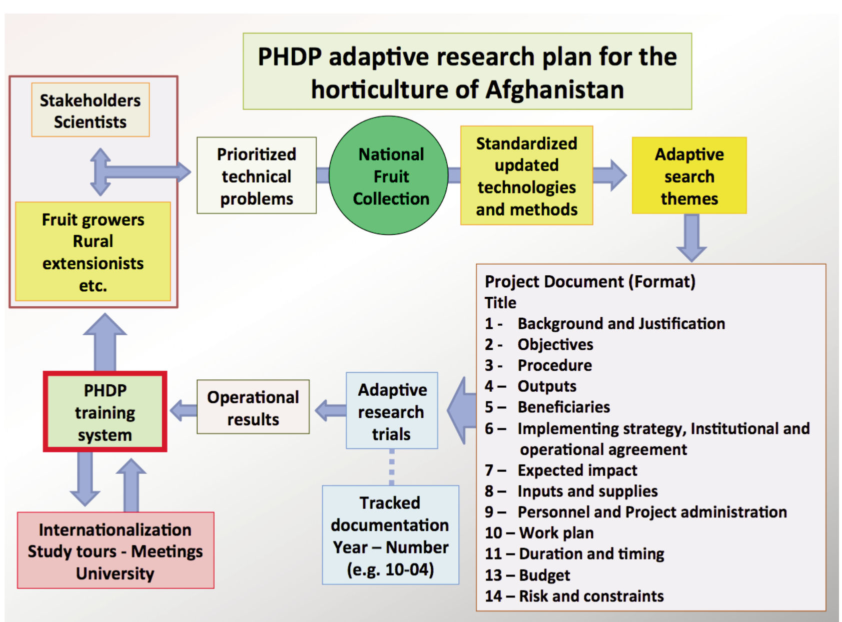

Fig. 3 - The circle of adaptive research within PHDP: from the definition of priorities to the impact of the achieved results. Source: PHDPII Report - Agriconsulting.

rootstocks (pear, cherry); chilling requirement; potential value for breeding and breeding activities (almond and apricot); micropropagation of rootstocks; pruning (grape) and training systems (apple). A plan to develop adaptive research has also been defined for upcoming years.

3. Conclusions

The pivotal role of the perennial horticulture development project

The EU-funded PHDP (2006-2015) has set the foundation for modern and sustainable development of commercial fruitculture in Afghanistan. The PHDP constituted the National Collection of Fruit and Nuts of Afghanistan and promoted a traceability system from the National Collection to the private nurseries organized in provincial associations and represented nationally by the ANNGO. For the first time in history, the orchard growers in Afghanistan could purchase labeled fruit saplings that certified the origin of the variety and rootstock and phytosanitary control of the mother stock material. As a result, thousands of new orchards are being planted with good marketable varieties, which will result in higher yield, better quality, and more income for the fruit-growers. The PHDP also conducted focused adaptive research, identifying the pollination interactions among plum and almond varieties and started a long term breeding program for almond and apricot. At this writing, all the above services have already been handed over to the Ministry of Agriculture of Afghanistan through a process of transition supported by the EU. At the same time, in the second phase, the PHDP promoted and developed the ANHDO which has become an independent and reputed organization, acting as a catalyst in the private sector, implementing projects to improve the value chain of the main fruit productions and work on post-harvest, quality standards and marketing. By addressing the foundation of horticultural development and having the strength of a strategic vision and the patience to work consistently for nine years, the PHDP took a pivotal role in the development of fruitculture in Afghanistan. The outputs of PHDP will have a relevant impact on development of the sector and on the Afghan economy as a whole for many years to come.

Future projections

Due to a lack of proper data and statistical treatment, it is extremely difficult to quote figures and attempt projections about future development of agriculture and horticulture in Afghanistan. However, based on the latest assessments (Altai Consulting, 2014) there has been a tremendous expansion in terms of the land invested in horticulture in the last 44 years, passing from 220000 ha in 1960 to the circa 340000 ha of today. But for the last 10 years yields had remained the same, indicating that the increase of horticultural production has been achieved almost exclusively at the expense of investing more irrigated land. Taking into account an estimated potential of 750000 ha of land invested in horticulture, a further 73% increase is expected in the next 10 years. This increased investment of land should be combined with extended irrigation schemes, and an upgrading and modernization of the extension and quality input and technical services provided to the farmers. As a result it is hoped that yields, quality standards, and marketability will also improve, thus generating income and jobs. This scenario is certainly possible, but will also require a modernization of the obsolete government institutions so that they become service oriented, providing needed policies and regulations and fostering partnerships with the private sector. This is perhaps the greatest challenge for the entire horticulture sector in Afghanistan.

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Phenotypic characterisation of almond accessions collected in Afghanistan

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Key words: genetic resources, germplasm, kernel, *Prunus dulcis.*

Abstract: Almond [*Prunus dulcis* (Mill.) D.A. Webb] accessions of Afghanistan have been surveyed, propagated, and grown in *ex situ* collections. Trees, leaves, flowers, and fruits were characterised following standardized procedures taking into account 48 phenotypic traits. The National Collection of Varieties of Almonds of Afghanistan showed a significant variability in terms of morphological traits, with a predominance of early flowering time accessions. Among the 56 accessions, Sattarbai, a unique group of cultivar typology characterized by crescent dry fruit, soft and thin "paper shell" and high kernel/dry fruit weight ratio (>0.65) was clearly represented by Cluster Analysis. Other accessions resulted closer to the international cultivars Lauranne, Carmel, Ferraduel and Ferragnes, considered as reference.

1. Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] presumably originated in mountain and desert areas of Central Asia and the Middle East (Martínez-Gómez *et al*., 2007; Gradziel, 2011; Zeinalabedini *et al*., 2012). After its domestication, which possibly occurred during the third millenium BC, it spread towards western areas via seeds carried by caravans along the old Silk Route (Fernandez i Martì *et al*., 2015). In Afghanistan, a geographical area criss-crossed by ancient trade routes and a cultural bridge between East and West, almond is called by its Persian name ''bādām'', similarly to Iran, India, Kashmir, Pakistan, Tajikistan, Tibet, and Turkey, or "badam-e-shirin". Almond is considered a species belonging to Afghan flora (Alam, 2011) and its cultivation has spread widely from ancient times. The presence of bitter tasting seeds is considered a relic of the domestication process (Rigoldi *et al*., 2015), hence the ancient presence of almond in Afghanistan can be corroborated by the occurrence of many bitter tasting seed specimens scattered throughout the Afghan territory. Furthermore, recent studies on almond evolution showed the existence of an unambiguous gene flow of wild almonds from the centre of origin to Iran (Fernandez i Martì *et al*., 2015), neighbouring to Afghanistan. On the other hand, *Amygdalus* species, among which *A. zabulica*, growing spontaneously in a restricted area of Afghanistan, was not considered by Ladizinsky (1999) as a possible almond progenitor because of its fleshy fruits, as well as *A. kuramica*, another species found only in Afghanistan and North West Pakistan; nevertheless Kester *et al*. (1991) reported that this species may bring sweet and "paper shell" fruit types, the latter trait being quite common in Afghan almond germplasm.

Although it has a cold, harsh continental climate, with hot dry summers and cold winters, Afghanistan is an important almond producing and exporting country; about 53,000 t of almonds in shell have been produced yearly in the last five years, representing about 1.8% of total world production (FAOSTAT, 2016). Almond kernels are an important source of nutritional and nutraceutical compounds for local populations, as well as a significant income for the

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country since a substantial percentage of almond nuts are exported to India and other countries of the region (Kaska *et al*., 2006; AAIDO, 2015).

The main production areas of export quality almonds are located in the regions of Samangan, Kunduz, Takhar, and Balkh (AAIDO, personal communication), and almonds are also traditionally grown in the "bagh" (home garden) and cultivated in orchards in almost all the valleys and flatlands of the country. The specific orography of the country, rich in narrow and isolated valleys, the multi-ethnic composition of the Afghan population, together with a primitive traditional agriculture, based in ancient times mostly on sexual reproduction of trees which is a suitable method of propagation for a species with a short juvenile period like almond, were factors that fostered the occurrence of a rich and differentiated local almond germplasm. Furthermore, almond is considered the most diverse species belonging to the genus *Prunus* (Socias i Company and Felipe, 1992) due to its self-incompatibility system (Socias i Company, 1990), the use of open pollinated seedlings in traditional fruitculture, and its adaptability to more diverse microclimates (Fernandez i Martì *et al*., 2015).

Within the framework of the Perennial Horticulture Development Project (PHDP - Phase I), several almond producing areas of the country were surveyed during the period 2007-2008 in order to collect the most valuable varieties (Giordani *et al*., 2014). This was achieved by following the indications of local fruit growers, scientists, and agronomists. The *in situ* collection was comprehensive of 82 local accessions which were propagated by budding. The obtained saplings were planted in *ex situ* replicated repositories in Kunduz and Mazar-i-Sharif, representing the National Collection of Fruits and Nut of Afghanistan.

Usually the primary evaluation of germplasm accessions of fruit tree species, including almond, is based on the observation and standardized description of phenotypic traits of the tree and its main organs, with the adoption of specific descriptor lists (Gülcan, 1985; Talhouk *et al*., 2000; De Giorgio *et al*., 2007; Chalak *et al*., 2006; UPOV, 2011; Rigoldi *et al*., 2015) and suitable uni- and multivariate statistical analyses to study the morphological variability, the relationships among the studied accessions and the correlation between the described traits (Lansari *et al*., 1994; Khadivi-Khub and Etemadi-Khah, 2015). Such approach is essential for germplasm characterisation and protection against bio-piracy, and it is a suitable method for the phenotypic distinction of collected varieties, a basic step for the further development of the almond industry given the high occurrence of synonyms and homonyms among the accessions indicated for commercial propagation and production purposes.

The present study aims to describe and analyse the morphological variability of a set of 56 accessions of almond described in the National Collection of Varieties of Fruits and Nuts of Afghanistan - Almond (Ministry of Agriculture, Irrigation and Livestock of Afghanistan, 2014), in relation to the results achieved by similar approaches on other almond gene pools.

2. Materials and Methods

Plant material

Flowers (n. 10), fully expanded leaves (n. 10), and fruits (n. 20) were randomly collected for two consecutive years from trees (n. 6) of the same accessions grown following traditional cultural practices in the orchards of the National Collection of Fruits and Nuts located in Kunduz (latitude 36.70893 N; longitude 68.86145 E; altitude 405 m a.s.l.) and Mazar-el-Shariff (latitude 36.73232 N; longitude 67.02216 E; altitude 353 m a.s.l.) in Afghanistan. Trees, grafted on almond seedlings, were four to five years old, productive and free of harmful viral diseases; off-type trees observed in the six planting plots were not included in the trial.

Data collection

Morphological and pomological characterisation of genotypes were performed following the Perennial Horticulture Development Project procedures, based on DUS-UPOV and IPGRI guidelines for almond (Gülcan, 1985; UPOV, 2011). Data related to tree characteristics were collected in the field; measurements and descriptions of the other organs were performed in the laboratory. Descriptive traits were determined based on rating and coding according to the adopted almond descriptor. Data were entered in Microsoft Excel® sheets, designed for the purpose with multiple choice notes for qualitative traits or open fields depending on the descriptor typology. Quantitative variables were measured and weighed adopting a manual calliper and a precision (0.01 g) electronic balance, respectively. Colour parameters were visually determined using a specifically designed colour chart; other qualitative characteristics such as organ shape were attributed by using illustrated charts.

Data processing and statistical analysis

Average and mode were calculated for all quantitative and qualitative parameters respectively; such values were used for the attribution of each accession to one class, regardless of the year and place of data collection. Classes and relative notes were defined for each measured quantitative trait. After mean values standardization, Multivariate Principal Component Analysis and Hierarchical Cluster Analysis (Euclidean distance and Ward's agglomeration method) were performed on XLS Stat software.

3. Results and Discussion

Studied accessions

The 56 studied accessions (Table 1) were initially collected in stands (orchards or scattered plants in home gardens) located in different areas distributed throughout the country, within latitudes 31° 36' 42.8400'' N - 36° 43' 42.9276'' and longitudes 65° 40' 51.9600'' E - 71° 9' 10.0008'' E. Altitudes ranged from 287 m to 1022 m a.s.l. Most of the accessions (>70%) were collected in various districts of northern provinces of Kunduz, Samangan and Balkh, considered the typical areas of almond production, although all accessions were susceptible in varying degrees to the risk of frost damage during the flowering time common in these locations. There were no late flowering varieties equivalent in flowering time to cultivars such as Lauranne, Ferragnes and Ferraduel.

Morphologic and phenological trait variation

Minimum, maximum, mean and standard deviation values for each analysed trait for the whole set of studied accessions is reported in Table 2. Among 140 possible notes attributable to 46 different internationally agreed morphological descriptors related to tree, leaf, flower, shell and kernel, only 17 (≈12 %) of them showed no cases, indicating a wide variability for almost all traits. The sole descriptor showing no variation is related to the number of pistils (always 1 in 100% of cases); colour of tip of flower, sepals and petals showed low variability as well. Among fruit traits, a special note, not present in the international standardised descriptor lists, was included to describe the "crescent" shape of green fruits and kernels, which showed a frequency of 1% and 16% of cases respectively for the whole collection.

The set of described accessions was characterised mainly by vigorous, open trees with dense foliage; medium size, mostly dark green leaves with short petiole and crenate margin. Flowers very frequently showed red brown sepals, broad elliptic white petals, stigmas above the anthers, and reddish stamen filaments. The predominant dry fruit traits were elongated and crescent shape, pointed apex, thick but medium-low resistant to cracking endocarps. Kernels were characterised by elliptic-narrow elliptic and crescent shapes, red brown colour, medium-weak rugosity and small-medium size. Kernel weight (1.1±0.4 g) was lower than the mean value (2.4 g) reported for a germplasm collection of Iranian, European and North American accessions, and similar to those observed in different almond collections of the Persian and Middle-East areas (Talhouk *et al.*, 2000; Chalak *et al.*, 2006; Zeinalabedini *et al.*, 2012; Khadivi-Khub and Etemadi-Khah, 2015) and Afghanistan (Kaska *et al.*, 2006). A distinctive trait of Afghan almonds is related to shell cracking resistance, a characteristic with relevant implications in value-added products (Ledbetter, 2008), which was found to be very low or low for 30% of accessions, indicating a consistent occurrence of "paper shell" and soft dry fruits. Conversely, a similar percentage of accessions with hard and very hard shell (28%) was observed. Kernel weight/dry fruit (nut + shell) weight ratio, sometimes indicated as shelling percentage or kernel yield, is another important commercial trait. Afghan accessions in the present study showed higher mean value (0.51±0.12) for this characteristic than those found by other authors on almond germplasm of the same geographic area (between 0.31 and 0.35 for the Persian area) (Sorkheh *et al*., 2010; Zeinalabedini *et al*., 2012; Khadivi-Khub and Etemadi-Khah, 2015), from Lebanon (from 0.30 to 0.41)(Talhouk *et al*., 2000; Chalak *et al*., 2006), and similar to the mean value (0.50±0.11) found in a previous study carried out on 17 local varieties of northern Afghanistan (Kaska *et al*., 2006). Lower mean values of kernel/dry fruit ratio were observed also on Moroccan almond germplasm (0.38)(Lansari *et al*., 1994), on European germplasm collections (Godini, 1984; Cordeiro *et al*., 2001; De Giorgio and Polignano, 2001), and on newly released cultivars (Vargas *et al*., 2008).

With regard to other fruit morphological traits linked to market value, the percentage of double kernel, a negative trait for international standardised trade but not very influent on domestic and subregional commerce, resulted absent or very low (0-2%

Table 1 - List of the described almond [*Prunus dulcis* (Mill.) D.A. Webb] accessions from Afghanistan, area of *in situ* collection, altitude and remarks on their commercial value

National Collection Code	Cultivar name	Area of collection (Province/Locality)	Remarks
AFG0153	Abdul Wahidi	Balkh - Khulm	Good quality
AFG0144	Belabai	Balkh - Khulm	Slight bitterness
AFG0167	Carmel	Kunduz - Char Darah (I)	International cultivar, later flowering than any native Afghan almond
AFG0527	Changaki	Kandahar - Kandahar	Low market value
AFG1008	Du Maghza Kulula	Kunduz - Char Darah	Low market value
AFG1007	Du Maghza Spin	Kunduz - Char Darah	-
AFG6308	Ferraduel	Kunar - Asad Bad (I)	International cultivar
AFG6206	Ferragnes	Nangarhar - Jalalabad (I)	International cultivar
AFG0793	Kaf	Samangan - Aybak	Medium market value
AFG0773	Kafmal	Kunduz - Char Darah	-
AFG4048	Kaghazi Du Posta	Herat - Guzara	Low market value
AFG4016	Kaghazi Gerd	Herat - Guzara	Medium market value
AFG0739	Kaghazi Herati	Herat - Enjil	Low market value (exceptionally large kernel)
AFG0532	Kaghazi Kalan	Kandahar - Kandahar	Valuable for kernel size (AFG0739 has 15% bigger kernels and half the percentage of doubles
AFG6040	Kaghazi Maida	Nangarhar - Muhmand Dara	Low market value; small kernel
AFG0535	Kaghazi Sia Dana	Kandahar - Kandahar	Good quality
AFG0847	Kajak Samangan	Samangan - Aybak	Good market quality
AFG0778	Kelk Arus	Kunduz - Char Darah	-
AFG0146	Khairodini	Balkh - Khulm	Moderately hard shell-
AFG6309	Lauranne	Kunar - Asad Bad (I)	International cultivar
AFG0173	Mahali Kunduz	Kunduz - Char Darah	Low market value; hard shell
AFG0147	Majidi Balkh	Balkh - Khulm	Small kernel
AFG2010	Majidi Samangan	Samangan - Aybak	Small kernel
AFG0166	Marawaja Doum	Kunduz - Char Darah	Low market value
AFG0165	Marawaja Du Maghdza	Kunduz - Char Darah	-
AFG0775	Marawaja Maida Dana	Kunduz - Char Darah	Low market value
AFG0534	Marawaja Safid Post	kandahar - Kandahar	Hard shell
AFG0151	Pista Badam	Balkh - Khulm	Low market value
AFG0160	Qaharbai	Samangan - Aybak	-
AFG1003	Qaharbai Allah Mir	Kunduz - Char Darah	-
AFG1006	Qaharbai Aykhanum	Kunduz - Char Darah	-
AFG0780	Qaharbai Hazratan	Kunduz - Char Darah	-
AFG0143	Qambari	Balkh - Khulm	-
AFG0142	Qambari 142	Kunduz - Char Darah	-
AFG0772	Qambari Kunduz	Kunduz - Char Darah	-
AFG0158	Sangak Dahum	Samangan - Aybak	Low market value
AFG0740	Sangak Haftum	Herat - Enjil	Low market value
AFG0519	Sangak Hashtum	Kandahar - Kandahar	Low market value
AFG0530	Sangak Nohum	Kandahar - Kandahar	Low market value
AFG0531	Sangak Shashum	Kandahar - Kandahar	Low market value
AFG0380	Sangi Du Maghza Kalan	Kandahar - Kandahar	Low market in shell
AFG0774	Satarbai Yaqubi	Kunduz - Char Darah	-
AFG0159	Sattarbai Bakhmali	Samangan - Aybak	High market value
AFG1002	Sattarbai Doum	Kunduz - Char Darah	High market value
AFG0157	Sattarbai Guldar	Samangan - Aybak	High market value
AFG0168	Sattarbai Mumtaz	Kunduz - Char Darah	Highest market value, intrinsically low yielding
AFG0154	Sattarbai No.4	Balkh - Khulm	High market value-
AFG2011	Sattarbai Sais Aybak	Samangan - Aybak	High market value
AFG0164	Sattarbai Sais Kunduz	Kunduz - Char Darah	High market value
AFG0156	Sattarbai Sais Talkhak	Samangan - Aybak	-
AFG0777	Sattarbai Sais Zuhrabi	Samangan - Aybak	High market value
AFG0145	Sattarbai Sufi	Balkh - Khulm	-
AFG1005	Shakh-i-Buz	Kunduz - Char Darah	-
AFG0155	Shakh-i-Buz Safid	Balkh - Khulm	-
AFG0162	Shokorbai	Samangan - Aybak	Acceptable market value
AFG0148	Zang Kafter	Balkh - Khulm	-

Table 2 - List of phenotypic traits, codes, states, classes and descriptive statistics adopted to describe the set of 56 accessions from the National Collection of Almond from Afghanistan. In brackets the percentage of cases observed for each note/descriptor

No.	Trait	Classes (% of cases)	Min.	Max.	Mean	SD
1	Juvenile tree vigour	1 - Weak (0); 2 - Medium (45); 3 - Strong (55)	2	3	2.55	0.81
2	Tree - Habit	1 - Up-right (3); 2 - Slightly open (19); 3 - Open (52); 4 - Spreading (24); 5 - Drooping (2)	1	5	3.00	0.63
3	Foliage - Density	1 - Loose (4); 2 - Medium (48); 3 - Dense (48)	1	3	2.41	0.29
4	Leaf blade - Length	1 - Short (12); 2 - Medium (88); 3 - Long (0)	1	2	1.91	0.42
5	Leaf blade - Width	1 - Narrow (9); 2 - Medium (91); 3 - Broad (0)	1	2	1.84	0.60
6	Leaf blade – Ratio length/width ratio	1 - Small (17); 2 - Medium (81); 3 - Large (2)	1	2	2.54	0.45
7	Leaf blade - Colour	1 - Light green (5); 2 - Medium green (36); 3 - Dark green (59)	1	3	1.73	0.47
8	Leaf blade - Incisions of margin	1 - Serrate (26); 2 - Crenate (74)	1	2	1.32	0.50
9	Leaf - Petiole length	1 - Short (67); 2 - Medium (33); 3 - Long (0)	1	2	1.41	0.36
10	Flower buds: - Shape	1 - Conical (59); 2 - Ovoid (41); 3 - Rounded (0)	1	2	2.02	0.69
11	Flower bud - Colour of tip of petals	1 - White (3); 2 - Pink white (93); 3 - Pale pink (2); 4 - Pink (2); 5 - Carmine (0); 6 - White, with carmine tip (0)	1	4	2.82	0.86
12	Flower bud - Colour of sepals	1 - Green (0); 2 - Brown green (34); 3 - Red brown (50); 4 - Dark red (16)	2	4	2.34	0.64
13	Flower bud - Hairiness of sepals	1 - Absent or very weak (24); 2 - Weak (22); 3 - Medium (52); 4 - Strong (2); 5 - Very strong (0)	1	4	1.80	0.82
14	Flower - Size	1 - Small (31); 2 - Medium (57); 3 - Large (12)	1	3	2.27	0.40
15	Flower - Shape of petals	1 - Narrow elliptic (24); 2 - Elliptic (26); 3 - Broad elliptic (50)	1	3	1.20	0.65
16	Flower - Colour of petals	1 - White (83); 2 - Pink white (17); 3 - Pink (0); 4 - Dark pink (0)	1	3	1.18	0.39
17	Flower - Number of stamens	1 - Few (31); 2 - Medium (55); 3 - Many (14)	1	3	1.84	0.53
18	Flower - Number of pistils	1 - Always 1 (100); 2 - Sometimes 2 (0); 3 - Frequently two (0); 4 - Few-medium (0); 5 - Medium many (0)	1	1	1.00	0.00
19	Flower - Position of stigmas compared to anthers	1 - Below (2); 2 - Same level (36); 3 - Above (62)	1	3	2.61	0.41
20	Stamen - Anthocyanin colouration of filaments	1 - Absent (22); 2 - Present (78)	1	2	1.79	0.81
21	Stigma - Size	1 - Small (26); 2 - Medium (65); 3 - Large (9)	1	3	1.73	0.63
22	Green fruit - Length	1 - Small (9); 2 - Medium (72); 3 - Large (19)	1	3	2.11	0.53
23	Green fruit - Shape	1 - Rounded (2); 2 - Ovate (9); 3 - Elliptic (2); 4 - Pointed (74); 5 - Crescent (1)	1	5	3.84	0.78
33	Green fruit - Pubescence	1 - Slight (29); 2 - Medium (59); 3 - Much (12)	1	3	1.82	0.64
34	Dry fruit - Shape	1 - Type 1 (5); 2 - Type 2 (0); 3 - Type 3 (48); 4 - Type 4 (28); 5 - Type 5 (19);	1	5	3.59	0.99
35	Dry fruit - Shape of apex	1 - Flat (7); 2 - Rounded (9); 3 - Pointed (84)	1	3	2.77	0.57
36	Dry fruit - Thickness of endocarp	1 - Thin (28); 2 - Medium (34); 3 - Thick (38)	1	3	2.09	0.82
37	Dry fruit - Resistance to cracking	1 - Very low (9); 2 - Low (21); 3 - Medium (42); 4 - High (19); 5 - Very high (9)	1	5	3.00	1.08
38	Dry fruit - Keel development	1 - Absent or very weak (9); 2 - Weak (28); 3 - Medium (37); 4 - Strong (24); 5 - Very strong (2)	1	5	2.79	0.97
39	Kernel - Shape	1 - Crescent (16); 2 - Narrow elliptic (31); 3 - Elliptic (34); 4 - Broad elliptic (17); 5 - Very broad elliptic (2)	1	5	2.57	1.02
40	Kernel - Size	1 - Small (64); 2 - Medium (36); 3 - Large (0)	1	3	1.36	0.48
41	Kernel - Thickness	1 - Thin (24); 2 - Medium (71); 3 - Thick (5)	1	3	1.82	0.51
42	Kernel - Main colour	1 - Yellow (2); 2 - Yellow brown (26); 3 - Light brown (16); 4 - Red brown (49); 5 - Dark chestnut brown (7)	1	5	3.32	1.01
43	Kernel - Intensity of colour	1 - Light (9); 2 .- Medium (74); 3 - Dark (17)	1	3	2.07	0.50
44	Kernel - Rugosity	1 - Very weak (3); 2 - Weak (34); 3 - Medium (58); 4 - Strong (5)	1	4	2.63	0.65
45	Kernel - Percentage of double kernel	1 - Absent/very low (23); 2 - Low (17); 3 - Medium (30); 4 - High (22); 5 - Very high (8)	1	4	2.66	1.21
46	Kernel/Dry fruit weight percentage	1 - Very low (0); 2 - Low (8); 3 - Medium-low (15); 4 - Medium (23); 5 - Medium-high (33); 6 - High (21); 7 - Very high (0)	1	6	4.48	1.19
47	Time of flowering	1 - Very early (48); 2 - Early (31); 3 - Medium (14); 4 - Late (5); 5 - Very late (2)	1	5	1.88	1.03
48	Ripening time (harvesting)	1 - Very early (0); 2 - Early (62); 3 - Medium (33); 4 - Late (5); 5 - Very late (0)	1	4	2.38	0.49

of fruit with double kernel) and low (3-5% of fruits with double kernel) in 29% and 20.5% of cases, respectively, with a general average of 4% of fruits with double kernels. This value is similar to the average value (4.5%) found by Sorkheh and colleagues (2010) in a germplasm collection composed of Iranian and European and North American accessions, but much lower than the findings of Kaska (Kaska *et al*., 2006) on a set of almond fruit collected mainly in northern provinces of Afghanistan (≈ 46 %) and those observed on Persian (39%)(Khadivi-Khub and Etemadi-Khah, 2015) and Lebanese (≈ 43%) germplasm (Chalak *et al*., 2006).

Time of flowering showed a high variation, with cultivars flowering from very early (before February 26; 49% of cases, among which many "Sattarbai" and "Sangak" type accessions) to very late (after March 12; ≈ 2% of cases, represented by the international cultivar Lauranne), which clearly indicates a prevalence of early flowering accessions in the almond germplasm from Afghanistan. Conversely, the National Collection of Almond lacks very early (before July 14) and very late (after 29 August) ripening accessions. The range of phenological and measured morphologic traits are shown in Table 3.

Relationship between the described accessions

Individual descriptive sheets were published in 2014 as The National Collection of Varieties of Fruits and Nuts of Afghanistan - Almond and are available online on the Internet (http://afghanistanhorticulture.org/pages/Germplasm.aspx), while the results of multivariate PCA and Cluster Analysis are reported in Table 4 and Figure 1. Principal Component Analysis

Table 3 - Scales defined for quantitative and phenological traits observed on the almond accessions from Afghanistan

Traits	Scales
Leaf blade	Length (mm): short (< 58); medium (58-83); long (>83)
Leaf blade	Width (mm): narrow (<20); medium (20-29); broad (>29)
Leaf blade	Ratio length/width ratio: small (<2.5); medium (2.5-3.5); large (>3.5)
Leaf	Petiole length (mm): short (<11); medium short (11-19); medium (>19-28); medium long (>28-36); long (>36)
Flower	Size (mm): small (<3); medium (3-4); large (>4)
Green Fruit	Length (mm): small (<32); medium (32-50); large (>50)
Kernel	Size (g): small (<0.7); medium (0.7-1.9); large (>1.9)
Kernel	Thickness (mm): thin (<5.5); medium (5.5-10.5); thick (>10.5)
Double kernel (%)	Very low (<15); low (15-27); medium-low (28-39; medium (40-51); medium-high (52-63); high (64-75); very high (>75)
Time of flowering	Very early (before February 26); early (February 27 - March 2); medium (March 3-6); late (March 7-11); very late (after March 11)
Time of maturity	Very early (before July 14); early (July 14-29); medium (July 30 - August 13); late (August 14-28); very late (after August 28)

Table 4 - Eigenvalues, expressed variability and correlations of the first seven Principal Components (accounting for 51.3% of variability) obtained from the whole accession/variable data set. The highest absolute correlation of each variable is in bold

Principal component	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	5.3	3.0	2.6	2.4	2.2	2.0	1.7
Variability (%)	14.0	7.9	6.8	6.5	5.9	5.3	4.6
Cumulative variability %	14.0	21.9	28.8	35.3	41.3	46.6	51.3
Variable correlation							
Juvenile tree - Vigour	0.19	0.13	**-0.27**	0.05	0.12	0.12	0.25
Tree - Habit	-0.28	-0.01	-0.03	0.01	-0.50	-0.11	**-0.43**
Foliage - Density	-0.01	-0.34	0.07	0.04	**0.55**	0.16	-0.07
Leaf blade - Length	-0.01	0.44	**0.47**	0.19	0.28	0.25	0.09
Leaf blade - Width	0.07	0.30	**-0.57**	0.03	0.16	-0.31	-0.21
Leaf blade - Ratio length/width	0.07	0.36	**0.62**	0.19	-0.03	0.19	0.25
Leaf blade - Colour	0.01	0.07	0.14	**0.50**	-0.23	-0.13	-0.03
Leaf blade - Incisions margin	-0.03	0.27	**0.41**	0.34	-0.15	0.41	-0.13
Leaf - Petiole length	0.03	0.42	0.26	**-0.36**	0.10	-0.33	-0.17
Flower bud - Shape	0.27	0.21	-0.22	-0.17	**0.31**	-0.20	0.26
Flower bud - Colour of tip of petals	0.21	0.17	-0.13	**0.46**	-0.26	-0.25	0.16
Flower bud - Colour of sepals	0.23	0.10	0.36	0.06	0.08	-0.26	-0.13
Flower bud - Hairiness of sepals	-0.04	**0.51**	0.05	-0.37	-0.08	0.06	-0.16
Time of beginning of flowering	-0.08	**0.39**	-0.33	0.21	-0.07	0.38	-0.07
Flower - Size	0.19	0.11	**0.49**	-0.38	0.13	-0.19	-0.10
Flower - Shape of petals	0.24	0.05	**0.28**	-0.24	0.22	0.11	0.13
Flower - Colour of petals	0.11	-0.23	0.14	0.16	**0.34**	-0.10	-0.29
Flower - Number of stamens	0.08	**0.55**	-0.13	-0.30	0.03	-0.11	-0.09
Flower - Position of stigmas compared to anthers	-0.31	0.32	-0.23	0.19	0.39	0.06	**-0.43**
Stamen - Anthocyanin colouration of filaments	-0.09	0.09	0.12	-0.38	-0.07	**0.48**	-0.18
Stigma - Size	0.23	**0.36**	0.11	-0.14	0.33	-0.18	0.29
Green Fruit - Size	**-0.66**	-0.15	-0.10	0.19	0.25	-0.08	0.15
Green Fruit - Shape	**-0.62**	-0.01	0.26	0.34	0.07	-0.39	0.08
Green Fruit - Pubescence	-0.04	0.12	-0.28	0.09	0.23	**0.47**	0.34
Time of Maturity	0.26	0.26	0.01	0.20	**-0.38**	0.09	-0.10
Dry Fruit - Shape	**-0.76**	-0.06	0.26	0.09	0.10	-0.31	0.04
Dry Fruit - Shape of Apex	**-0.50**	0.18	0.01	0.10	-0.17	0.09	0.48
Dry Fruit - Thickness of Endocarp	**0.71**	-0.16	0.14	0.23	0.03	-0.03	0.04
Dry Fruit - Resistance to Cracking	**0.81**	-0.38	0.08	0.09	0.09	-0.10	0.04
Dry Fruit - Keel Development	-0.27	**0.45**	-0.06	-0.05	-0.07	-0.14	0.21
Fruit - Percentage of Double kernels	-0.07	-0.30	-0.01	**-0.36**	0.19	0.01	-0.12
Kernel - Shape	**0.74**	0.07	-0.11	0.09	-0.07	0.25	-0.25
Kernel - Size	-0.09	0.01	**-0.44**	0.18	0.28	-0.01	0.06
Kernel - Thickness	**0.63**	0.24	-0.20	-0.26	-0.06	-0.10	0.26
Kernel - Main Colour	**0.35**	0.31	-0.01	0.32	0.20	-0.27	0.01
Kernel - Intensity of Colour	0.28	0.41	-0.03	**0.46**	0.31	-0.05	-0.35
Kernel - Rugosity	-0.16	-0.13	0.11	0.16	**0.48**	0.32	-0.11
Kernel Dry/Fruit ratio	**-0.73**	0.31	-0.09	-0.21	0.08	0.07	-0.14

based on the averages of the discrete scores of the 48 morphological and phenological variables for each accession indicated a low correlation between the utilized variables, with 15 PCAs with eigenvalue higher than 1, accounting for 75.8% of variability within the whole set. Such result can be attributed to the adoption of a multi-state discrete scoring methodology but also to a generalized low correlation between variables.

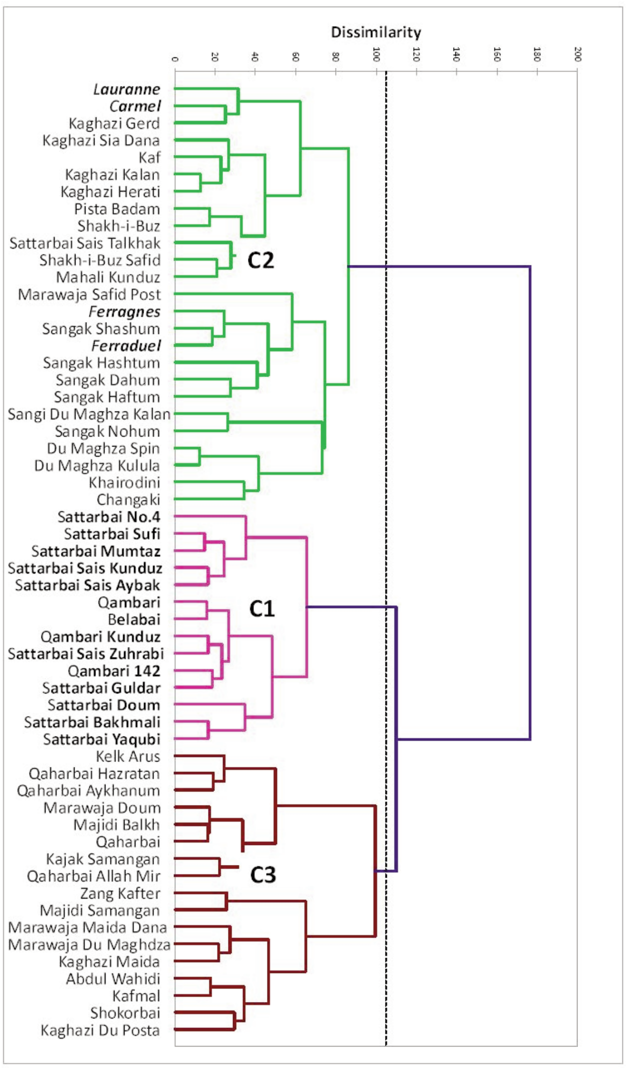

Fig. 1 - Dendrogram (Euclidean distance; Ward's agglomerating method) obtained by analyzing 48 multi-state, discrete morphological variables of 56 almond accessions belonging to the National Collection of Fruits and Nuts of Afghanistan (bold cursive: International cultivars; bold: accessions belonging to C1 cluster "Sattarbai group").

The average Euclidean distance of the generated proximity matrix between the studied accessions resulted equal to 8.46±1.21, ranging from 4.94 (minimum value) observed in the pair "Kaghazi Kalan - Kaghazi Herati" (collected from Kandahar - Dehe Kochi Village and Herat Province, Enjil District, Kahdestan village, respectively) up to 12.59 (maximum value) for the couple "Sattarbai N.4 - Marawaja Safid Post". The dendrogram generated by Cluster Analysis is reported in Figure 1, where three main significant clusters (C1, C2 and C3) can be observed. The first cluster C1 (7.35±0.92 average distance; 5.43 minimum distance and 9.53 maximum distance for pairs "Sattarbai Sufi - Sattarbai Mumtaz" and "Sattarbai N.4 - Sattarbai Doum", respectively) is almost completely composed by "Sattarbai" denominated accessions (10 of 14 accessions), while only one "Sattarbai" type ("Sattarbai Sais Talkhak") belongs to a different cluster (C1). Table 5 reports the representative characteristics of the "Sattarbai" almond type, considered the best in terms of fruit quality and market value, as resulted from the analysis of the 48 morphological and phenological variables of the 10 "Sattarbai" accessions included in cluster C1. Even if a certain degree of variation within single traits of leaves (colour), flowers (petal shape), dry fruits (shape, thickness of endocarp, keel development and percentage of double kernel) and kernels (shape and main colour) was observed, as a whole, these accessions are very closely related and they constitute a clearly defined special subset within the Afghan almond germplasm (Fig. 1). The accession Sattarbai Sais Talkhak, the only "Sattarbai" named cultivar outside cluster C1, showed six characteristics (namely leaf colour, flower size, shell resistance to cracking, dry fruit shape and kernel main colour) with significant differences and 16 traits slightly differing from the "Sattarbai" standard type; this fact substantiates its location in a different cluster. On the other hand, Qambari, Qambari Kunduz, Qambari 142 are varieties often confused with "Sattarbai" type also by expert traders, which confirms the high phenotypic similitude between "Qambari" and "Sattarbai" groups and justifies their presence in cluster C1. Conversely, Belabai, albeit showing many traits of the "Qambari/Qaharbai" type, differentiates for the distinctive and sometimes bitter flavour of the kernel which detracts from its otherwise medium value.

Cluster C2 is formed by the four international cultivars (Lauranne, Carmel, Ferragnes and Ferraduel) belonging to the National Collection and by 21 local accessions, among which all "Sangak" (*sangak* meaning "stone" in local languages) accessions, a part of "Kaghazi" types and other unique varieties. Broad elliptic sepals, Type 3 dry fruit shape, thick endocarp,

Table 5 - Morphological and phenological characteristics of "Sattarbai" almond type

No.	Trait	Note (mode)	Range
1	Juvenile tree - Vigour	Medium	Medium-Strong
2	Tree - Habit	Open	Open-Spreading
3	Foliage - Density	Dense	Medium-Dense
4	Leaf blade - Length	Medium	Medium-Long
5	Leaf blade - Width	Medium	Medium
6	Leaf blade - Ratio length/width	Medium	Small - Medium
7	Leaf blade - Colour	Medium green	Medium green - Dark green
8	Leaf blade - Incisions margin	Crenate	Crenate - Serrate
9	Leaf - Petiole length	Short	Short-Medium
10	Flower bud - Shape	Conical	Conical - Ovoid
11	Flower bud - Colour of tip of petals	Pink white	Pink white
12	Flower bud - Colour of sepals	Red brown	Red brown - Brown green
13	Flower bud - Hairiness of sepals	Medium	Medium - Weak
14	Flower - Size	Small	Small - Medium
15	Flower - Shape of petals	Narrow elliptic	Narrow elliptic - Elliptic
16	Flower - Colour of petals	White	White
17	Flower - Number of stamens	Few	Few - Medium
18	Number of pistils	Always 1	Always 1
19	Flower - Position of stigmas compared to anthers	Above	Above - Same level
20	Stamen - Anthocyanin colouration of filaments	Present	Present - Ansent
21	Stigma - Size	Small	Small - Medium
22	Green Fruit - Size	Large	Large - Medium
23	Green Fruit - Shape	Pointed	Pointed - Crescent
33	Green Fruit - Pubescence	Medium	Medium - Slight
34	Dry Fruit - Shape	Type 5 - Crescent	Type 5 - Type 4
35	Dry Fruit - Shape of Apex	Pointed	Pointed
36	Dry Fruit - Thickness of Endocarp	Thin	Thin - Medium
37	Dry Fruit - Resistance to Cracking	Very low	Very low - Low
38	Dry Fruit - Keel Development	Strong	Medium - Strong
39	Kernel - Shape	Narrow elliptic	Narrow elliptic - Crescent
40	Kernel - Size	Small	Small - Medium
41	Kernel - Thickness	Thin	Thin - Medium
42	Kernel - Main Colour	Yellow brown	Yellow brown - Light brown
43	Kernel - Intensity of Colour	Medium	Medium - Light
44	Kernel - Rugosity	Medium	Medium - Weak
45	Fruit - Percentage of Double kernels	Medium high	Absent - very low- High
46	Kernel - Dry/Fruit ratio	High (0.64)	High (0.58-0.72)
47	Time of beginning of flowering	Very early	Very early - Early
48	Ripening time (harvesting)	Early	Early - Medium

medium resistance to cracking, red brown kernel, medium percentage of double kernels and medium kernel/dry fruit weight ratio (average 0.45, about 30% less that C1 mean value) were the traits differentiating this group of varieties from those of cluster C1. The last cluster (C3) held 17 local accessions mostly belonging to “Qaharbai”, “Marawaja” and “Majidi” types and other unique varieties; such accessions resulted characterised by medium size flowers, dry fruits with weak keel development, high percentage of double kernels, and medium high kernel/dry fruit weight ratio (average 0.53).

4. Conclusions

This first comprehensive study of morphological and phenological traits of almond accessions from Afghanistan clearly reveals an important level of variation among traits of the local germplasm. Analogous findings were reported for Iranian and Lebanese germplasm (Talhouk *et al*., 2000; Chalak *et al*., 2006; Zeinalabedini *et al*., 2012; Khadivi-Khub and Etemadi-Khan, 2015). Furthermore, a special subset of local varieties, to the best of our knowledge not yet reported by scientific international literature and unique in its kind because of dry fruit and kernel crescent shape and very soft "paper shell" endocarp, denominated "Sattarbai" type, has been clearly highlighted. The best Sattarbai types are extremely crescent shaped, with very narrow, elongated kernels and a very clear fissure in the "paper shell" along its length. The Qambari types have less exaggerated length and crescent shape nuts, but clearly have "paper shell". Qaharbai types are less elongated and tend to have more thickening of the semi "paper shells". Shakhe Buz (="Goat's horn") has an exaggerated "S" shape. Other Kaghazi or "paper shell" types may be downgraded because of untypical shape, but the accession Kaghazi Herati AFG0739 has kernels some 40-50% heavier than the typical Sattarbai or Qambari kernel. The Sattarbai, Qambari, Qaharbai and some other types of almond were found exclusively in the north of Afghanistan, indicating a probable connection to the central Asian germplasm, particularly to the famous almond growing areas of the Fergana Valley.

Nevertheless, a very early flowering time represents a limit to the expansion of these cultivars, and breeding programs are already being developed to overcome this obstacle. Specific morphological studies associated to DNA fingerprinting would be the next step in order to better clarify the identity of individual local accessions, to reveal the relationship of the Afghan almond germplasm with *Prunus dulcis* genetic resources of other areas, and to study the role of Afghanistan, an intersectionbetween East and West, in the flow of almond around the world.

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Short Note

Implications of investigating pollination and cross compatibility in the almond varieties of Afghanistan

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Key words: breeding, incompatibility groups, national collection, *Prunus amygdalus, Prunus dulcis.*

Abstract: Survey and collection of almond accessions for a National Collection of Fruit and Nuts of Afghanistan began in 2007. Investigations into cross compatibility of almond accessions began in 2008 on *in situ* collected exemplar trees, and in 2010 on trees in the *ex situ* collections. The methods varied in relation to specific trials, nevertheless as an average 150 flower buds were isolated ahead of flowering on 7 one-year-old shoots per tree, and used as pollen donors or receptors. The initial trials on *in situ* accessions were performed on one single tree per variety, while six trees were used for the tests carried on in the *ex situ* collections. Fruit set percentage from self-pollination, cross-pollination and open pollination was calculated. All native Afghan varieties tested were shown to be self incompatible. Various problems related to weather and other conditions worked against a comprehensive testing of all combinations of varieties, although many useful and surprising conclusions were reached.

1. Introduction

It is known that most almond [*Prunus dulcis* (Mill.) D.A. Webb, syn. *Prunus amygdalus* Batch] varieties in the world, and until relatively recently, all the major commercial varieties, are self incompatible. This means that each variety needs to be grown with another variety to ensure pollination. Not only that, each variety has its own specific incompatibility genes, and has to be pollinated by another variety with a different set of incompatibility genes (Micke, 1996).

In 2007, the Perennial Horticulture Development Project (PHDP), funded by the European Commission (EC) for the rehabilitation and development of the horticulture industry in Afghanistan, began the collection of varieties of fruits and nuts in Afghanistan, with a view to characterisation of those varieties and promotion of the best varieties for the development of commercial horticulture. The programme envisaged the distribution of varieties through a system of certified tree production with information provided as to how to plant and grow superior orchards. Since every almond orchard that would be planted in Afghanistan would need to be planted with a combination of two or more inter compatible varieties, it seemed essential that work be done on identifying suitable combinations of varieties.

The PHDP programme also included the development of the production of the superior Afghan almond types, which include a range of paper shell types that are very much appreciated in the Indian market, and which command very high prices. These are exemplified by the Sattarbai and Qambari types, which are elongated crescent shaped nuts with a paper shell that in the most prized varieties opens along a lateral fissure to expose the kernel inside. Since there was no way of predicting the composition of the Afghan germplasm in respect of incompatibility genes, a start was made in 2008 on testing the various combinations of varieties.

This paper reports on the performed activities and

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the first results achieved related to self- and inter-compatibility of almond varieties of Afghanistan, together with the some notes on the gained experience and the implications of these findings in the almond industry of Afghanistan. As illustrated in the following paragraph, different trials have been defined and set up in different locations taking into account farmer knowledge and tradition, local operative conditions and environmental aspects.

2. *In situ* tree pollination trials in the Kunduz, Samangan and Balkh provinces

During the 2007 season, the PHDP had collected budwood from some 84 separate accessions from around Afghanistan, and budded these onto almond seedling rootstocks at six different centres with a view to planting out duplicate collections in the northern Afghanistan centres of Mazar-e Sharif and Kunduz in spring 2009. It had already been ascertained by PHDP, in particular through a comprehensive nursery survey, that the main centre of the important almond varieties was in the north of Afghanistan, especially in the provinces of Kunduz, Samangan and Balkh provinces.

While it was obvious that testing almond pollination attributes would be much easier when the germplasm was collected into the variety collections, it was considered an urgent priority for PHDP to find out about the pollination compatibilities of the main varieties. Many stories were being reported from the field by the PHDP staff and by associated projects, such as Roots of Peace Almond Industry Development Project, concerning the lack of knowledge among many Afghan farmers about pollination requirements of almonds. In general, the need for pollination was unknown, and some farmers were insecticide spraying bees that were in the almond blossom, on the assumption that bees were "eating the flowers". While traditionally many orchards included a range of varieties, they did not necessarily overlap in flowering time, nor were they necessarily compatible. One grower was supposedly so impressed with the results of the Nonpareil variety that he planted a whole large block to that single variety, and then wondered why results were disappointing.

Not only was solving the almond pollination problem considered an urgent priority for PHDP because of the lack of knowledge of most small farmers, but as a donor funded project working within the Ministry of Agriculture, PHDP had to demonstrate meaningful results ahead of planting out the national collections of the different species of fruits and nuts. So in 2008, a simple trial at three sites was designed, using the original *in situ* trees from which the budwood for the germplasm collection was collected.

Materials and Methods

The first experiment to study the self and cross incompatibility of almond accessions in the northern part of Afghanistan took place from February 2008 (prior to flowering) to August, 2008 (harvesting of fruit). Three groups of six in situ trees were selected for their proximity in single orchards or in closely nearby orchards, in Khulm (Balkh province), in Aybak (Samangan province) and Chardara (Kunduz province). Each tree was selected before flowering and lengths of branch with 100-200 flower buds were selected and marked. Branches used to donate or receive pollen were bagged ahead of flowering using cotton muslin cloth to avoid ingress of bees or other pollinating insects. All the trial was done using single *in situ* trees, so on each tree there were bags for ten sites (branch) to donate pollen of uncontaminated flowers, bags for ten sites to receive pollen from five other trees (including two replicates for each cross), bags for two sites for self pollination (two replicates), and two sites marked for natural pollination (open pollination). So there were 7 treatments on each tree. Pollination, either self or cross pollination, was done on three successive days, to cover the period of flowering, by bringing flowers from the reserved branches to the receptor branches. Protective bagging was removed only to allow the hand pollination, until a few days after flower fall, when the protection was removed.

The structure of the trial was based on the hypothesis that all the varieties were self incompatible, so that any fruit set was due to the pollen transferred by the hand crossing. For each tree there was also two self pollination replicates, where the tree's own pollen was used to pollinate flowers. If there had been any self pollination, this would have invalidated the results of the crossing between different trees.

Results and Discussion

It was noted that the general level of fruit set on the control sections of branch (open pollination) was generally quite low. Hand pollination as practised in this trial would increase the setting of fruit up to ten fold. This indicated a lot of problems with the current practices, probably mostly to do with the absence of pollinating insects. The condition of the flower buds after what had been a very hard winter, the fertility status, the temperatures and humidity at flowering could all have had an effect. Presentation of data to farmers and discussion with them indicates a lack of knowledge of the need for pollination which results in the deliberate killing of bees, as they think the bees eat the flowers, and the planting of large areas to single varieties, with resulting nil crop.

The data obtained is reported in Table 1. Comparing the fruit set percentage obtained by selfing and intercrossed pairs or the average of all cross-combinations. It was shown that all six almond successions were self incompatible. On the other hand, the basic criteria for a successful cross- combination was fruit set with hand pollination equal to or higher than fruit set with natural background pollination (open pollination). The criteria for a non-successful combination was fruit set equal or lower than that with self pollination. The low fruit set with natural pollination brought the boundary line between successful/unsuccessful cross to be too near, so there were a lot of inconclusive results. Hence, the information from this trial is generally disappointing. Fruit set was low in the orchards, but occasionally there were some interesting results. The Carmel flowered much later than other varieties, including Nonpareil. The variety Carmel 167 was too late flowering to be of use with the local varieties and Nonpareil 171. Nonpareil 171 was pollinated by Abdul Wahidi 1003, but the reverse cross had nil pollination.

3. Pollination trials in the *ex situ* collections

The almond pollination trials in PHDP were done in 2008 with *in situ* trees at three locations in Kunduz, Samangan, and Khulm. For the 2010 and 2011 trials, the same principles were followed, with cross pollination in all combinations of varieties in sets of six varieties. In the national collections, which for almonds are situated in the centres in Balkh and Kunduz, there are six identical trees of each almond accession, and this layout was exploited to greatly simplify the trials. Out of the six trees, one tree was used to provide pollen to the other five varieties, and for self pollination, and each of the other received pollen from one of the other five trees in that trial set.

In 2010, the numbers of flowers available for the trials were in many cases too few to permit valid results to be drawn, and most of the varieties in Balkh and Kunduz suffered damage from a late frost. In 2011, many varieties set reasonable amounts of fruit for drawing conclusions, but other varieties

Table 1 - Pollination data from mature *in situ* exemplar trees of the national collection of almond varieties, khulm, Balkh province, 2008

Female (Receptor)	Male (Pollinizer)/Fruit Set %					
Location: Khulm, Balkh Province	*Qambari 143*	*Bellabai 144*	*Sattarbai sufi 145*	*Zang kaftar 148*	*Sattarbai bakhmali 149*	*Sattarbai no.4 154*
Qambari 143	0.2	12	10	4	13	2
Bellabai 144	30	1	17	8:05	0	4
Sattarbai sufi 145	13	10	0	8	11	2
Zang kaftar 148	9	3	0	2	3	2
Sattarbai bakhmali 149	14	0	3	16	0	0
Sattarbai no.4 154	2	10	3	7	2	0.4
Cross-pollination average	13.6	7	6.6	8.75	5.8	2
Location: Kunduz	*Marawaja kaghazi 166*	*Carmel 167*	*Sattarbai 168*	*Qaharbai 170*	*Nonpareil 171*	*Abdul wahidi 1003*
Marawaja kaghazi 166	0	0	0	1	0	1
Carmel 167	0	1	11	3	0	0
Sattarbai 168	2	0	0	0	2	0
Qaharbai 170	1	0	1	0	0	6
Nonpareil 171	2	2	7	4	1	8
Abdul wahidi 1003	1	0	0	15	0	0.3
Cross-pollination average	1.2	0.4	3.8	4.6	0.4	3
Location: Aybak, Samangan province	*Sattarbai 156*	*Sattarbai guldar 157*	*Sattarbai bakhmali 159*	*Qaharbai 160*	*Khairodini 161*	*Shokorbai 162*
Sattarbai sais 156	3	42	42	35	57	26
Sattarbai guldar 157	1	0	0	2	1	0
Sattarbai bakhmali 159	5	3	6	3	24	9
Qaharbai 160	22	15	27	0	20	6
Khairodini 161	16	32	8	11	2	6
Shokorbai 162	12	1	3	8	8	1
Cross-pollination average	11.2	18.6	16	11.8	22	9.4

Summery results, based on final percentage.

seemed to still remain in a juvenile phase. This did not allow for maximum values to be extracted from the trials.

Additional pollination trials were undertaken in the Badam Bagh Kabul centre, in the plots of the almond variety demonstration, which included a limited number of accessions.

It was considered important that information was collected about the condition of the trees for setting fruit. This is done by allowing a number of flowers to be open to natural pollination from bees and other insects. Bees were introduced to the national collections to maximise natural pollination. The proposition was that if any variety was not showing good fruit set with this natural open pollination, then any cross pollination efforts by hand would also not show good fruit set. Results from those varieties could clearly not then be used to indicate incompatibility in that cross.

After the mostly rather inconclusive pollination trials in 2010 and 2011, by 2012 the trees were quite large, and only individual branches needed to be marked before flowers opened.

Materials and Methods

A straight length of branch with an estimated 200+ flowers were chosen for the trial. The beginning and end of the selected length of branch were marked and any flower buds above and below the marked length of branch could be stripped off to allow easy counting. For each variety, the enumerators were asked to pick two trees at either end of the plot, to get a good average of the open pollinated count.

The layout of the pollination trial is shown for blocks of six trees, which is the standard layout in the National Collections. By using all six trees available in the accession, it can be seen that a set of six varieties can easily be managed (Fig. 1).

The varieties for the pollination trials were every year chosen based on the relative importance of their nuts in the commercial market. A meeting was held with almond traders in October 2008, where the traders were asked to assign monetary values to a range of samples of almonds in shell collected from the exemplar trees of the national collection of almonds. This selection of the most important varieties was important, as the number of possible combinations of almond accessions to be tested ran into many thousands.

Results and Discussion

The 2012 trials repeated some combinations which did not give clear results in 2010 or 2011, and quite a lot of useful information was obtained. The larger number of combinations tested also allowed for progress in identifying varieties that did not combine with each other, and the start of working out some incompatibility groups. Once a variety could be allocated to an incompatibility group, predictions can be made on what would be suitable combinations, without actually doing a field test.

Ahead of the 2010 trials it was suggested that two replicates should be made. However, for each combination there is the reverse cross, which makes a second replication. To obtain reasonable numbers for estimation of pollination compatibility, it was arranged that at least 100 flowers should be pollinated in each combination, but it was not considered necessary to pollinate more than 200 flowers for each combination. After 2010, the flowers were protected from bee pollination by nylon netting rather than cotton muslin. The nylon netting did not absorb moisture during rain, thus avoiding damage to flowers in wet and windy conditions.

A substantial number of results were obtained from the 2012 trials. Unfortunately in 2013, there were severe late frosts in the north of Afghanistan,

BLOCK Variety A

Give pollen Self pollen	Receive from D
Receive from B	Receive from E
Receive from C	Receive from F

BLOCK Variety B

Give pollen Self pollen	Receive from D
Receive from A	Receive from E
Receive from C	Receive from F

BLOCK Variety C

Give pollen Self pollen	Receive from D
Receive from A	Receive from E
Receive from B	Receive from F

BLOCK Variety D

Give pollen Self pollen	Receive from C
Receive from A	Receive from E
Receive from B	Receive from F

BLOCK Variety E

Give pollen Self pollen	Receive from C
Receive from A	Receive from D
Receive from B	Receive from F

BLOCK Variety F

Give pollen Self pollen	Receive from C
Receive from A	Receive from D
Receive from B	Receive from E

Fig. 1 - Layouts of almond national collection blocks for cross pollination. Each box in the diagram represents one tree in each variety block. Each variety is coded by a letter, in order to show the systematic layout of the trial. The code sheets would represent a different accession in each year's trials.

which meant that no results were obtained that year. Late frosts also affected pollination trials in the north of Afghanistan in 2014, and results were limited to the almonds in demonstration plots in Kabul, which is a much later flowering site and has not lost a crop due to frost since planting out almond varieties in 2009.

4. Successful combinations when tested for cross pollination compatibility 2008-2012

Some 93 different combinations of Afghan almond accessions (186 combinations when the reverse cross is taken into account) have been shown to be successful. This is calculated before the accession names have since been updated/rationalised in the National Register of Almond Varieties published in 2014. No further information can be deduced from a list of compatible varieties. What is more useful for making further deductions about the possibilities of successful combinations is the classification of pollination groups (Kester *et al.*, 1994), which is derived from information about combinations of varieties which do not successfully cross pollinate, as below.

From the information about incompatible combinations above, the following attempt was made to classify the different varieties in Incompatibility groups, based on results up to 2012.

Only later was it decided to work on the assumption that Sattarbai accessions 168, 771 and 1001 were all the same variety. It was also decided without the incompatibility data that Sattarbai 142 should be reclassified as Qambari, thus the incompatibility information would indicate that accessions 142 and 143 can be treated as different clones of the same variety.

The results are reported in Table 2 and 3, where the confirmed incompatible combinations and the individuated incompatibility groups are indicated. At this regard, it is worth noticing that Group A can't be said to be definitively separated from the others, due to non sufficient data at the end of the 2012 trials. Conversely, groups B, C and D are considered different.

Table 2 - List of incompatible combinations of almond accessions belonging to the National Collection of Aghanistan

Site	Year	Accessions
Khulm	2008	Sattarbai Bakhmali 149 x Bellabai 144
Khulm	2008	Sattarbai Bakhmali 149 x Zang Kaftar 148
Khulm	2008	Qambari 143 x Sattarbai No. 4 -154
Aybak	2008	Shokurbai 162 x Sattarbai Guldar 157
Kunduz	2011	Qambari 143 x Sattarbai 142
Kunduz	2011	Qambari 143 x Qambari 2009
Kunduz	2011	Sattarbai 142 x Qambari 143
Kunduz	2011	Sattarbai 142 x Qambari 2009
Kunduz	2011	Qambari 2009 x Sattarbai 142
Kunduz	2011	Sattarbai Bakhmali 2008 x Sattarbai No.4 -154
Kunduz	2011	Sattarbai 168 x Sattarbai 771
Mazar	2012	Sattarbai 168 x Sattarbai 1001
Mazar	2012	Sattarbai 771 x Sattarbai Sufi 145
Mazar	2012	Sattarbai 771 x Sattarbai Bakhmali 159
Mazar	2012	Sattarbai Sais 777 x Sattarbai Guldar 157
Kunduz	2012	Khairodini 846 x Abdulwahidi 153

5. Cross pollination for variety verification

A range of similar Sattarbai accessions, collected under accession number 168, 771 and 1001 from different growers had proven to be very similar when characterised across a range of leaf, flower and fruit characters per the UPOV standards (UPOV, 2011). Accession numbers 168 and 1001 were also included in the set of nut samples to be assessed by the almond traders in October 2008, and were given almost identical market values ahead of all the other almond varieties. It seemed too much of a coincidence when such similar and high value varieties proved to be incompatible with each other. It is difficult to imagine a scenario whereby one of those three accessions is the parent of the other, because

Table 3 - Incompatibility groups defined for some almond accessions of the National Collection of Almond of Afghanistan

A	B	C	D	E
Sattarbai Bakhmali 149	Qambari 143	Shokorbai 162	Sattarbai 168	Khairodini 846
Bellabai 144	Sattarbai No.4 -154	Sattarbai Guldar 157	Sattarbai 771	Abdulwahidi 153
Zang Kaftar 148	Sattarbai Bakhmali 2008	Sattarbai Sais 777	Sattarbai 1001	
	Sattarbai 142		Sattarbai Sufi 145	
	Qambari 2009		Sattarbai Bakhmali 159	

This designation of incompatibility group does not refer to any other classification of almond incompatibility groups that might have been used by other authors.

of the intrinsic nature of the incompatibility genes. The most logical explanation, because it is also the simplest, is that the three accession numbers 168, 771, and 1001 were just three different samples (clones) of the one variety that had spread across the north of Afghanistan by vegetative propagation by different nursery growers. The three different clones had already been distributed to registered mother stock growers, and all three continue to be maintained under their accession numbers, but the final saplings are all marketed under the new name of "Sattarbai Mumtaz" (="Superior Sattarbai")(Ministry of Agriculture, Irrigation and Livestrock, 2014).

Following this rationalisation of the Sattarbai accessions, it was decided to use this approach to try to identify other duplicates in the national collections. The accession Cardinal seemed to be phenotypically very similar to the accession of Carmel in the collection. Since no other record of an almond variety called Cardinal could be found, and because it was known that variety names gradually changed after introduction into Afghanistan with unmanaged distribution of varieties, the hypothesis that Cardinal was actually a misnamed Carmel was tested by attempting cross pollination of the two accessions in the demonstration orchard in Kabul.

Using the same protocol as with the other pollination testing, it was shown that Cardinal and Carmel would not cross with each other, with pollination in either direction. This was taken as confirmation that Cardinal was the same as Carmel and was therefore removed from the national collection.

Other pollination tests were done with the same objective in mind. An accession collected as Nonpareil 6041 appeared to be very similar to Carmel, but cross pollination with Carmel set seed, indicating that 6041 was possibly an offspring of Carmel, perhaps from a cross between Nonpareil and Carmel, which two varieties would have been introduced together into orchards in Afghanistan in the 1990s. This accession, 6041, was also included in the sets of six varieties as was normally used in the cross pollination trials, and some of its offspring from crosses with superior Afghan almond types continue to be grown and tested in the almond breeding programme.

Another accession, collected as Sattarbai 6038, was considered to be very similar with the varieties Ferragnes and Ferraduel. Again, cross pollination trials with all combinations of Ferragnes, Ferraduel and 6038 showed that accession 6038 was not either Ferragnes or Ferraduel. Again, Ferragnes and Ferraduel were also introduced into Afghanistan in the 1990s as a combination to be planted together, so accession 6038 could be from a seedling resulting from a cross of those two varieties.

6. Incompatibility in almond varieties of Afghanistan: final remarks

All the trials on pollination in Afghanistan have been done within the context of a development project, the Perennial Horticulture Development Project (PHDP) and its successor project PHDPII. The projects have been successful in developing perennial horticulture from the very foundations of the industry, that is from deriving knowledge about how to use the native and imported germplasm to develop sustainable economic production systems. Further development continues with continuing private and public sector programmes financed by the European Union, within a programme framework that is now attracting other donors.

The adaptive research programmes have been based on very simple and basic concepts that are implemented in the context of a country devastated by more than 35 years of almost continuous warfare and internal strife.

Almonds have been a traditional export for many years, and if a connection can be shown with the Fergana Valley to the north in Tajikistan, then the Afghans can claim an almond export industry to India and the Persian Gulf region for at least five hundred years (Hiro, 2006).

In terms of planting almond orchards, it is now possible to plant just two varieties in pairs that cross pollinate each other, in the same way there are the famous combinations of Nonpareil and Carmel in California, and Ferragnes and Ferraduel in France. A good combination for Afghanistan could be Nonpareil planted with Sattarbai Mumtaz. What is clear however, is that there must be quite a few different incompatibility genotypes among the Afghan almond germplasm, so the growers can be quite confident, that in the absence of any specific data on varietal combinations, the planting of four different varieties will almost certainly give good fruit set. It should also be noted that there is no reason to think that these four different varieties could not all be different Sattarbai types.

Future work is provide information on the recommended combinations, to demonstrate the importance of pollination with bees, to ensure orchard growers understand the importance of planting mixtures of varieties, and taking remedial measures where they have single variety orchards. More combinations of varieties need to be tested for pollination compatibility, until recommendations can be made for all required commercial varieties. However, the testing of further combinations can probably wait until capacity in the Plant Biotechnology Laboratory in Kabul is increased to allow for direct examination of the incompatibility factors within the DNA of each accession.

The testing of almond pollination within the context of a development project has been simplified because of necessity, such that emasculation was not practised (Kester *et al*., 1994). The theory of incompatibility has been extended to encompass pollination testing as an extra verification method in the identification of varieties. The results of the pollination trials, that are the seeds from the inter variety crosses, have been planted out and formed the beginnings of a selection process for almond crosses which is expected to result in the release of several improved lines to growers by 2017. The breeding programme has taken on a life of its own, and is now focused on achieving late flowering, productive Afghan almond types. Innovative methods of cross pollinating early and very late flowering almond lines have been developed, to ingress late flowering characters into the local germplasm.

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Short Note

Selected pomegranate germplasm from Afghanistan: morphological variability and relationship among collected accessions

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Key words: genetic resources, phenotype, *Punica granatum*.

Abstract: During the period 2008-09 and in the frame of PHDP 58 Afghan and 18 foreign pomegranate accessions have been collected and grown under homogeneous environmental and cultivation practice conditions in *ex situ* collections. Standardized procedures were adopted to describe mature trees, leaves, flowers and fruits for a total of 30 phenotypic traits. Within the National Collection of Pomegranate of Afghanistan, coefficients of variability ranged from 8.8 to 31.7% for fruit diameter and weight of non edible part, respectively. Principal component analysis revealed the absence of correlated variables among different organs. The whole set of accessions resulted discriminated on the basis of the studied morphological parameters and all the accessions were grouped into 3 sub-sets by hierarchical cluster analysis. Local accessions resulted distributed in the 3 clusters, nevertheless the largest one held all the foreign varieties while the second one included all the accessions collected under the putative name of 'Bedana'. The adopted morphological studies allowed to identify one true-to-type 'Bedana' accession, considered the best Afghan variety for fresh consume due to its very soft seed, and to solve the cases of homonymy. Analogously, various accessions originally collected from different regions of Afghanistan under the name of 'Kandahari' were identified and renamed.

1. Introduction

The market of pomegranate fruits is noticeably expanding worldwide. The increasing interest towards this photophilous and moderately xerophytic species relies essentially on it nutritional and nutraceutical properties, being considered a functional product of relevant benefit for the prevention of human diseases (Martinez *et al.*, 2012; Kotsiou and Tesseromatis, 2015). *Punica granatum* L. is native to Iran (Teixeira da Silva *et al.*, 2013), nevertheless a western area (which includes part of Anatolia, the Caucasus and Iran) and an eastern area (southeastern Turkmenistan, southern Tajikistan, Afghanistan, northern Pakistan, Kashmir, northern India and Nepal) are generally recognized (Alam, 2011). Due to its hardiness pomegranate naturalizes quite easily and many local landraces and varieties can be found in the area of the Mediterranean basin, in Middle east and in the Arabic peninsula (Al-Sadi *et al.*, 2015). Iran embrace a rich germplasm of pomegranate, with 760 specimens, 24 genotypes and various commercially important cultivars listed in different studies, nevertheless also India, Israel, Egypt, Tunisia, Morocco, Spain and Italy hold valuable genetic resources of *Punica granatum* (Holland *et al.*, 2009).

More than 20 varieties of pomegranate (locally called 'anar'), among which 'Bedana' (soft seeded), 'Khog Dandar', 'Tarwah', 'Boocha', 'Pastakai', and 'Soor', were grown in Afghanistan during the second half of the XX century (Alam, 2011). More recently, 48 cultivars of pomegranate from various provinces of Afghanistan were described and characterized in relation to fruit colour, flavour and seed hardiness

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(Samadi, 2008); among them, 'Kandahari' and 'Bedana' were considered excellent cultivars (Glozer and Fergusson, 2008).

In the frame of the Perennial Horticulture Development Project (PHDP - Phase I), several pomegranate producing areas of Afghanistan were surveyed during the period 2007-2008 and a set of the most valuable varieties was collected (Giordani *et al.*, 2014). The *in situ* pomegranate collection resulted comprehensive of 59 local accessions which were propagated by cuttings. The obtained saplings were planted in *ex situ* collections, together with 19 imported cultivars in Kandahar and Jalalabad, thus representing the National Collection of Fruits and Nut of Afghanistan (Saeedi *et al.*, 2012).

Morphological characterisation of native germplasm accessions is the first step for their rational introduction in modern and efficient production chains and a standardized description of phenotypic traits based on the adoption of specific descriptor lists is a pre-requisite for this purpose (Frankel, 1970).

The present study aims at describing and analysing the pomegranate accessions belonging to the National Collection of Varieties of Fruits and Nuts of Afghanistan.

2. Materials and Methods

Plant material

Data were collected during 2013 and 2014 from self-rooted 4 years old trees grown in the repository of the National Collection of Fruits and Nuts of Afghanistan located in Kandahar (latitude 31°36′ N and longitude 65°42′ E; altitude 1009 m a.s.l.). For each accession flowers (10 samples), fully expanded leaves (10 samples) and fruits (20 samples) were randomly collected for two consecutive years (2013-2014) from trees (6 samples) grown following standardized cultural practices.

Data collection

Traits were described by rating and coding procedures based on the EC 29 GENRES Project Pomegranate descriptor list and guidelines (GENRES, 1998; Bellini *et al*., 2008). Quantitative variables were measured and weighed adopting a manual calliper and a precision (0.01 g) electronic balance, respectively. Colour parameters were visually determined using a specifically designed colour chart; other qualitative characteristics were attributed by using illustrated charts. Data of 13 qualitative characteristics (tree habit, vigour and suckering tendency; shoot thorniness; leaf apex shape; number of flowering periods; flower calyx and corolla colour; colour change of calyx after petal fall; fruit juiciness and fruit skin colour and thickness; seed colour) and 17 biometrical variables (Table 1) were collected for all the studied accessions.

Table 1 - Mean values, ranges and coefficients of variation for 17 quantitative traits of pomegranate accessions of the National Fruit and Nuts Collection of Afghanistan

Variable	Mean	Range	CV (%)	Standard deviation
Leaf - Total length (mm)	68.4	52.6-86.2	11.5	7.9
Leaf - Blade length (mm)	63.2	48.0-78.5	11.5	7.3
Leaf - Blade width (mm)	20.7	16.2-26.7	11.7	2.4
Flower - Petal length (mm)	26.1	19.2-30.8	9.1	2.4
Flower - Petal width (mm)	19.9	14.3-30.2	13.4	2.7
Flower - Length of calyx (mm)	38.5	29.5-46.7	10.2	3.9
Flower- Width of calyx (mm)	13.8	9.1-24.3	14.4	2.0
Fruit - Equatorial diameter (mm)	84.5	64.9-99.2	8.1	6.8
Fruit - Diameter of calyx (mm)	21.1	11.6-35.3	19.6	4.1
Fruit - Height without calyx (mm)	76.4	56.7-94.0	8.8	6.7
Fruit - Calyx height (mm)	23.2	14.8-34.6	18.6	4.3
Fruit - Total weight (g)	309.3	134.8-490.1	24.3	75.1
Fruit - Weight of non edible part (g)	144.6	76.6-323.1	31.7	45.8
Fruit - Edible/Total weight (%)	53.8	18.7-70.8	13.5	7.3
Seed - Weight (g)	0.3	0.2-0.4	18.8	0.1
Seed - Length (g)	10.3	8.0-13.6	8.9	0.9
Seed - Width (mm)	6.8	4.9-8.4	12.2	0.8

Data processing and statistical analysis

Average and mode were calculated for all qualitative and quantitative parameters respectively; such values were used for the attribution of each accession to one class, regardless the year of data collection. Classes and relative notes were defined for each quantitative measured trait. After average value standardization, Multivariate Principal Component Analysis and Hierarchical Cluster Analysis (Euclidean distance and Ward's agglomeration method) were performed on XLS Stat software.

3. Results and Discussion

Studied accessions

The survey allowed to collect 58 Afghan accessions (Fig. 1) in stands represented by orchards and home gardens distributed in the Eastern (Kapisa, Nangarhar; 29 accessions), Southern (Kandahar and Farah; 16 accessions) and Northern (Takahr, Balkh and Sar-e-Pul; 13 accessions) areas, at altitudes ranging from 422 m a.s.l. to 1513 m a.s.l. Such accessions were grown together with 19 imported varieties including the international reference cultivar 'Wonderful'.

Morphologic and phenological trait variation

Descriptive statistical values for continuous biometric data of the whole set of 77 accessions are reported in Table 1. Leaf size showed a relatively low variation (CV% ≈ 11.5 for total leaf length, length and width blade), as well as flower biometric traits, while higher values of variations were observed in fruit and seed traits. Namely, minimum and maximum mean of fresh fruit weight (≈ 134-490 g) fall within the range of analogous values observed by Okatan *et al.* (2015), Caliskan and Bayazit (2013) in pomegranate collections from Turkey and by Mars and Marrakchi (1999) in a set of 16 accessions collected in Tunisia. On the other hand, Orhan *et al.* (2014), Mondragòn Jacobo (2012), Bartual *et al.* (2012), Ferrara *et al.* (2012), Russo *et al.* (2012), and Legua *et al.* (2012) observed higher mean values of fruit weight in accessions collected in Coruh Valley (Turkey), in Mexico, in Elche (Spain), in Apulia (Italy) and in Morocco, respectively. A narrower range of variability for this parameter (204-288 g) was observed in a study on Iranian accessions (Theranifar *et al.*, 2010). Seed (aril) fresh weight (mean 0.33 g; range 0.17-0.33 g), resulted higher than the values reported by Okatan *et al.* (2015) (0.28 g; range 0.14-0.41 g) for Turkish accessions and by Mondragòn Jacobo (2012)(0.20 g; range 0.12-0.26 g) for Mexican accessions, but lower than those found out by Caliskan and Bayazit (2013) in Turkish pomegranate accessions (0.42 g; range 17-67 g) and by Ferrara *et al.* (2012) (0.38 g; range 0.27-0.51) in Apulian pomegranates in Italy. The ratio between the edible part of the fruit (arils) and the total fresh fruit weight ranged from 18.7% to 70.8%, with an average of 53.8%, lower than the value (55.6%) found by Legua *et al.* (2012) in accessions from Morocco.

The beginning of flowering time ranged from 104 to 115 days from the beginning of the year while day 253 resulted the earliest date of the beginning of ripening time (Fig.1 A, B). The range of flowering and ripening time of the investigated accessions resulted of 11 and 44 days, with a discontinuous frequency for ripening time between days 269 and 284 (Fig. 1 B). The ripening time period (end of September-October) resulted similar to the Spanish and Turkish one; no significant correlation has been found between fruit size and ripening time, a pattern observed by different authors in the germplasm of other areas (Holland *et al.*, 2008; Martínez *et al.*, 2012; Caliskan and Bayazit, 2013).

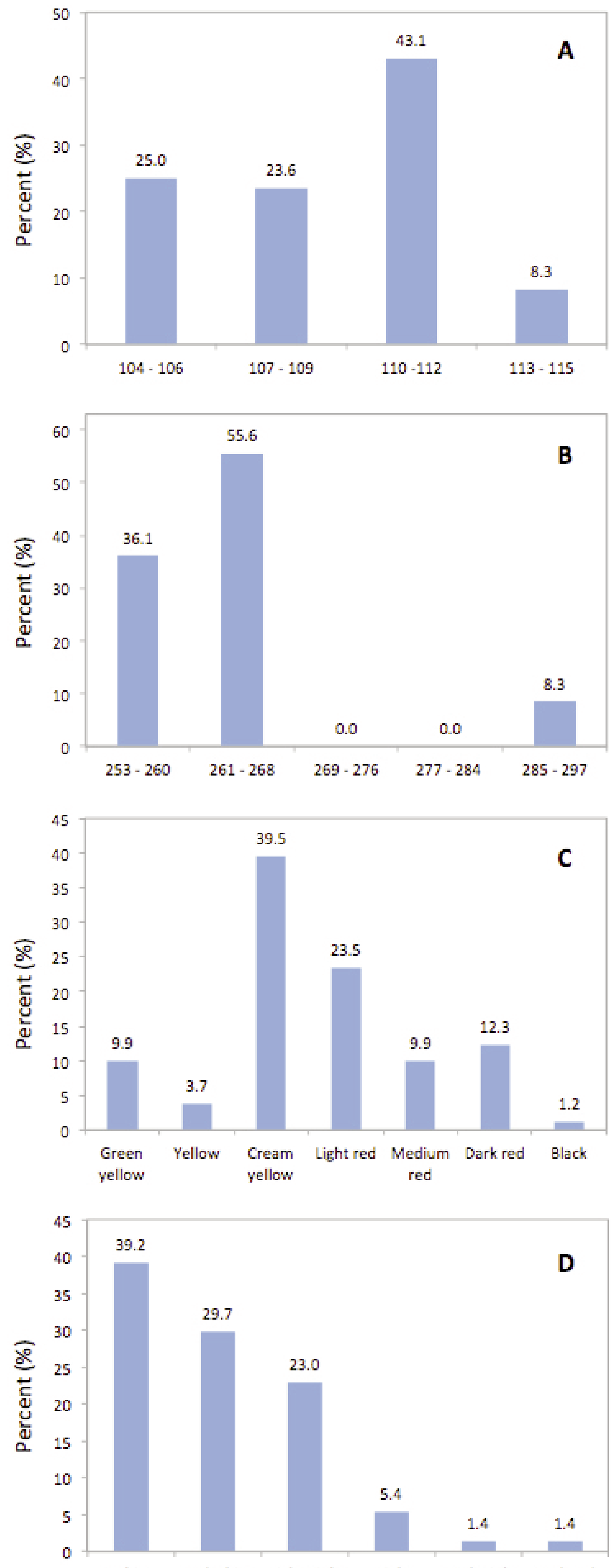

Fig. 1 - Distribution (%) of the collected pomegranate accessions in relation to: a) beginning of flowering time (day of the year); b) beginning of ripening time (day of the year); c) fruit skin color and d) fleshy part of seed (aril) color.

Attaining to colour fruit traits, which are important quality attributes in pomegranate marketing (Mena *et al.*, 2011), most of accessions presented fruits with yellowish skin (53.1%), followed by reddish colouration (45.7%), and only 1.2% of accessions showed totally black skin colour. Fleshy part of seeds was in most cases white (39.2% of accessions), while pinkish and red-white colour shared the same percentage (≈30%) of accessions and only 1.4 % of varieties showed dark red arils (Fig. 1 C, D).

Relationship between the described accessions

Principal Component Analysis based on the averages of the discrete scores of the morphological qualitative and quantitative variables showed a low correlation between the utilized variables, with 12 principal components with eigenvalue higher than 1, all together accounting only for 76.8% of the variability. Taking into account the sub-set of 19 biometric continuous variables regarding leaves, fruits, and seeds, the 6 principal components with eigenvalue>1 accounted for 78% of the whole variability, confirming a very low correlation among variables for the same set of accessions. Higher levels of correlation among the biometric variables have been reported for different groups of Turkish (Caliskan and Bayazit, 2013; Okatan *et al*., 2015) and Tunisian (Mars and Marrakchi, 1999) local varieties.

The average Euclidean distance of the generated proximity matrix based on the 17 biometric variables resulted equal to 5.96±1.21, ranging from 1.99 observed in the pair 'Bedana Samashkeli - Jan Mohammadi' up to 11.67 for the couple 'Sorkhak-Sakerdze'. The dendrogram generated by the Cluster Analysis is reported in figure 2, where three main significant clusters (C1, C2 and C3) can be observed. Cluster C1 resulted the largest with 35 accessions, 19 of which represented by foreign non Afghan accessions, including the international cultivar 'Wonderful'.

Cluster C2 was constituted by 19 accessions, 6 of which were originally collected as 'Bedana', a typical Afghan variety appreciated by the presence of very soft seeds. Albeit this easily appreciable and discriminating trait, the standardized characterisation of the presumed 'Bedana' accessions showed that only one accession could be identified as belonging to the true 'Bedana' type. The accession collected in Kandahar and entered in the collection as 'Bedana-AFG0383' showed heavy and hard seeds and other relevant differences from the 'Bedana' type, such as big calyx, pale skin colour and low juiciness, and it has been

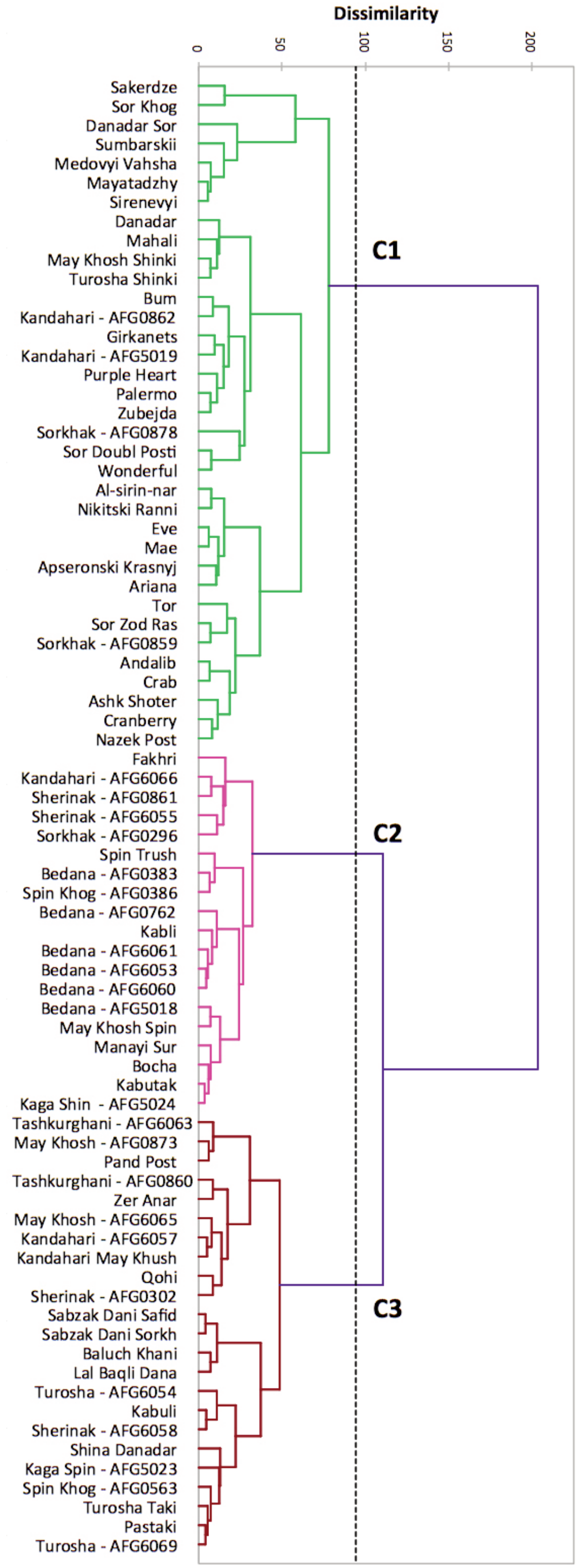

Fig. 2 - Dendrogram (Euclidean distance; Ward's agglomerating method) obtained by analyzing 30 multi-state discrete morphological variables of 58 pomegranate accessions of Afghan origin and 19 imported varieties belonging to the National Collection of Fruits and Nuts of Afghanistan (bold: imported cultivars; bold cursive: putative 'Bedana' type; cursive: putative 'Kandahari' type).

identified as ‘Mirwais Khani’ (Fig. 2). Similarly the accession originally denominated ‘Bedana-AFG0762’, brought medium hard seeds, white-pink arils, and was renamed as ‘Abasi’. Also ‘Bedana-AFG5018’, collected in Tabag district of Kapisa Province, and ‘Bedana - AFG6061’, originated from Sherzad district of Jalalabad Province, did not show the typical characteristics of the true-to-type ‘Bedana’, and were renamed as ‘Ghani Kheli’ and ‘Jan Mohammadi’ respectively. Finally, the presumed ‘Bedana’ type ‘AFG6053’, originated in the district of Jalalabad, notwithstanding its high morphological similarity with the true-to-type ‘Bedana’ (soft seeds, juicy and good taste), showed low productivity and was denominated ‘Bedana Samaskheli’.

Cluster C3, holding 23 local accessions, is not evidencing any special relationship among previously known cultivars.

During the survey, a part from ‘Bedana’ name, another recurrent denomination attributed by farmers to local accessions was ‘Kandahari’ (from Kandahar). Indeed ‘Kandahari’ accessions were collected from different provinces of Afghanistan and they showed different and unique phenotypic traits during the characterisation hence they have been considered as different varieties. For instance, ‘Kandahari - AFG6066’ originated from Sher Zad district of Nangarhar Province, had thin fruit skin and hard seeds, and it was renamed as ‘Rahmani’; ‘Saifi’ was the accepted name for the former ‘Kandahari - AFG5019’, collected in Tagab district (Kapisa province) producing fruits with hard seeds, reddish arils and low juiciness. ‘Mukhtari’ was originally ‘Kandahari - AFG0862’ from Khulam district (Balkh Province) with medium hard seeds and white arils. Actually ‘Kandahari’ name was kept only for the accession collected as ‘Kandahari - AFG6057’ originated from Tagab district (Kapisa Province) and producing large fruits with medium hard skin, medium hard seeds and red arils.

4. Conclusions

This study was the first of its kind carried on for pomegranate in Afghanistan in terms of standardized methodology; comparative analysis with previous data collected from local Afghan varieties of pomegranate trees grown in different environmental conditions and as unique individuals resulted not completely reliable. The results revealed a wide variability of morphological traits within the Afghan pomegranate collected accessions; this finding is consistent with similar researches carried on local pomegranate accessions in countries where *P. granatum* is present since ancient time. Such variation can be considered as a result of the combined effect of sexual reproduction and asexual propagation. On the other hand, many cases of homonyms have been observed, hence confirming a common trend in the denomination of varieties by farmers of countries where perennial species are grown traditionally.

More specific morphological studies associated to the evaluation of tree productivity and fruit biochemical and commercial traits would be the next step in order to better classify the Afghan pomegranate accessions in relation to their main suitability for different uses (fresh fruit consume, juice production, food supplements, etc.).

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Introduction of determination of optimum harvest date in Afghanistan. Sweet cherry: a case study

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Key words: harvest date, maturity index, sweet cherry.

Abstract: In the last decade, Afghanistan has been under the political attention by several Institution and Organizations whose invested millions of dollars for the reconstruction of the country. Part of such efforts were focused on the agricultural sector, as the principal source of income of Afghanistan. Mainly, perennial horticulture represents the one of the most challenging opportunity for the developing economies of Afghanistan, and the high contribute of EU in such direction, shown significant results. However, the level of the export is still neglected and the reputation of Afghan' products has lost the ancient brittleness. The generic low hygienic conditions and poor quality of the products are the primary cause of such situation. In this scenario, PHDP (Perennial Horticulture Development Project) first and ANHDO (Afghanistan National Horticulture Organization) then, tried to improve the quality of perennial horticulture crops. PHDP has, for the first time created a certification scheme for the production of certified perennial plants, ANHDO has the main goal of improving the quality of the fruit value chain of the principal crops in Afghanistan. In this study the first protocol for the determination of the Optimum Harvest Date (OHD) for Cherry var. Burlat is given, as a case study of the new course of Horticulture in Afghanistan.

1. Introduction

During the last decades, after over ten years of reconstruction projects, Afghanistan horticulture has grown to the pre-war level. In 2014, the estimated horticulture crops extended over 340,000 ha that represents the 14% of the total irrigated land. The overall contribution of horticulture is valued at 1.4 billion (6.7%, to the total GDP) and the 34% of the agriculture GDP contribution with a crops employment generated estimated in 2 million people involved to various degrees. Principal crops are grapes ($330m), almonds ($120m), pomegranates ($100m), all of which are largely exported to the neighbour countries. By comparison, illicit crops (opium poppy, cannabis) in the same period are grown on 220,000 ha with an estimated value of about $1 billion. Many important fruit and vegetables are origin from Afghanistan (e.g. pistachio, walnut and pomegranate) and Afghanistan has favourable climate condition suitable for high quality horticulture. The hypothetically development of the horticulture sector is huge as well as the potential international interest for their quality (pomegranates, apricots, almonds, raisins). Despite the improvements, especially the road network, the horticulture sector claims a lack of massive interventions. As a consequence, the overall quality is still low due to a well-known list of constraints: small land holdings, small commercial orchards, lack of standardized product, poor orchard management, lack of infrastructure and substandard storage, sorting, packaging, marketing, transport facilities.

Afghan quality control system

In general, the quality of the agriculture products is the final result of a long list of interventions, controls and standards adopted by a nation, generically define as quality control system.

The quality control system has the primary function of protecting its citizen by selecting active controls of the imported commodities at the borders and promote the safety of the exported goods. The lack

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of an efficient quality control system, affects the horticulture sector in many ways. The general situation in Afghanistan is critical in both the import/export directions: international requirements (rules and consumers education) on food are extremely rigid and difficult to overcome for Afghan traders. From the import side, the situation is without control. The absence of an explicit laws with a specific phytosanitary standards regulation, allows to the neighbour countries to freely provide goods and services without any reliable control. The consequence is a risk for the food safety and human health aspects (e.g. OGM) and besides that also the religious point of view is involved. The respect of Halal rules is in fact often not certified and the customs authority has technical difficulties to check the provided certificates.

For the above mentioned reasons, the national quality control system of Afghanistan is now under a deep revision: Afghanistan has started the World Trade Organization (WTO) membership path.

This is a very important step forward for the quality control of the country, especially for the agricultural commodities: join the WTO will allow to Afghanistan to implement non-tariff barriers useful to protect his local market from the massive import from neighbours countries i.e China, Pakistan and India on top (WTO, 1995 a). Beyond WTO, the gap between the Afghan food industries and the international competitors, is consistent. Afghan industries may require large investment to make them efficient and competitive in a reasonable period of time. Applying a protection policy is probably, the best way for Afghanistan to fill the gap with its competitors. Protection would act as incentive for such industries, the introduction of Technical Barriers agreements (TBT) may establish a more transparent trade with the neighbour countries and allow to Afghanistan a better control of the imported commodities based on harmonised rules. Application of TBT required the WTO membership and the main obstacle to this is the lack of a Food Law that will clarify the different responsibilities among the Ministries and will produce the conditions to the creation of the National Quality Control System. The responsibilities on the food safety are now divided between the Ministry of Public Health (MoPH) and the Ministry of Agriculture Irrigation and Livestock (MAIL) but such division is not clear, with the consequence of an inefficient Sanitary and Phytosanitary System, with respect to the requested SPS agreement (WTO, 1995 b).

At the best of our knowledge, the Food Law is still stuck in the Parliament and Afghanistan doesn't adopts any procedures and a clear set of harmonized standards. Such a law represents a necessary milestone for the future of agriculture sectors, but it is not sufficient to promote a real food quality control in Afghanistan. The first goal of such law must be the protection of human health from risks created by uncontrolled trading, tools as quarantine measures, certification, borders checks, inspections network and laboratory tests are the pillars of the quality control. In the last ten years, the PHDP has successfully developed a certification scheme for the horticulture crops and keep the horticulture to an upper level of quality in the whole Afghanistan. Moreover, PHDP developed the national collection orchards (NC) where the local and international varieties were tested for their adaptive level in the specific Afghanistan climate. Within its activities, PHDP has also set-up six pomology laboratories where, for the first time, local and introduced horticulture varieties were characterized under many aspects (e.g. UPOV, grafting, etc.). The PHDP project ends up with a great numbers of varieties, however, not all these varieties were fully characterized and some basic information are still missing. Specifically, the harvesting date and maturity index were not included as tested parameters because out of the scope of the project. Horticulture Private Sector Development Project (HPS) has, in one specific subjective (SO3), as primary goal, the evaluation and the promotion of the fruit commercial varieties. ANHDO is the implementing partner of HPS and in close collaboration with MAIL (that has inherited the ownership of the Pomology laboratories) has succeeded to the PHDP cultural heritage and is developing the missing horticulture parameters: in the next two years at least 60 varieties of the major commercial horticulture crops will be fully characterized and studied for the pomology basic tests, including post-harvest tests, maturity indexes and harvesting date by HPS specific sub objective (SO2 - Adaptive research).

ANHDO/HPS ambitions are high and even if the pomology laboratories are well equipped and the technical staff has been trained, the overall technical level is still critical due to the general environmental situations and only basic tests are possible.

Fruit quality in Afghanistan: Cherries

As least development country, Afghanistan has a total potential catchment not depending on the quality of the products and the domestic market has the peculiarity of accepting every type of fruits. The wide range of the existing social classes creates consumers with different tolerance and allow traders to sell all the product without any formal losses. The well-known effect of the extended cherry shelf-life over the 7-10 days (loss of firmness, color and flavour, stem discoloration, desiccation and mould growth) will not affect the trade. However, such condition is changing with the increase of the consumer awareness. Soon, farmers and traders will face, for the first time, the necessity of an improvement in the cherries quality. To be prepared, for the very first time in Afghanistan, HPS has set-up a formal protocol to start the characterization of the main commercial fruit varieties, with the final aim of helping farmers to harvest the crops in the optimum period for domestic consumption or international export.

Aim of the work

The aim of the study is to provide a simple and pragmatic in-field tool for the ANHDO/HPS beneficiaries farmers, to identify the appropriate harvesting date and suitable maturity index for cherry cv. Burlat. The proposed approach is a compromise between scientific research (that requires at least twenty single observations) and the real condition of Afghanistan that forced to reduce such observations to five. PHDPII project provided initial data on the harvesting period of many varieties, based on visual observations. This study will confirm the hypothetical harvesting date and will provide (among different existing parameters) one or two easy and quick maturity indexes for the Afghan farmers.

Determination of optimum harvest date of sweet cherry cv. Burlat grown in kabul

Optimum Harvest Date (OHD) is defined as the date in which harvesting results in optimum fruit quality of the produce after long-term storage (Baumann, 1998). Typically, OHD is predicted using calculation models or through direct measurement of certain physiological fruit parameters and quality characteristics during a given period until harvest (Çalhan *et al*., 2014). The first method required historical series of meteorological and phenological data that are not available in Afghanistan. On the contrary, direct measurements of fruit characteristics, in specific intervals before the estimated harvest date, are more reliable for specific cultivars and applicable in Afghanistan. This study was conducted to evaluate OHD through physical and chemical measurements of fruit quality as a maturity index for estimating proper harvest time of Burlat cherries, in Kabul. Among the exiting maturity indexes, sugar content (SSC), titratable acidity (TA), SSC/TA ratio, skin color, and firmness have all been used as indices of cherry fruit maturity. However, skin color has long been accepted as the best indicator for the appropriate harvest maturity of sweet cherries (Drake and Elfving, 2002).

2. Materials and Methods

Cherry cv. Burlat has leaves sawed, average green, slightly lengthy and pointed. Burlat fruit has a dark red or purple skin, slightly flat and mean sized. The flesh is dark red, with sweet taste, juicy and firm. It is defined as early ripening, it is self-incompatible and it needs a pollinizer.

Fruit from the sweet cherry cv. Burlat was harvested during 5 different dates (between 18.05.2015 and 27.05.2015) from the National Collection in Badam Bagh, Kabul, Afghanistan. Full bloom date of Burlat was at 19.04.2015. The harvesting period was concentrated in 10 days due to existing data obtained from previous tests on the same station. In the National Collection each varieties is replied 6 times in a row. Fruits were collected early in the morning from branches with the same orientation (South-East) and all the fruit from the selected branches were immediately transferred to the pomology laboratory. In the laboratory, samples with defects (split, diseased or damaged fruit) were annotated and then rejected. The collected cherries were then divided into 5 maturity class according to their visual skin color:

a) Yellowish - immature;
b) Light red - nearly mature;
c) Red - mature;
d) Dark red - ripe;
e) Overripe.

The number of cherries for each class was annotated. In this study only Red and Dark groups were considered. Fruits parameters were collected by 24 fruits per class for a total of 48 samples. Weight was measured by an electronic balance (Kern EMB-200) to an accuracy of 0.01 g; results were expressed as gram (g). Fruit width and length was measured using manual calliper (Verinier caliper 0-150 mm). Results were expressed as millimeter (mm). Total soluble solids concentration (TSS) was determined with a temperature compensated digital refractometer (ATAGO Pal-1) previously calibrated with distilled water and results expressed as °Brix. Titratable acidity (TA), was estimated by juice titration with 0.1 N NaOH to the titration end point of pH 8.1, monitored with a pH meter (HANNA Instruments HI3221) and expressed as malic acid content (mg/100 mL). Flesh firmness was defined as the maximum load required to push the 6 mm diameter probe into the fruit firmness one side of each fruit, to a depth of 6mm with fruit texture analyser (Fruit Pressure Tester FT 327). The results were expressed in Newton (N). Skin color was measured on 24 fruits per class using a Minolta Chromometer Model CR 400 and average readings at six pre-determined points on the circumference of the fruits were recorded. The instrument was calibrated against a standard white color Plate (Y=93,9, x=0,313, y=0,321) (Konika Minolta, 2013), Color space (XYZ) (Batu, 2004; Žnidarčič and Požrl *et al.*, 2006).

All the above mentioned parameters were measured between 18.05.2015 and 27.05.2015. The final dataset was statistically analysed by Pearson correlation test, ANOVA test and PCA using Systat 12 software and Minitab 17.

Part of the graphs do not shown Red group values at the latest observation. This happened because during the division in maturity classes - immediately done after the harvest - in the last part of the maturity process, the Red group was without samples. Such a situation is obviously due to the ripening of cherries and the lack of non-mature fruits.

3. Results

As reported in figure 1, Red groups weight increase at maximum rate to 24/05 and then starting decreasing. On the contrary, the Dark group results do not shown significant variation of weight. This suggest that the maturity weight was reached before the start of the observations, and is confirmed by the red group trend that increases as confirmation of the maturity development. As reported in figure 2, height increase during the observation period. However, as expected, the height/diameter ratio is not changing (Table 1). The difference between the first and the last observations reports an increase of 7.56%. This result is clearly explained by the shorter observation period where the contiguous observations are not significant.

Firmness (Fig. 3), as expected, decrease during the observations period and drastically dropped between 24/05 and 26/05. Considering the Dark red and Red Groups separately (Fig. 3), the decrease is 31.89% and 50.23% respectively. Sugar content (Fig. 4) is constantly increasing for both Dark and Red groups.

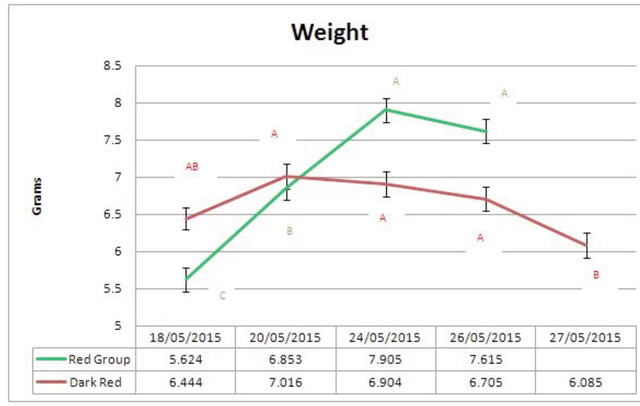

Fig. 1 - Effects of different picked dates on 48 fruit weight (g) of sweet cherry cv. Burlat fruits. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

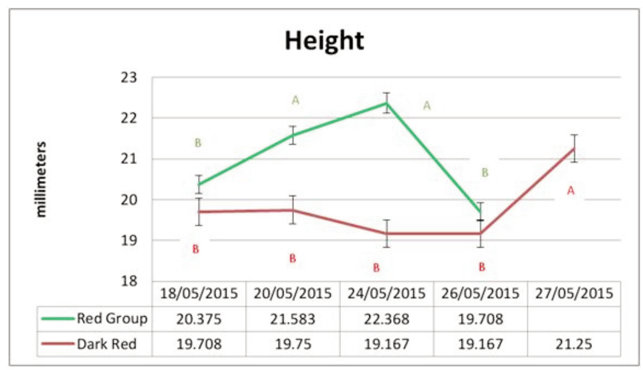

Fig. 2 - Effects of different picked dates on 48 fruit height (mm) of sweet cherry cv. Burlat. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

Table 1 - Effects of different picked date of ratio between height and diameters (H/D) on 48 fruits of sweet cherry cv. Burlat

Date	Height-diameter ratio					
	Dark red group		Red group		Error D.R.	Error R.
5/18/15	0.852	A	0.893	A	0.017	0.018
5/20/15	0.859	A	0.879	A	0.018	0.018
5/24/15	0.833	A	0.839	A	0.018	0.019
5/26/15	0.834	A	0.861	A	0.018	0.018
5/27/15	0.984	B			0.018	

Means with different letter are significant different (Tukey's HSD p<0.05).

The most significant increase is visible between 24/05 and 27/05, in which the sugar content passes from 20.27 °Brix to 24.73 °Brix for the Dark group. In the last day of observation (27/05), none of the sampled cherries was classified as red group, as consequence of the ripening process. The pH values, in this study, do not provide a reliable maturity index, as the variation observed during the experimental period were significant but ranged within the instrument

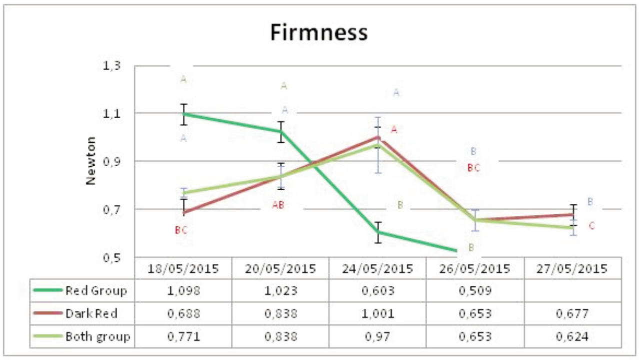

Fig. 3 - Effects of different picked dates on 48 fruit firmness of sweet cherry cv. Burlat compared with Red and dark red group. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

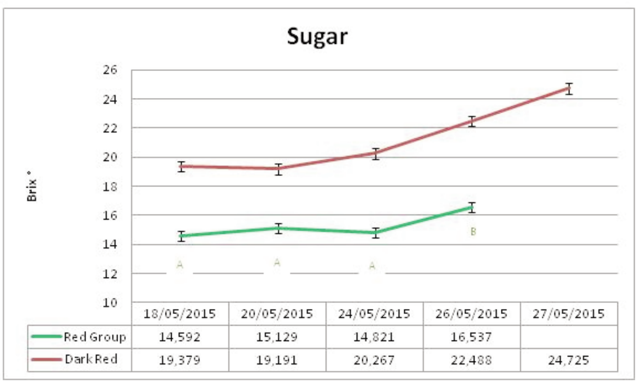

Fig. 4 - Effects of different picked dates on 48 fruit sugar content (Brix°) of sweet cherry cv. Burlat for both Dark and Red groups. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

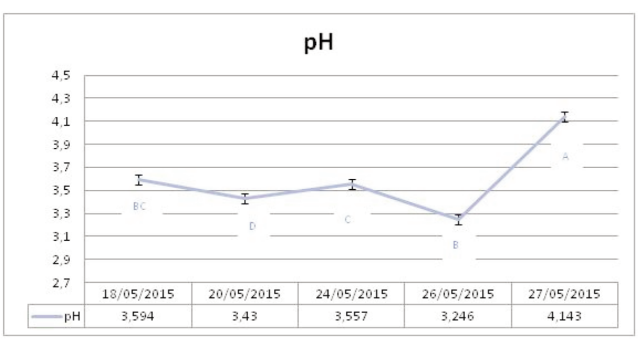

Fig. 5 - Effects of different picked dates on 48 fruit pH of sweet cherry cv. Burlat. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

error (Fig. 5). The graph reported in figure 6, perfectly, explain and reflects the Burlat maturity pattern of TA: the total acidity starts decreasing from the 26/05 in the Dark Group. Such a result, compared with sugar content clearly states that cherry acidity is decreasing, while the sugar content is still growing (Fig. 4). Considering the path of the sugar content

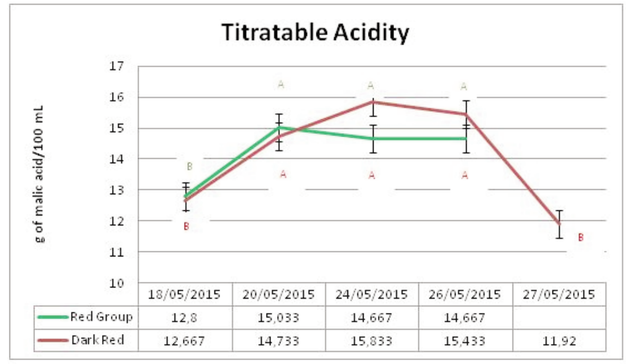

Fig. 6 - Effects of different picked dates on 48 fruit sugar content and tritatable acidity (TA) of sweet cherry cv. Burlat fruits for both Dark and Red groups. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

and the TA result, is possible to set the maturity days between 26/05 and 27/05. The same conclusion is obtained by looking the color XYZ-CIE results (Fig. 7), in which the variation of color is stable in the same period. The identification of the most suitable parameter used as easy maturity index, passed through a first analysis of the data set using a PCA analysis. However, the interpretation of a PCA with more than 8 factors and at least three principal components, is not easy. To overcome such problem the Pearson correlation matrix was used to reduce the number of parameters analysed with PCA. The main correlations among X, Y, Z parameters are reported in Table 2. The color components are very well correlated, and such strong correlations justify to use only one of the

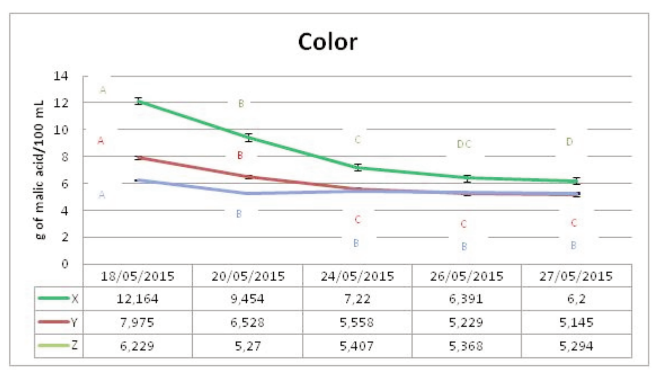

Fig. 7 - Effects of different picked dates on 48 fruit XYZ CIE color of sweet cherry cv. Burlat for both Dark and Red groups. Vertical bars represent standard error. Means with different letter are significant different (Tukeys's HSD p<0.05).

Table 2 - Pearson correlation for the XYZ-CIE parameters

	X	Y	Z
X	1.000		
Y	0.993	1.000	
Z	0.834	0.883	1.000

three components in the PCA (Tabachnick and Fidell, 2006). Therefore, only X factor has been considered in the simplified PCA test, because from the statistical analysis (Fig. 7) such variable appears more sensible to the ripening process compared to Y and Z. Table 3 reported all the other correlations. Between diameter and weight the correlation is sufficient to justify the usage of the height and diameter ratio (H/D) to get an unique shape factor that will reduce the variability in the data set (Tabachnick and Fidell, 2006). Sugar content and pH are well positively correlated (0.694), as expected. Applying the above mentioned consideration, the simplified PCA is reported in figure 8. The first three components explain 71.8% of the variability. Table 4 reports loadings values for the first three components. SSC and pH have the highest loadings values and are positively correlated, thus able to explain almost all the variance of the dataset. Also color has a high loading value (Table 3) that suggests it as one of the possibly maturity index suitable for the Afghan farmers. The parameter weight explains the remaining variance. Weight, as very easy and cheap measure can be used as additional maturity index with sugar content, pH and color.

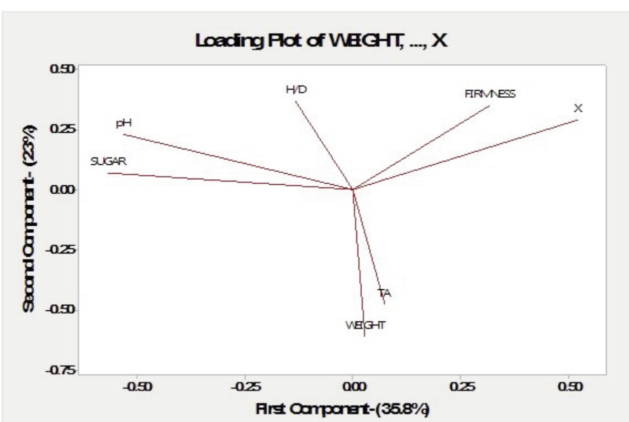

Fig. 8 - Simplified PCA using the following parameters: Weight, SSC, TA, pH, H/D, Firmness, X.

Table 4 - Variance explained by components

Variable	PC1	PC2	PC3
Weight	0.027	-0.609	0.166
H/D	-0.133	0.367	-0.724
Firmness	0.317	0:35	-0.001
SSC	-0.568	0.069	0.003
pH	-0.532	0.733	0.166
TA	0.072	-0.477	-0.645
X	0.520	0.291	0.070

4. Conclusions

The Cherry cv. Burlat OHD is set for the year 2015 at 26th of May. Such result must be verified in the next coming years and correlated with the meteorological situation. Based on the whole results dataset, the suggested 'Burlat' characteristic at the OHD are: height: 19.167±0.254 mm, weight: 6.705±0.126 mm, pH: 3.246±0.009, sugar content: 22.488±0.5°Brix, TA: 15.433±0.450 g/ml, firmness 0.653±0.037 N, X: 6.391, Y: 5.229, Z: 5.368 (RGB=6B3540 HTML color).

As explained, the aim of the study was providing a simple tool applicable in the field as maturity index to the Afghan farmers. As reported, sugar content, pH, color and weight have very good chances to be used as maturity indexes. However, for the color determination, the required instrument is very expensive and not suitable for the Afghan condition, in which the gross national income per capita is 640 USD (World Bank, 2004). A similar conclusion is valid for the pH. Sugar content is, thus, the suggested solution for farmers. It is relatively cheap and extremely easy to use. However, based on the laboratory results, HPS may provide color gauge specific for each single varieties to the HPS beneficiaries and confirm with SSC, weight, pH and color tests the validity of sugar content as maturity index.

The same methodology applied for 'Burlat', and explained in this study, was used with four other

Table 3 - Pearson correlation for all paramenters

	Weight	Height	Diameter	Grade	Firmness	SSC	X	pH	TA
Weight	1								
Height	0.274	1							
Diameter	0.599	0.485	1						
Grade	0.540	0.121	0.423	1					
Firmness	-0.207	0.053	0.119	-0.098	1				
SSC	-0.134	-0.259	-0.339	0.035	-0.251	1			
X	-0.302	0.073	0.023	-0.382	0.417	-0.704	1		
pH	-0.199	-0.207	-0.315	-0.037	-0.260	0.694	-0.481	1	
TA	0.129	0.045	0.153	0.262	-0.193	0.152	-0.306	-0.126	1

cherry cultivars: Balck Star, Cherry Pie, Santina and Stella Compact, following the estimated OHD:
'Black Star': 6 June, 'Cherry Pie': 30 May-6 June, 'Santina': 4 June, 'Stella Compact': 6-11 June.

Acknowledgements

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The phytosanitary status of the National Collection of fruits and nuts of Afghanistan and the private Mother Stock Nurseries: a virus survey

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Key words: germplasm, plant pathology, virus disease.

Abstract: The horticultural industry is a vital component of the agriculture sector of Afghanistan, the primary engine of the country's recovering economy which engages approximately 80% of the working population. This sector was thriving in the 1970s, but is today incapable of competing in the international market. To recover and develop the horticulture of the country, the European Community (EC) supports the PHDP (Perennial Horticulture Development Project), to provide true to type/ecotype and healthy planting materials, and the Plant Biotechnology Laboratory, to ensure the health status of local germplasm. This laboratory started screening the health status of the Afghan Germplasm National Collections in order to ensure the multiplication of not only the best-selected varieties or ecotype, but also to avoid production and distribution of virus-infected trees. Inspection for symptoms and sample collection for viral diseases was carried out in all the National Collection fields, including cherry, pear, peach, plum, apricot, almond, apple, grape and citrus plants, located in different areas of the country. Stone fruit plants infected by *Apple chlorotic leaf spot virus* or *Prunus necrotic ringspot virus* have been identified in the National Collection experimental farms located in different provinces of Afghanistan. Moreover, many grape plants included in the National Collection located in Herat and Kandahar resulted infected by *Grapevine fanleaf virus*, but only few imported plants by *Grapevine leafroll associated virus 1*, *Grapevine leafroll associated virus 3* or *Grapevine virus A*. Finally, in Jalalabad (Nangarhar province) citrus plants showing vein flecking, yellowing and plant decline symptoms were found to be infected by *Citrus tristeza virus*. Some of the identified viral isolates have been characterized molecularly, amplifying a fragment corresponding to the coat protein gene from a selection of positive samples. The presence of those viruses in different accessions of the national collection is of concern for Afghan horticulture. Implementation of the certification schemes is therefore necessary to quarantine the production and for the employment of virus-free propagating material.

1. Introduction

A variety of horticultural products is grown in Afghanistan with the potential to be (re)developed into valuable export commodities and brands. The primary engine of the Afghan economy is its agriculture industry, which engages approximately 80% of the working population. Fruit trees, such as peach, plum, apricot or almond and grape are therefore of significant economic importance to the country, with importing or local exchange of plant material for propagation or commercial growing of these fruits likely. The absence of certain viruses is an essential pre-requisite for virus-free certification of plant material in general in many parts of the world (Golino and Savino, 2005; Barba *et al.*, 2015). In Afghanistan, there is a tremendous increase in fruit tree nursery production and regulation of phytosanitary certification of plant propagation material is therefore of major importance.

In 2005 the Ministry of Agriculture, Irrigation and Livestock (MAIL) stated in the agriculture master plan that the rejuvenation of the horticulture sector would be a government priority. Thriving in the 1970s, this sector has become incapable of competing in the international market. Three interconnected problems have been identified for the industry: 1) the existing orchards are run-down and production is

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dwindling in quantity and quality; 2) nursery owners lack access to quality inputs and services; and 3) the regulatory framework to develop and support a competitive horticulture industry is minimal.

To support and develop the horticultural industry of the country, a nursery certification system was started in 2006 from the complete collection Afghanistan's fruit and nut trees crop varieties by the Perennial Horticulture Development Project (PHDP) funded by European Union (EU). One of the main causes for the decrease in production is deleterious pests and diseases, especially plant viruses and virus-like diseases present in Afghanistan.

Virus infection in plants is particularly difficult to detect as viral infection may be sectorial or latent, with symptoms masked or even absent under certain conditions. Virus-free certification is currently implemented using conventional techniques for virus detection in vegetal material such as biological indexing, serological or molecular assays. Biological indexing requires, in many instances, grafting in greenhouse facilities which is expensive and time consuming. Serological assays and molecular techniques, particularly ELISA and RT-PCR, have been tested in several laboratories and shown to be efficient for detection and quantification of virus infection in pome and stone fruits (Gambino and Gribaudo, 2006; Faggioli *et al.*, 2013). In a country such as Afghanistan, the establishment of a laboratory where sensitive, reliable and specific techniques for routine detection of selected viruses of fruit trees can performed is therefore essential for virus-free certification.

Many viral diseases can affect fruit trees, resulting in latent or symptomatic infection. According to their effect on the cultivated host, presence of the most important viruses in propagation plant material is regulated at international level. In particular, *Plum pox virus* (PPV) is the causal agent of Sharka, the most damaging virus disease of stonefruits. Some strains of *Apple chlorotic leafspot virus* (ACLSV) can also induce serious diseases in stone fruits. *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) are of considerable economic significance in stonefruits as well, while *Citrus tristeza virus* (CTV) is considered to be the most destructive virus of citrus crops. *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV) and *Apple stem pitting virus* (ASPV) are spread worldwide and able to causes significant losses in mixed infections (Hadidi *et al.*, 2011; Barba *et al.*, 2015).

Arabis mosaic virus (ArMV), *Grapevine fleck virus* (GFkV). *Grapevine fanleaf virus* (GFLV), *Grapevine virus A* (GVA) and "grapevine leafroll-associated viruses" (GLRaVs) such as *Grapevine leafroll associated virus 1, 2* and *3* (GLRaV-1, GLRaV-2 and GLRaV-3) are of great economic impact in grape and their absence in propagation material is an essential requirement in the certification schemes of most countries (Martelli, 2014).

National collection centres were established in six agro-ecological zones in Afghanistan to collect germplasm. After registration and cataloguing, the mother stock nurseries were established from the same planting material to ensure the availability of high quality and healthy planting material to nursery growers and farmers.

Theoretical and practical knowledge are essential to develop a complete profile for viral diseases in order to improve the quality of planting material within the national fruit tree germplasm (Rizzo *et al.*, 2012, 2015). We therefore developed an in-depth study regarding the detection and identification of viral pathogens through the use of advance and sensitive methodologies and techniques to characterize identified plant viral pathogens and to develop control strategies for the management of diseases.

These efforts are aimed at improving the quality of planting material and will ensure access to quality and certified planting material for nursery growers and orchard owners for the establishment of new orchard/motherstock nurseries, as well as for the rehabilitation of existing orchards. Screening agricultural products according to the protocols and regulations of global plant quarantine will also positively affect the import and export of Afghan agricultural entities. Following the standards of international sanitary and phytosanitary rules and regulations is the only way to compete in the international market.

2. Materials and Methods

Plant material

Plant material was collected from the National Collection Centres (NCCs) and Mother Stock Nurseries (MSNs) from 2009 to 2015. In total, more than 12,000 samples were collected from different species and tested for different viruses. In particular, almond, apricot, cherry, peach and plum samples were tested for the presence of ACLSV, ApMV, PDV, PNRSV and PPV; apple and pear samples for ACLSV, ApMV, ASGV and ASPV; citrus samples for CTV; and grape samples for ArMV, GVA, GFkV, GFLV, GLRaV-1, GLRaV-2 and GLRaV-3.

About 32% of the fruit tree samples were collected from the six NCCs located in Kabul (apricot and plum), Balkh (apricot and almond), Kunduz (almond), Herat (grape and plum), Kandahar (grape and pomegranate) and Nangarhar (citrus and pomegranate). Moreover, samples were also collected from MSNs (26%) and from private orchards (nearly 40%) (Fig. 1).

Each year stone fruit samples were collected during the vegetative stage in spring or summer, between March and September, according to the climatic conditions of each location. Similarly, apple and pear samples were collected in May, June, July or September; citrus in March, May or November; and grape in dormant stage in January, October or December.

The collecting method consisted of sampling leaves (in the case of stone and pome fruits), shoots or canes (grape) or leaves and flowers (citrus) homogeneously distributed around the canopy of the plant. All collected material was maintained under refrigeration (below 10°C) during transport from the field to the laboratory, then kept at -20°C until analyses.

Serological and molecular analyses

All samples were ground in "Universal" extraction bags (12x15 cm) using the HOMEX 6 homogenizer (Bioreba) then analysed by DAS-ELISA using reagent kits from Bioreba (Reinach, Switzerland) specific for each virus assayed and following the manufacturer's instructions.

Samples that resulted positive by DAS-ELISA test were subsequently analysed by RT-PCR reaction using specific primer pairs (Table 1).

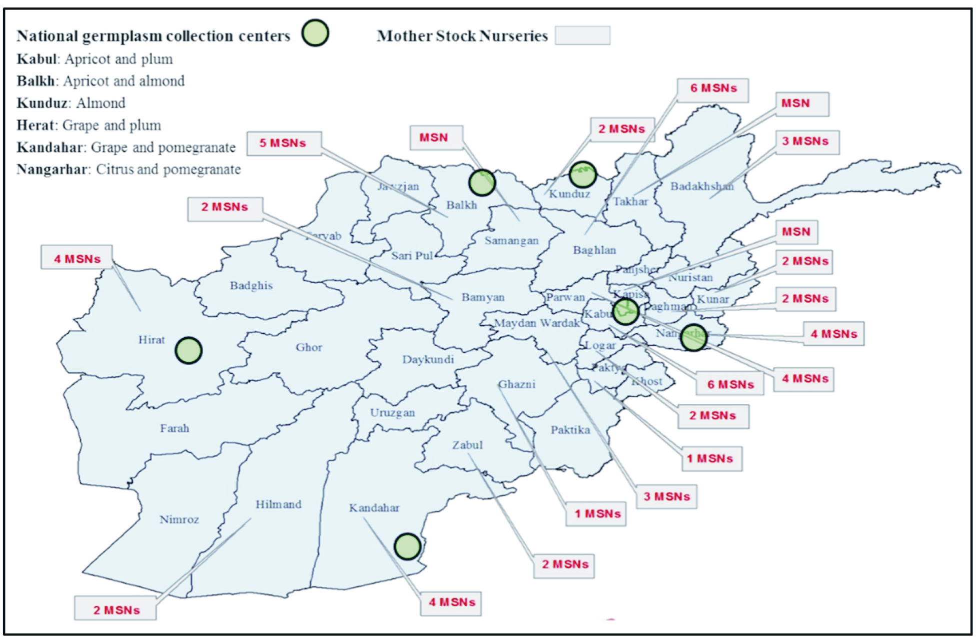

Fig. 1 - Location of the six national collection centres and of the 57 mother stock nurseries in Afghanistan.

Table 1 - List of primer pairs, size of amplicons, and RT-PCR conditions used to confirm viral infection in all samples resulting positive to the DAS-ELISA test

Virus	Primers	Primers sequences (5' - 3')	Size of amplif. (bp)	Cycling (temp./time)*			N° of cycles	Reference
				Denat.	Anneal.	Exten.		
ACLSV	Sense	TTCATGGAAAGACAGGGGCAA	677	94°C/20 s	58°C/20 s	72°C/20 s	35	Menzel *et al.*, 2002
	Antisense	AAGTCTACAGGCTATTTATTATAAGTCTAA						
PNRSV	MG1	ATGGTTTGCCGAATTTGC	675	94°C/60 s	55°C/45 s	72°C/60 s	35	Glasa *et al.*, 2002
	MG2	ACTCTAGATCTCAAGCAGGTC						
GFLV	M3	ATGCTGGATATCGTGACCCTGT	118	94°C/10 s	54°C/10 s	72°C/45 s	35	Gambino and Gribaudo, 2006
	M4	GAAGGTATGCCTGCTTCAGTGG						
CTV	CTVF	TAATGGACGACGAACAAAGA	655	94°C/40 s	56°C/40 s	72°C/60 s	30	Rehman *et al.*, 2012
	CTVR	CCAAGCTGCCTGACATTAGT						

* PCR amplifications were performed at 94°C for 5 min for initial denaturation and 72°C for 5 min for final extension.

Total RNAs were extracted from each sample using CTAB RNA extraction method, as previous reported (Ratti *et al.*, 2004). Complementary DNA (cDNA) was synthesized using M-MLV reverse transcriptase (Promega, USA) in a final volume of 5.0 µl. RNA (1.0 µl), mixed with M-MLV 5x buffer, 0.5 µl 10 mM dNTPs, 1.0 µl 10nmol/ml specific reverse primer, 0.25 µl of 200 U/µl M-MLV and 2.25 µl of nuclease-free water, then incubated at 4 2°C (or 37°C for ACLSV and PNRSV) for 1 h. For PCR amplification, 5.0 µl of cDNA template, 5.0 µl of 5x Green GoTaq Buffer, 1.5 µl of 25 mM $MgCl_2$, 1.0 µl 10 nmol/ml of specific forward primer, 0.5 µl of 10 mM dNTPs and 0.2 µl of 5 U/µl GoTaq Polymerase (Promega, USA) were mixed in a total volume of 25 µl. The number of cycles and cycling conditions for each primer set are given in Table 1. The amplified PCR products were analysed in 1% agarose gel stained by ethidium-bromide and visualized under UV light after electrophoresis.

3. Results

DAS-ELISA analyses

All apple and pear plants analysed (180 samples) resulted free from ACLSV, ApMV, ASGV and ASPV. Symptoms such as yellowing, leaf distortion and puckering were observed in some stone fruit plants during collection of the 5521 samples that were tested by DAS-ELISA (Fig. 2). Among them, 23 (0.4%) samples collected from several MSNs in Kabul, Khandahar, Parwan, Herat and Baghlan provinces resulted positive to ACLSV. In particular, there were two plants of European plum (Clone 364/local name Alu BokharaShalili), one cross of Myrobalan and Japanese plum (4031/Grangej Zard), 17 plants of peach (five from 804/Turki Sorkh, five from 812/Dir Ras and seven from 811/TurkiZard), one plant of almond (159/Sattarbai Bakhmali) and two plants of apricot (373/Shakarpara and 4037/Aqa Banu). Two (0.03%) almond samples, both collected from the NCC in Balkh province, resulted infected by ApMV; in was interesting that one of them also tested positive to PNRSV. Analyses against PNRSV evidenced 38 samples (0.6%), collected from NCCs or MSNs located in Balkh, Herat, Kabul, Khandaharand Paktya as infected by the virus (13 almond, five plum, nine peach, and 11 cherry plants). Finally, one plum sample (cv. Alu Bokhara Shalili), from a MSN in Kabul, resulted infected by PDV while none of the stone fruit samples tested resulted positive to PPV (Tables 2-6 and Fig. 3).

Fig. 2 - Yellowing observed in an apricot accession during sample collection in a National Collection Centre.

Among the 895 grape samples analysed, one plant from the NCC in Kandahar (cv. Shir Ahmadi Herat) resulted infected by GVA and only one sample of the cv. Pizzutello bianco, imported from Italy and collected in the NCC in Herat, tested positive to GLRaV-1 (it also resulted as infected by GLRaV-3). Moreover, GLRaV-3 was detected in two samples (cv. Pizzutello bianco and Uva Palieri both imported from Italy), collected from the NNC in Herat. Regarding GFLV, all the 62 samples which resulted infected by the virus (6.9%) belong to Afghan cultivars, with the exception of the cv. Regina dei vigneti imported from Italy. All grape samples tested resulted negative to ArMV, GFkV and GLRaV-2 (Table 7) (Fig. 3).

In addition, the Plant Biotechnology Laboratory analyzed 5400 citrus plants and 318 of them (5.9%) resulted infected by CTV. In particular, among the positive samples, eight plants, showing vein clearing and flecking, yellowing and plant decline symptoms (Fig. 4), were collected from the NCC in Jalalabad and belong to six different accessions: Kumquat cv.

Table 2 - Almond samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
PPV	North	174	0	0	0	205	0	174	0	53	0	82	0	78	0	766	0
	Central	2	0	0	0	24	0	136	0	103	0	69	0	42	0	376	0
	South	0	0	0	0	0	0	0	0	47	0	0	0	0	0	47	0
PDV	North	174	0	0	0	205	0	174	0	53	0	82	0	78	0	766	0
	Central	2	0	0	0	24	0	136	0	103	0	69	0	42	0	376	0
	South	0	0	0	0	0	0	0	0	47	0	0	0	0	0	47	0
PNRSV	North	174	**6 (Balkh)**	0	0	205	**5 (Balkh)**	174	0	53	0	82	0	78	0	766	11
	Central	2	**1 (Herat)**	0	0	24	0	136	0	103	0	69	0	42	**1 (Herat)**	376	2
	South	0	0	0	0	0	0	0	0	47	0	0	0	0	0	47	0
ApMV	North	174	0	0	0	205	0	174	0	53	0	82	0	78	0	766	0
	Central	2	0	0	0	24	0	136	0	103	0	69	0	42	0	376	0
	South	0	0	0	0	0	0	0	0	47	0	0	0	0	0	47	0
ACLSV	North	0	0	0	0	205	0	174	0	53	0	82	0	78	0	592	0
	Central	0	0	0	0	24	0	136	0	103	0	69	0	42	**1 (Herat)**	374	1
	South	0	0	0	0	0	0	0	0	47	0	0	0	0	0	47	0
Total		176	7	0	0	229	5	310	0	203	0	151	0	120	2	1189	14

Northern regions: Badakhshan, Baghlan, Balkh, Faryab, Jowzjan, Kunduz, Samangan, Sar-e Pol and Takhar. Central regions: Badghis, Bamiyan, Farah, Ghor, Herat, Kabul, Kapisa, Kunar, Laghman, Logar, Nangarhar, Nurestan, Panjshir, Parwan and Wardak. Southern regions: Daykundi, Ghazni, Helmand, Kandahar, Khost, Nimruz, Orūzgān, Paktia, Paktika, Zabul.

Table 3 - Apricot samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
PPV	North	0	0	0	0	0	0	68	0	0	0	40	0	80	0	188	0
	Central	278	0	833	0	0	0	221	0	141	0	69	0	48	0	1590	0
	South	0	0	0	0	0	0	0	0	29	0	0	0	0	0	29	0
PDV	North	0	0	0	0	0	0	68	0	0	0	40	0	80	0	188	0
	Central	278	0	833	0	0	0	221	0	141	0	69	0	48	0	1590	0
	South	0	0	0	0	0	0	0	0	29	0	0	0	0	0	29	0
PNRSV	North	0	0	0	0	0	0	68	0	0	0	40	0	80	0	188	0
	Central	278	0	833	0	0	0	221	0	141	0	69	0	48	0	1590	0
	South	0	0	0	0	0	0	0	0	29	0	0	0	0	0	29	0
ApMV	North	0	0	0	0	0	0	68	0	0	0	40	0	80	0	188	0
	Central	278	0	833	0	0	0	221	0	141	0	69	0	48	0	1590	0
	South	0	0	0	0	0	0	0	0	29	0	0	0	0	0	29	0
ACLSV	North	0	0	0	0	0	0	68	0	0	0	40	0	80	**2 (Baghlan)**	188	2
	Central	0	0	0	0	318	0	221	0	141	0	69	0	48	0	797	0
	South	0	0	0	0	0	0	0	0	29	0	0	0	0	0	29	0
Total		278	0	833	0	318	0	289	0	170	0	109	0	128	2	2125	2

See legend of Table 2 for provinces included in Northern, Central and Southern regions.

Table 4 - Cherry samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
PPV	North	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7	0
	Central	0	0	0	0	0	0	1	0	93	0	58	0	35	0	187	0
	South	0	0	0	0	0	0	0	0	18	0	0	0	0	0	18	0
PDV	North	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7	0
	Central	0	0	0	0	0	0	1	0	93	0	58	0	35	0	187	0
	South	0	0	0	0	0	0	0	0	18	0	0	0	0	0	18	0
PNRSV	North	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7	0
	Central	0	0	0	0	0	0	1	0	93	**6 (Kabul)**	58	**1 (Kabul)**	35	**3 (Herat)**	187	10
	South	0	0	0	0	0	0	0	0	18	**1 (Paktya)**	0	0	0	0	18	1
ApMV	North	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7	0
	Central	0	0	0	0	0	0	1	0	93	0	58	0	35	0	187	0
	South	0	0	0	0	0	0	0	0	18	0	0	0	0	0	18	0
ACLSV	North	0	0	0	0	0	0	7	0	0	0	0	0	0	0	7	0
	Central	0	0	0	0	0	0	1	0	93	0	58	0	35	0	187	0
	South	0	0	0	0	0	0	0	0	18	0	0	0	0	0	18	0
Total		0	0	0	0	0	0	8	0	111	7	58	1	35	3	212	11

See legend of Table 2 for provinces included in Northern, Central and Southern regions.

Table 5 - Peach samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
PPV	North	0	0	0	0	0	0	75	0	41	0	9	0	65	0	190	0
	Central	4	0	0	0	267	0	374	0	186	0	21	0	13	0	865	0
	South	0	0	0	0	0	0	0	0	75	0	0	0	0	0	75	0
PDV	North	0	0	0	0	0	0	75	0	41	0	9	0	65	0	190	0
	Central	4	0	0	0	267	0	374	0	186	0	21	0	13	0	865	0
	South	0	0	0	0	0	0	0	0	75	0	0	0	0	0	75	0
PNRSV	North	0	0	0	0	0	0	75	0	41	0	9	0	65	0	190	0
	Central	4	0	0	0	267	0	374	**7 (Herat)**	186	**1 (Kabul)**	21	0	13	0	865	8
	South	0	0	0	0	0	0	0	0	75	**1 (Kandahar)**	0	0	0	0	75	1
ApMV	North	0	0	0	0	0	0	75	0	41	0	9	0	65	0	190	0
	Central	4	0	0	0	267	0	374	0	186	0	21	0	13	0	865	0
	South	0	0	0	0	0	0	0	0	75	0	0	0	0	0	75	0
ACLSV	North	0	0	0	0	0	0	75	0	41	0	9	0	65	0	190	0
	Central	0	0	0	0	267	0	374	0	186	**17 (Kabul)**	21	0	13	0	861	17
	South	0	0	0	0	0	0	0	0	75	0	0	0	0	0	75	0
Total		4	0	0	0	267	0	449	7	302	19	30	0	78	0	1130	26

See legend of Table 2 for provinces included in Northern, Central and Southern regions.

Table 6 - Plum samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
PPV	North	0	0	0	0	0	0	48	0	31	0	0	0	45	0	124	0
	Central	160	0	0	0	190	0	168	0	140	0	31	0	15	0	704	0
	South	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PDV	North	0	0	0	0	0	0	48	0	31	0	0	0	45	0	124	0
	Central	160	0	0	0	190	0	168	0	140	0	31	0	15	0	704	0
	South	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PNRSV	North	0	0	0	0	0	0	48	0	31	0	0	0	45	0	124	0
	Central	160	**5 (Herat)**	0	0	190	0	168	0	140	0	31	0	15	0	704	5
	South	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ApMV	North	0	0	0	0	0	0	48	0	31	0	0	0	45	0	124	0
	Central	160	0	0	0	190	0	168	0	140	0	31	0	15	0	704	0
	South	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACLSV	North	0	0	0	0	0	0	48	0	31	0	0	0	45	0	124	0
	Central	0	0	0	0	190	0	168	0	140	**2 (Kabul)**	31	0	15	0	544	2
	South	0	0	0	0	0	0	0	0	33	**1 (Kandahar)**	0	0	0	0	33	1
Total		160	5	0	0	190	0	216	0	204	3	31	0	60	0	861	8

See legend of Table 2 for provinces included in Northern, Central and Southern regions.

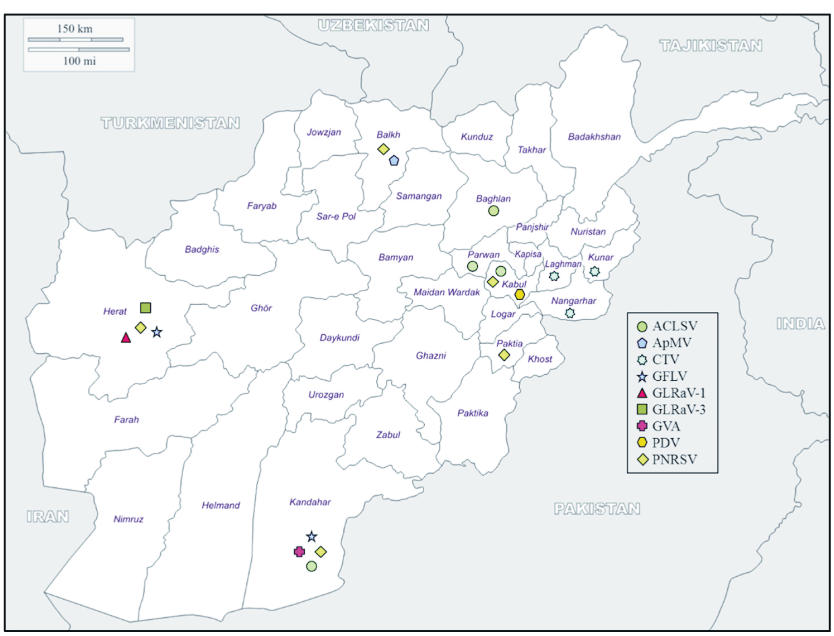

Fig. 3 - Distribution, among Afghan provinces, of samples resulting positive to ACLSV, ApMV, CTV, GFLV, GLRaV-1, GLRaV-3, GVA, PDV or PNRSV.

Margarita (isolates J4 and J8), Orange cv. Mahali (J61), Rough lemon cv. Mahali (J101) and Mandarin Group cvs. Fruter (J76), Tangelo Mapo and Clementine Di Nules. The other 312 plants positive to CTV were collected from private orchards located in the provinces of Kunar, Laghman, and Nangarhar (Table 8, Fig. 3).

RT-PCR and molecular characterization

Samples from stone fruit plants which resulted infected by ACLSV, or PNRSV following DAS-ELISA technique were analysed also by RT-PCR reaction. The results obtained confirmed the presence of each viral agent assayed in the samples tested.

In particular, regarding ACLSV isolates, a 677-long nucleotide fragment, corresponding to partial coat protein gene, was amplified from all ELISA-positive samples by RT-PCR using ACLSV sense and antisense primers (Menzel *et al.*, 2002). Eleven of the identified isolates of ACLSV were then characterized molecularly. Sequence analysis revealed similarity ranging from 83.6 to 100.0% within ACLSV isolates detected in Afghanistan. Blast analysis showed that sequences of two peach isolates, 812/Dir Ras, shared the highest nucleotide similarity (95.8 and 96.2%) with the GenBank ACLSV isolates Apr 3 from Jordan (AJ586631). Moreover, the same Afghan isolates showed nucleotide identity between 95.0 and 95.7% with isolates S4, PP23, PP63, and HL2 (JN849008, GU327991, GU328003 and GQ334211, respectively) from China. Sequence analysis of isolate 364/Alu

Fig. 4 - Vein clearing and yellowing symptoms observed in citrus accessions collected from the National Collection Centre in Jalalabad (Nangarhar province).

Table 7 - Grape samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
GFLV	Herat	0	0	0	0	156	**19**	117	**13**	271	**22**	0	0	0	0	544	54
	Kandahar	0	0	0	0	0	0	0	0	268		0	0	0	0	268	0
ArMV	Herat	0	0	0	0	156	0	117	0	271	0	0	0	0	0	544	0
	Kandahar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GVA	Herat	83	0	0	0	156	0	117	0	271	0	0	0	0	0	627	0
	Kandahar	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	1
GFkV	Herat	83	0	0	0	156	0	117	0	271	0	0	0	0	0	627	0
	Kandahar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLRaV-1	Herat	83	0	0	0	156	0	117	**1**	271	0	0	0	0	0	627	1
	Kandahar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLRaV-2	Herat	83	0	0	0	156	0	117	0	271	0	0	0	0	0	627	0
	Kandahar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GLRaV-3	Herat	83	0	0	0	156	0	117	**2**	271	0	0	0	0	0	627	2
	Kandahar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		83	0	0	0	156	19	117	16	539	23	0	0	0	0	895	58

Table 8 - Citrus samples tested and resulting positive by DAS-ELISA during the first seven years of activity of the Plant Biotechnology Laboratory based in Kabul.

Virus	Region	2009		2010		2011		2012		2013		2014		2015		Total	
		Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
CTV	Nangarhar	0	0	193	6	263	2	1276	153	1554	110	131	0	492	0	3909	271
	Laghman	0	0	0	0	0	0	180	9	200	4	0	0	296	3	676	16
	Kunar	0	0	0	0	0	0	318	21	246	9	0	0	249	1	813	31
Total		0	0	193	6	263	2	1774	183	2000	123	131	0	1037	4	5398	318

Bokhara Shalili, 804/Turki Sorkh and 811/Turki Zard proved that they are 99.6 to 100.0% identical with each other and share high (94.3 to 94.7%) nucleotide identity with the Iranian isolate T12Ja (KM586376).

Lower nucleotide similarity (86.2%) was shown by one 804/Turki Sorkh isolate with the isolate 274-Chal (FN391009) from Greece. Finally, analysis of the sequence of isolates 804/Turki Sorkh, 811/Turki Zard and 812/Dir Ras revealed a high degree of identity (86.2 to 88.4%) with the corresponding nucleotide sequences of the isolate SK-92-pl (HQ398253) from Slovakia.

RT-PCR reaction performed on samples infected by PNRSV, according to DAS-ELISA results, amplified a fragment of 675 nucleotides in length when PNRSV specific primer pair MG1/MG2 was used (Table 1).

Regarding grape samples, specific PCR products of 118 nucleotides where also observed on DAS-ELISA positive samples, using specific primers pair M3/M4 against GFLV as reported in Table 1.

Some of the citrus samples tested positive by DAS-ELISA were also tested using primers CTVF and CTVR allowing amplification of specific PCR products 655 nucleotides in length (Table 1). Moreover, six CTV isolates collected at the Jalalabad station were characterized. Sequence analysis revealed high similarity, ranging from 91.1 to 99.8%, within the CTV isolates detected. In accordance with the previously defined phylogenetic groups (Zemzami *et al.*, 2002), nucleotide sequences of Afghan CTV isolates cluster in Group 1 (J4 and J8), Group 4 (J61 and J76) and Group 5 (J101). In particular, J4 and J8 isolates show, respectively, 99.4 and 99.2% identity with reference isolate T36 (M76485) from the USA (Florida). Furthermore, in Group 4, isolate J61 and J76 were more similar to ANO-1 isolate (DQ211658) from Egypt (98.5 and 98.0% identity, respectively) than to isolate 443-4 (AY791844) from Croatia (97.4 and 97.5%, respectively). Finally, isolate J101, in Group 5, shows 95.6% identity with isolates C268-2 (AY750770) and C269-6 (AY750775) from Argentina.

4. Discussion and Conclusions

To improve Afghanistan's agricultural sector, the EC supports the PHDP (Perennial Horticulture Development Project), for true to type/ecotype and healthy planting materials, and the Plant Biotechnology Laboratory, to ensure the health status of local germplasm. The laboratory started screening the health status of the Afghan Germplasm National Collection in order to ensure the multiplication of not only the best-selected varieties or ecotype, but also to avoid production and distribution of virus-infected fruit trees. Symptom inspection and sample collection for viral diseases was carried out in all the National Collection fields, including peach, plum, apricot, almond, apple, grape and citrus plants, located in different areas of the country.

During the first seven years of its activity, the Plant Biotechnology Laboratory based in Kabul, played a key role by satisfying the increasing need for certified propagation plant material to establish new plantations in Afghanistan. Moreover, it contributed to protecting the country from undesirable introduction of pathogens due to increasing exchanges of propagation plant material among different countries and between different continents.

Until now, the Plant Biotechnology Laboratory has verified the absence of PPV (the most devastating virus for stone fruit) in the Afghan germplasm and detected and verified the presence of several important plant viruses in stone fruits, citrus and grapevines. Further activity will be addressed to confirming, by RT-PCR, infection by ApMV, PDV, GVA, GLRaV-1 and GLRaV-3 in almond, plum and grape accessions which resulted positive by DAS-ELISA, using specific primer pairs (Parakh *et al.*, 1995; Menzel *et al.*, 2002; Goszczynski and Jooste, 2003; Osman *et al.*, 2008).

The Plant Biotechnology Laboratory first reported ACLSV occurrence in peach, plum, almond and apricot plants in Afghanistan. According to the preliminary results of sequence analysis, due to the relative low identity with other ACLSV isolates present in GenBank, our mother stock nurseries appear to be infected by several Afghan isolates of the virus, but further studies will be addressed to understanding if ACLSV has been introduced into the country through infected propagation material. In any case, the presence of ACLSV in important cultivars of peach and plum is a worrying aspect of the Afghan certification program.

Similarly, our results identified, for the first time, PNRSV, ApMV, and PDV infected plants in Afghanistan. Even if the latter two viruses were detected by DAS-ELISA test, the presence of the viruses in accessions of the National Collection field is cause for worry for Afghan horticulture as all three viruses spread through infected propagating material and nursery productions (Mink, 1992). Moreover, PNRSV and PDV are also seed- and pollen-transmitted and can be spread by pollinating insects (Kelley and Cameron, 1986; Aparicio *et al.*, 1999; Glasa *et al.*, 2002; Amari *et al.*, 2007) while seed transmission of ApMV is known only in hazelnuts (Cameron and Thompson, 1985). A comparative sequence and phylogenetic analysis will be performed (Zindović *et al.*, 2015) to show if clustering of various isolates is associated or not with geographic and host origin.

Regarding the detection of viruses in grape, according to our results the presence in the NCC in Kandahar of GVA and GLRaV-1, and GLRaV-3 in Herat seems to be restricted to only a few accessions. Early detection of these viruses by the Plant Biotechnology Laboratory avoided multiplication and therefore the spread of infected grape material in Afghanistan. In contrast, the noticeable presence of GFLV (nearly 7%) in local cultivars maintained at the NCC in Herat suggests the presence of the virus in Afghan germplasm of grape for some time. This hypothesis should be confirmed by studying the genetic variability of Afghan isolates in order to investigate the relationship among their geographical origin, sequence variability, and grapevine cultivar (Meng *et al.*, 2006; Vigne *et al.*, 2009; Terlizzi *et al.*, 2015).

Finally, our analyses identified, for the first time, CTV infected plants in Afghanistan (Rehman *et al.*, 2012). The presence of a dangerous viral disease such as CTV in many citrus trees (nearly 6% of the assayed plants) from NCCs, MSNs and private orchards represents a serious problem for the Afghan citrus industry.

High sequence identity with many isolates from USA, Egypt, Croatia, and Argentina together with the large spread of the virus in Afghan citrus-cultivating areas suggests the introduction of the virus into the country a long time ago. The presence of this virus seems to be, therefore, endemic; further investigations showed that infected plants did not exhibit quite as dramatic symptoms as those usually found in other citrus cultivation areas (e.g. Spain, Brazil, Argentina etc.) where the virus is extremely dangerous and causes in some cases plants death (Rocha-Peña *et al.*, 1995). A specific research program is in progress to investigate if a mild strain of CTV is present in the eastern region (Nangarhar, Kunar, Laghman) environment, if tolerant/resistant sour orange rootstock clones exist, or if the environmental (climate and soil conditions) influences the infectivity of CTV.

Due to the economic importance of these crops, implementation of the certification schemes is therefore necessary in Afghanistan in order to guarantee the production and employment of virus-free propagating material.

It is well known that data regarding the presence and spread of different plant viruses are important for individual countries, their neighbours, and for the whole agricultural community where different viruses can be spread by vegetative propagation via plant material. A continuous survey is therefore necessary to protect Afghanistan from the introduction and/or spread of dangerous pests. As previously experienced, most effective management programs require prompt removal of infected trees and replacement with healthy planting material from certified nurseries (Hadidi *et al.,* 2011; Pallas *et al.*, 2012). Establishment of rigid phytosanitary controls related to the importation of propagation material, as well as the eradication of infected trees, is therefore of great importance in Afghanistan.

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