Adv. Hort. Sci., 2016 30(4): 217-223 DOI: 10.13128/ahs-20347

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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Received for publication 11 January 2016
Accepted for publication 13 July 2016

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,

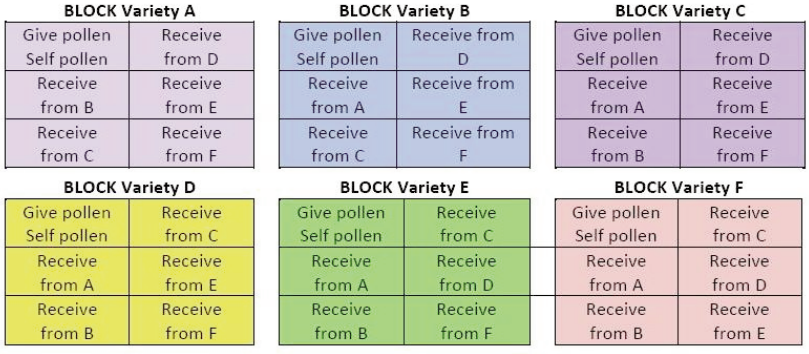

Fig. 1 - Layouts of almond national collection blocks for cross pollination. Each box in the diagram represents one tree in each variety block. Each variety is coded by a letter, in order to show the systematic layout of the trial. The code sheets would represent a different accession in each year's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

tance 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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