

PHYTOCHEMICAL SCREENING, BOTANICAL STUDY AND DNA FINGERPRINTING OF *PRUNUS AMYGDALUS* BATSCH "UMM ALFAHM" CULTIVAR CULTIVATED IN EGYPT

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ABSTRACT

Objective: The present study was designed to throw light on the phytochemical screening, macro and micro morphological studies, as well as, DNA fingerprinting of *Prunus amygdalus* Batsch Umm alfahm cultivar with the aim of plant authentication.

Methods: Phytochemical screening of the stems, leaves and pericarps was carried out using standard procedures. DNA fingerprinting was carried out using randomly amplified polymorphic DNA (RAPD) using ten primers for the analysis. In addition, seed protein was analyzed using continuous polyacrylamide gel electrophoresis in vertical slab apparatus in the presence of sodium dodecyl sulfate (SDS-PAGE).

Results: Phytochemical investigation of *P. amygdalus* revealed positive results for the presence of steam volatile substances and saponins in leaves, while pericarp contains traces of saponins. Whereas, sterols and/or triterpenoids, tannins, carbohydrate and/or glycosides, and flavonoids were detected in leaves and stems and as traces in pericarp.

Conclusion: From the present study, macro and micro morphological characters, as well as, DNA fingerprinting can be considered as the identifying parameters to authenticate the plant.

Keywords: Botanical study, *Prunus amygdalus*, DNA fingerprinting, Phytochemical screening.

INTRODUCTION

Almond (*Prunus amygdalus*, syn., *Prunus dulcis* or *Amygdalus communis*) is a small deciduous tree belonging to the subfamily, Prunoideae of the family, Rosaceae. Almond is one of the major tree crops of the world, and is one of the oldest commercial nut crops of the world; from the middle and west Asia, it has diffused to other regions and continents which include the Middle East, China, the Mediterranean region and America. Beside its commercial use as a nut crop, the almond can be used for ornamental planting because of its beautiful white or pale pink flowers [1].

According to Hartwell [2], the seed and/or its oil is used in folk remedies for cancer (esp. Bladder, breast, mouth, spleen, and uterus), carcinomata, condylomata, corns, indurations and tumors. Also Almond is reported to be astringent, carminative, demulcent, diuretic, emollient, laxative, nervine, sedative, stimulant and tonic. It is a folk remedy for asthma, cold, corns, cough, dyspnea, eruptions, gingivitis, heartburn, itch, lungs, skin sores, spasms, stomatitis, and ulcers. The kernels are valued in the diet and for peptic ulcers [3]. Reports on the chemical composition and biological activities of Almond were traced [4] and nothing could be found on this cultivar grown in Egypt. This encouraged the authors to perform the present study aiming to throw light on the preliminary phytochemical screening of the title plant. Macro and micro morphological studies, as well as, DNA fingerprinting was carried out with the aim of authenticating the plant.

MATERIAL AND METHODS

Plant material for phytochemical investigation

Air-dried stem, leaves and pericarp of *P. amygdalus* Batsch Umm alfahm cultivar were coarsely powdered and packed in dark-colored, tightly closed containers for phytochemical study. The 70 % ethanolic extracts were analyzed for the presence of phytochemical constituents using standard procedures [5-10].

Plant material for botanical study

Samples of *P. amygdalus* Batsch, Umm alfahm cultivar, family Rosaceae, used in this study were collected since September 2011 from farmer

(MAFA 1) on Albasateen region at West Nubaria, Egypt. Plant identity was kindly authenticated by Dr. Reem Sameer Hamdi, Assistant Professor of plant classification at the Department of Plant, Cairo University herbarium. Voucher sample no. 2014.09.22 was deposited at the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Methods for botanical study

Fresh samples were fixed for at least 48 hrs in formalin-acetic acid-70% alcohol (5:5:90 v/v) and dehydrated, paraffin infiltrated and embedded in wax using customary techniques. Transverse sections were cut on a rotary microtome to a thickness of 15 microns and stained with safranin and fast green [12]. A light microscope was used to view the slides. Microphotographs were obtained using image analyzer at the central laboratory on the faculty of pharmacy, Cairo University.

Plant material for molecular investigations

Samples of fresh leaves of the *P. amygdalus* Batsch Umm alfahm cultivar (C1) and other two hybrid species 53(C2) and 54(C3) cultivated in Egypt were obtained in September 2011 for DNA fingerprinting. They were stored at -70°C, freeze-dried and ground to a fine powder prior to DNA isolation.

Buffers and agarose gel

Extraction buffer and agarose gel were prepared according to the reported method [11]. Agarose gel: 1.5% containing ethidium bromide (0.5ug/ml) in 1X TBE buffer.

Primers

Ten primers were used for randomly amplified polymorphic DNA (RAPD) analysis obtained from Operon Technologies Inc., Alameda, California, USA with the following sequence:

OPA-03: 5'- AGTCAGCCAC -3'; OPA-04: 5'- AATCGGGCTG -3'; OPA-06: 5'- GGTCCCTGAC -3'; OPA-11: 5'- CAATCGCCGT -3'; OPA-17: 5'- GACCGTTGT -3'; OPC-01: 5'- TTCGAGCCAG -3'; OPC-07: 5'- GTCCGACGA -3'; OPC-09: 5'- CTCACCGTCC -3'; OPO-02: 5'-

ACGTAGCGTC -3'; OPO-14: 5'- AGCATGGCTC -3'. Molecular weight markers were obtained from New England Biolab Co., UK.

Apparatus

DNA thermocycler (Hybaid PCR Express) was used for amplification of DNA, agarose gel electrophoresis tool (Biorad Wide Mini Sub Cell) was used for the separation of RAPD fragments according to size and an ultraviolet polaroid camera was used for the visualization of RAPD fragments.

Material for seed protein profiling

DNA fingerprinting kits

The DNA isolation kit (Qiagen DNeasy kit), including Cell lysis buffer (AP1), RNase Protein-depleting buffer (AP2), AP3/E Buffer, AW buffer, AE buffer, TBE buffer (Qiagen Santa Clara, CA) were used

Amplification reagent

The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs (Pharmacia, Sweden), 1 µM primer, 1 U Taq DNA polymerase (Perkin - Elemer / Cetus, USA; Advanced Biotechnologies, UK) and 25 ng template DNA.

SDS-Polyacrylamide Gel electrophoresis (PAGE)

Monomer solution (30 %)

Acrylamide (29.2 g), N-N-bis-methylene-acrylamide (0.8 g) and 100 ml distilled water were mixed, filtered and stored at 4 °C in dark (30 days maximum)

Buffer

Tris- base (1.5 M) was prepared as follows: Tris base (18.15 g) in 100 ml distilled water, the pH was adjusted to 8.8, and stored at 4 °C and used as separating buffer. While Tris base (0.5M) (Tris base 6g, pH 6.8) was used as stacking buffer.

SDS (10 %)

Ten grams SDS were dissolved in 100 ml distilled water.

Ammonium persulfate (APS) (10 %)

100 mg ammonium persulfate were dissolves 1 ml distilled water.

Staining solution

Staining solution consists of Coomassie brilliant blue R-250 (0.1 %), Methanol (40 %) and Glacial acetic acid (10 %).

Destaining solution

Consists of Methanol (40 %) and Glacial acetic acid (10 %).

Sample buffer (62.5 mM Tris, 20% Glycerol, 2% SDS & 5% β-ME)

Consists of distilled water (4 ml), 0.5 M Tris-HCl (1 ml), Glycerol (0.8 ml), 10% SDS (1.6 ml), β- mercaptoethanol (0.4 ml), and Bromophenol blue (0.2 ml).

Electrode buffer 5 X (1X= 25 mM Tris, 192 mM glycine and 0.1% SDS)

Consists of Tris base (15 g), Glycine (72 g), SDS (5 g) and 1L distilled water, stored at 4 °C, the working concentration is 1X and the pH was 8.3.

Protein tracking dye

Bromophenol blue (0.05 g) in 100 ml distilled water.

Methods for protein extraction

Seed protein was analyzed using continuous polyacrylamide gel electrophoresis in vertical slab apparatus in the presence of sodium dodecyl sulfate (SDS-PAGE). The mature seeds (0.1 g) were powdered and mixed with 1 ml sample buffer. The slurry was centrifuged at 6000 rpm for 10 min. The supernatant was used immediately for electrophoresis.

Gel preparation

The gel used for separation was prepared and poured between the glass plates immediately after adding ammonium persulfate. After polymerization of the gel (11 cm), the stacking gel (3 cm) was poured into the glass plates and then comb was inserted to form sample wells.

Gel electrophoresis [14]

1-Sample preparation

Protein extracts were diluted with sample buffer (1:3 v/v), then 500 µl of 10% SDS were added followed by 25 µl of mercaptoethanol. They were then placed in a boiling water bath for 5 min and 5 µl of bromophenol was added as a tracking dye. Ten µl of the sample was loaded and 10 µl of marker protein mixture was used as standard.

2-Running conditions

Runs were carried out at a constant voltage of 200 Volt. Usual runs took approximately 120 min.

3-Gel staining and destaining

The chromatograms were stained with an excess of coomassie brilliant blue stain R-250 for about one hr. After gel staining, the gel was transferred to destaining solution to remove excess stain.

Methods for molecular investigations

DNA extraction and quantification, amplification of RAPD markers and analysis of RAPD data were performed according to [11]

RAPD procedures

RAPD profiling was performed as recommended by William *et al.* (1990) [13] with some modifications. It was carried out using oligonucleotide sequences of ten 10-mer random primers; the sequence of these ten primers was used for randomly amplified polymorphic DNA (RAPD) analysis obtained from Operon Technologies Inc., Alameda, California, USA with the following sequences: OPA-03: 5'- AGTCAGCCAC -3'; OPA-04: 5'-AATCGGGCTG-3'; OPA-06: 5'-GGTCCCTGAC-3'; OPA-11: 5'-CAATCGCCGT-3'; OPA-17: 5'-GACCGCTTGT-3'; OPC-01: 5'-TTCGAGCCAG-3'; OPC-07: 5'-GTCCCCGACGA-3'; OPC-09: 5'-CTCACCGTCC-3'; OPO-02: 5'-ACGTAGCGTC-3'; OPO-14: 5'-AGCATGGCTC-3'. The primers were synthesized at the Agricultural Genetic Engineering Research Institute (AGERI) in ARC, Giza, Egypt on an ABI 392 DNA/RNA synthesizer and used to screen the ten *Artemisia* populations. Polymerase chain reaction (PCR) was made in 50µl reaction mixture composed of 2.5µl of the appropriate template DNA, 0.5µl Taq polymerase, 0.5µl primer, 6.0µl MgCl₂, 5.0µl of NTP's mix, 0.5µl 10x reaction buffer and 35.0µl distilled water. Amplification of DNA was carried out in a thermal cycler (Perkin Elmer Cetus 480) as follows: 1 cycle at 94 °C for 5 min. followed by 40 cycles each for 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C with the final extension phase of seven min at 72 °C and storage at 4 °C. Amplified DNA fragments were separated in 1.4% agarose gel. After electrophoresis, the RAPD profiles were visualized using UV trans-illuminator and photographed for analysis.

Data analysis [15]

The relationship among the examined populations was estimated based on differences among them in both RAPD finger printing and protein electrophoresis separately and in combination. Qualitative traits were all two-state characters and were coded as 0 or 1. For the data analysis, the presence or absence of unique and shared polymorphic as well as monomorphic products was recorded as 1 for presence and 0 for absence. However, only polymorphic bands were used to calculate the genetic Euclidean similarity coefficient among the examined populations and were used to construct distance trees that illustrate the genetic diversity using the un-weighted pair group method.

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids and / or nitrogenous bases, while anthraquinones, cardiac glycosides and oxidase enzymes are absent in all studied organs of *P. amygdalus* Batch. Steam volatile substances and saponins were present in leaves

only. While pericarp contains traces of saponins. Whereas sterols and/or triterpenoids, tannins, carbohydrate and/or glycosides and flavonoids were present in leaves and stem but as traces in pericarp.

Results of molecular investigations

The banding profiles produced by the ten decamer primers used in RAPD analysis of *P. amygdalus* were: OPA-03, OPA-04, OPA-06, OPA-11, OPA-17, OPC-01, OPC-07, OPC-09, OPO-02 and OPO-14.

Genetic diversity revealed by RAPD data

RAPD-PCR was performed with ten random primers using genomic DNA samples extracted from the three *P. amygdalus* cultivars. DNA fingerprinting and reproducible RAPD profiles generated were pictured in (Fig. 1).

Distribution of RAPD markers among *Prunus* cultivars

Reproducible patterns and amplification profiles were screened for the polymorphism among the three cultivars.

The total number of amplified product was 101 fragments generated by the ten used primers with an average of 10.1 fragments per primer. The total number of polymorphic bands was 28 ranging from 14 as maximum amplified fragments and 6 as minimum amplified fragments and the total numbers of monomorphic bands were 73 as dictated in table 1. The largest size of the amplified product was 2500 bp while the smallest size of the amplified product was 150 bp.

The primer OPC-01 produced 14 bands as the highest number of fragments with 28.57 percent polymorphism. The primer OPA-11 generated 10 amplified fragments and produced 6 bands as the highest number of polymorphic bands recording 60.00 percent polymorphism as well as the highest number of unique bands. The percentage of polymorphism revealed by the different primers ranged from 9.10 percent for primer OPA-10 and OPA-03 to 60.00 percent for primer OPA-11-01. The proportion of total polymorphism (27, 72 %) indicates that the examined cultivars did not exhibit high level of polymorphism.

Table 1: The total number of RAPD-PCR fragments, distribution of unique and polymorphic bands, monomorphic bands and percentage of polymorphism generated by ten decamer arbitrary primers in three *Prunus amygdalus* cultivars

Primer	Size of amplification products (bp)	Number of bands	Mono-morphic bands	Unique bands	Polymorphic bands	Percentage of Polymorphism
OPA-04	1300-300	6	5	1	1	16.67
OPA-11	6500-150	10	4	6	6	60
OPA-17	1500-450	8	5	3	3	37.5
OPA-06	1200-250	11	10	1	1	9.1
OPC-01	2500-180	14	10	4	4	28.57
OPO-02	1000-250	10	5	5	5	50
OPO-14	1400-300	7	6	1	1	14.28
OPC-09	1500-200	12	10	2	2	16.67
OPA-03	750-200	11	10	1	1	9.1
OPC-07	1100-220	12	8	4	4	33.33
Total		101	73	28	28	27.52
Mean		10.1	7.3	2.8	2.8	
Percentage						27.72

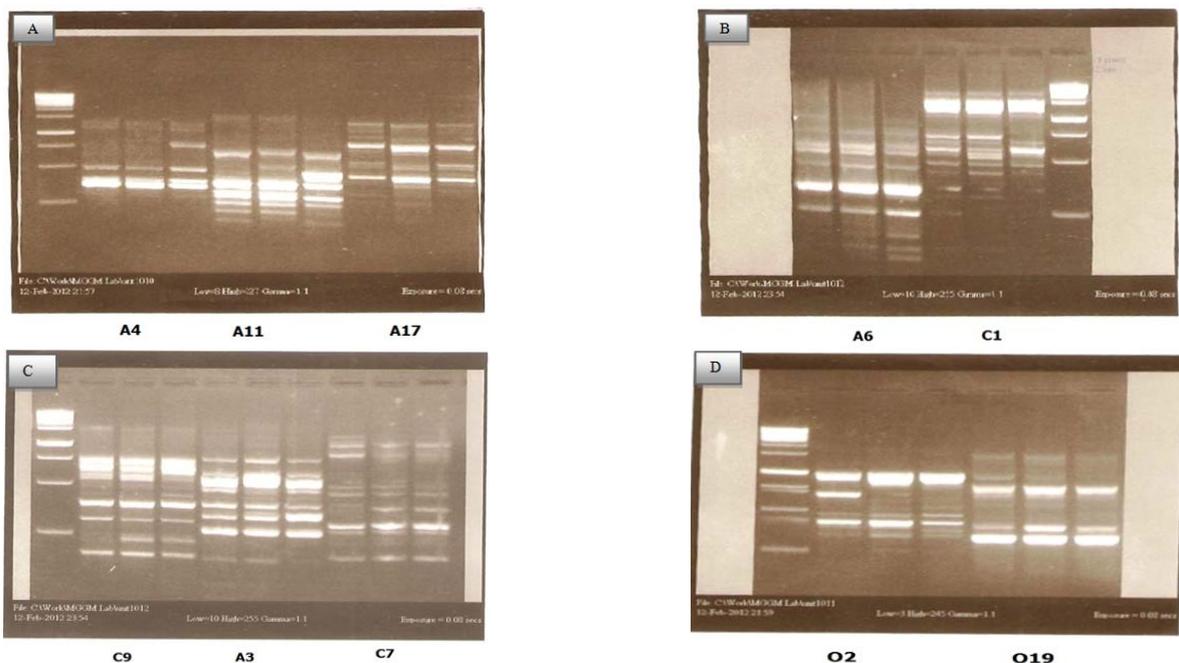


Fig. 1: DNA fingerprinting and reproducible RAPD profiles using genomic DNA samples extracted from three *Prunus amygdalus* cultivars. (A) The primers OPA-04, OPA-11, OPA-17; (B) The primers OPA-06, OPC-01; (C) The primers OPC-09, OPA-03, OPC-07; (D) The primers OPO-02, OPO-19

Table 2: Protein banding pattern of seeds storage protein: C1 represent *P. amygdalus* Batsh; C2 and C3 represent two *Prunus* hybrids.

Molecular Weight, KDa	Marker	<i>Prunus</i> (C1)	<i>Prunus</i> (C2)	<i>Prunus</i> (C3)
250.00	+	-	-	-
130.00	+	-	-	-
107.75	-	+	+	+
95.00	+	-	-	-
72.00	-	-	-	-
60.64	-	+	+	+
55.00	+	-	-	-
37.50	-	+	+	+
36.00	+	-	-	-
30.75	-	-	+	+
28.00	+	-	-	-
Total		4	4	3

Protein electrophoresis data

Seed protein SDS PAGE banding profiles revealed the production of bands with molecular weight ranging from 107.75 and 30.75 KDa in *P. amygdalus* and the two hybrids, (Fig. 2). The highest number of protein bands was 4. Three bands were monomorphic and only one band was polymorphic giving 25% polymorphism. The polymorphic band was produced by C2 and C3 and has a molecular weight of 30.75 KDa, table 2.

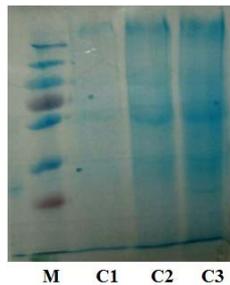


Fig. 2: Electrophoretic banding profile of seed protein, C1, *Prunus amygdalus* "Umm alfahm" cultivar; C2, *Prunus* hybrid 53; C3, *Prunus* hybrid 54; M, marker

Numerical Data analysis

Relationships of *Prunus* cultivars based on RAPD data

Phenogram was constructed using the UPGMA method based on the molecular analysis of RAPD data and has been estimated in terms of similarity using Jacquard's coefficients is presented in (Fig. 3). This phenogram is based on information given in table 1 for measuring the genetic distance among the examined *Prunus* cultivars. The phenogram figured the three studied cultivars of *P. amygdalus* and have the highest average distance 1.30. At this level where accession C1 is separated off from all *Prunus* accessions. The nearest average between C2 and C3 accessions was 1.11.

Relationships of *Prunus* accessions based on protein electrophoresis data

Phenogram was constructed using the UPGMA method based on the molecular analysis of protein electrophoresis data and estimated in terms of similarity using Jacquard's coefficients is presented in (Fig.3). This phenogram is based on information given in table 2 for measuring the genetic distance among the examined *Prunus* cultivars. The phenogram figured the three studied cultivars of *P. amygdalus* and have the highest average distance 1.65. At this level where accession C1 is separated off from all *Prunus* accessions. The nearest average between C2 and C3 accessions was 1.20.

Relationships of *Prunus* accessions based on both DNA and protein electrophoresis data

Phenogram was constructed using the UPGMA method based on the molecular analysis of RAPD data and protein electrophoresis data

estimated in terms of similarity using Jacquard's coefficients are presented in (Fig.3). This phenogram is based on information given in Tables 1& 2 for measuring the genetic distance among the examined *Prunus* cultivars. The phenogram figured that the three studied cultivars of *P. amygdalus* are having the highest average distance 1.35. At this level where accession C1 is separated off from all *Prunus* accessions. The nearest average between C2 and C3 accessions was 1.15. It can be concluded from the UPGM analysis of genetic diversity based on RAPD and protein electrophoresis that C2, C3 are closely related cultivars and may be identical. The C1 cultivar has split as separate identity [16] [17].

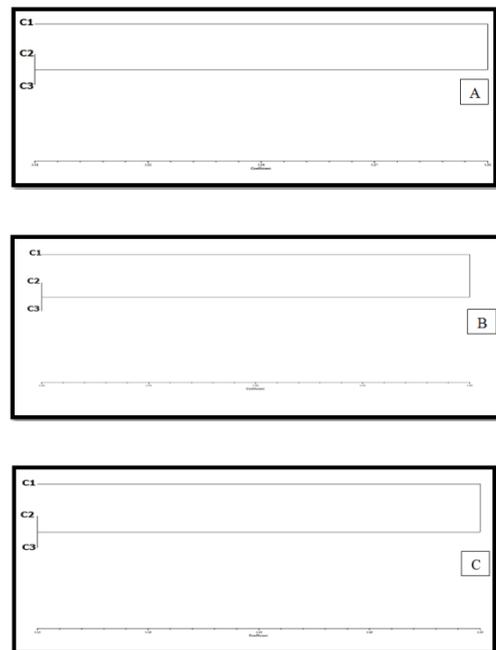


Fig. 3: UPGMA Phenogram illustrating the relationships between the three *Prunus* cultivars (A) Based on DNA data; (B) Based on protein electrophoresis data; (C) Based on both DNA and protein electrophoresis data

Botanical study

Macro-morphology

P. amygdalus (Fig. 4) is an evergreen tree, cultivated in some places of Egypt such as, North Sinai, West Nubaria, Matrouh, South Sinai and Alexandria. Trees or shrubs are 3–6 m in height. Branches are erect or horizontally spreading, unarmed, with many short branchlets, glabrous. Branchlets are brownish, grayish brown to grayish black with age. Winter buds are brownish, ovoid, 3–5 mm, glabrous.

Leaves

Those on branches approximate and often fascicled; petiole 1–2(–3) cm, glabrous, with 2 to 4 nectaries at apex or at base of leaf blade; leaf blade is lanceolate to elliptic-lanceolate, 3–6(–9) × 1–2.5 cm, slightly pilose when young, glabrescent, base is rounded, margin is entire to shallowly serrate, apex is acute to shortly acuminate (Fig. 5).

Flowers

Flowers are solitary, opening before the leaves. Pedicel is 3–4 mm, 4–10 mm in fruit, glabrous. Hypanthium is cylindrical, (5–)6–8 × 3–5 mm, glabrous. Sepals are broadly oblong to broadly lanceolate, 5–6 mm, glabrous, margin is pubescent, and apex is obtuse (Fig. 5).

Petals

They are white or pinkish, oblong to obovate-oblong, 1.2–2 × 0.7–1.1 cm, base is tapering to a narrow claw, and apex is obtuse to emarginated (Fig. 5).

Stamens

Stamens are unequal in length. Ovary is densely tomentose and style is longer than stamens (Fig. 5).

Drupe

It is obliquely ovoid to oblong-ovoid, compressed, 3–4.3 × 2–3 cm, densely pubescent, base is mostly subtruncate, apex is pointed to somewhat obtuse; mesocarp thin, splitting at maturity; endocarp is yellowish white to brown, ovoid, broadly ellipsoid, or shortly oblong, asymmetric on both sides, 2.5–3(–4) cm, hard to fragile, ventral suture is curved and ± acutely keeled, dorsal suture is generally straight, surface is smooth and pitted with or without shallow furrows, and base is obliquely truncate to orbicular-truncate (Fig. 5).



Fig. 4: A photograph of the tree of *Prunus amygdalus* Batsch (x=0.043)

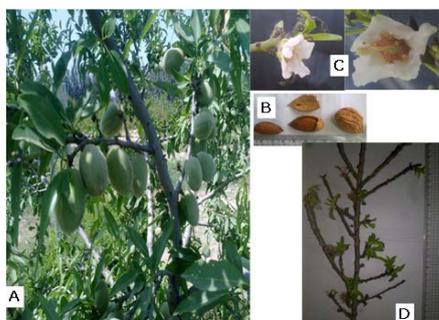


Fig. 5: A photograph of different parts of *Prunus amygdalus* Batsch, (A) Large leaves, stem and fruit (x=0.22); (B) A photograph of seed With/ without pericarp (x= 0.73); (C) Young branches and flowers of tree showing stamens (x=1); (D) Young leaves and young branched stems (x=0.41)

Micro-morphology

The leaf lamina

A transverse section of the leaf shows upper and lower epidermises enclosing a dorsiventral mesophyll. The palisade tissue consists of two rows of columnar cells in the lower epidermis and three rows in the upper one, and is interrupted in the midrib region with collenchymatous cells. The midrib is more prominent on the lower side and shows collateral arc-shaped vascular bundle which is surrounded by a discontinuous band of fibres, while the lower part of the ring is formed of patches of fibres interrupted with parenchymatous cells (Fig. 6).

The epidermis

The upper epidermis of the lamina consists of polygonal, slightly isodiametric and tangentially elongated cells with straight slightly thick anticlinal walls, covered with a thin, smooth cuticle and devoid of stomata. The lower epidermis consists of nearly isodiametric or slightly elongated cells with slightly curved anticlinal walls, covered with thin, smooth cuticle. Stomata are present on the lower epidermis only. Stomata are of anomocytic type being surrounded by 4-5 cells and they are oval in shape.

Trichomes are non-glandular, unicellular, and straight, curved or spirally twisted similar to those present in the young stem. Hairs are having acute apices, covered with smooth cuticle with narrow lumina. Hairs are present on the upper surface more than on the lower one and arising from a cicatrix. In the neural region, the cells of the upper and lower surfaces are polygonal, axially elongated, having straight thin walls, covered with thin, smooth cuticle, and devoid of stomata. Neural trichomes are non glandular, unicellular, straight, curved or spirally twisted, and covered with smooth cuticle. The lower neural cells are more elongated than the upper (Fig. 6).

The mesophyll

It is dorsiventral, the palisade is continuous over the midrib region, and formed of 1-2 rows of columnar cells, closely packed, having straight anticlinal walls, the lower one is shorter than the upper one and both containing green plastids. The spongy tissue is formed of 6-8 rows of rounded to irregular parenchyma cells with wide intercellular spaces containing scattered prisms, clusters of calcium oxalate and starch granules. Small vascular bundles may be embedded within the spongy tissue (Fig. 6).

The midrib

The cortical tissue of the midrib consists of 6 rows of collenchymatous cells beneath the upper epidermis and 2-3 rows abutting the lower epidermis, followed by 3-4 layers of thin walled parenchyma showing narrow intercellular spaces and containing prisms, clusters of calcium oxalate and few scattered starch granules. The endodermis is indistinct. The pericycle is formed of discontinuous ring of lignified fibres interrupted by parenchymatous cells from the lower side (Fig. 6).

The vascular tissue

It consists of an arc-shaped collateral vascular strand which is formed of xylem towards the upper side and phloem towards the lower side separated by uniseriate medullary rays consisting of polygonal or slightly elongated cells containing tannins (green color with ferric chloride T. S.). The xylem consists of lignified spiral, annular vessels and wood parenchyma. The phloem consists of sieve elements and phloem parenchyma. The phloem parenchyma contains scattered prisms of calcium oxalates and tannins (Fig. 6).

The powdered leaf

The powdered leaf is dark green in color, odorless with an astringent taste. The leaf powder is characterized microscopically by the following elements (Fig. 7):

1. Fragments of the upper epidermis consist of isodiametric cells with straight and slightly thick anticlinal walls, covered with thin, smooth cuticle, and devoid of stomata.

- Fragments of the lower epidermis consist of nearly isodiametric or slightly elongated cells, having curved anticlinal walls, covered with smooth cuticle, and showing anomocytic stomata.
- Fragments of upper and lower neural epidermal cells with isodiametric elongated cells which are more elongated in the lower one and having thin walls and covered with smooth cuticle. They are devoid of stomata.
- Fragments of palisade cells, which are columnar, thin walled cells containing green plastids.
- Fragments of lignified pericyclic fibres, which are pitted having narrow and moderate lumina and acute apices.
- Fragments of lignified spiral, annular and pitted xylem vessels.
- Fragments of lignified spiral, annular and pitted xylem vessels.
- Fragments of pitted lignified tracheids and tracheidial vessels.
- Numerous prisms and clusters of calcium oxalates.

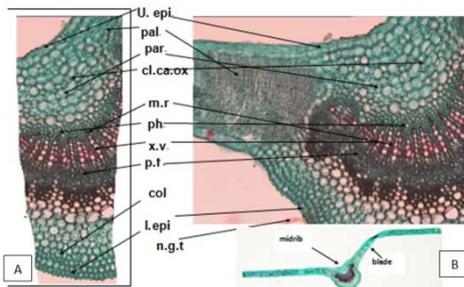


Fig. 6: Photograph of the transverse sector of the leaf of *Prunus amygdalus* Batsch. (A) Low power (x=25); (B) High power (x=200) cl. ca. ox, cluster of calcium oxalate; col., collenchyma; l. epi., lower epidermis; m. r., medullary ray; n. g. t., non glandular trichome; par., parenchyma; pal., palisade; p. f., pericyclic fibres; ph. Phloem; s., stomata; u. epi., upper epidermis; x. v., xylem vessel

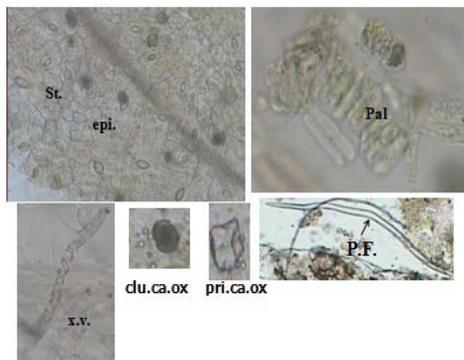


Fig. 7: Photographs of the powder of *Prunus amygdalus* Batsch leaves (x=200) clu. ca. ox, cluster of calcium oxalate; epi., epidermis; pal., palisade; p. f., pericyclic fibres; pri. ca. ox, prisms of calcium oxalate; st., stomata; x. v., xylem vessel

The leaf petiole

A transverse section of the petiole is nearly rounded on the lower side and grooved on the upper one. It is formed of an epidermis followed by the cortex which is formed of collenchymas followed by a parenchymatous tissue containing clusters of calcium oxalate. It is traversed by three crescent-shaped vascular strands consisting of a main one accompanied by two smaller ones (Fig. 8).

The epidermis

It consists of polygonal, isodiametric or slightly elongated cells with straight slightly thick anticlinal walls and covered with a smooth cuticle. Stomata are absent. Trichomes are numerous, non-glandular,

unicellular arising from a cicatrix. The trichomes are more abundant on the grooved side (Fig. 8).

The cortex and ground tissue

It is formed of about 2-3 rows of collenchymatous cells, neighboring the upper epidermis and 4-5 rows neighboring to the lower epidermis. The parenchyma cells are rounded or oval in shape, showing narrow intercellular spaces, containing clusters of calcium oxalates and scattered starch granules (Fig. 8).

The pericycle

The pericycle is formed of a continuous band of fibres above the vascular bundle and of patches of fibres below the vascular bundle interrupted with parenchymatous cells (Fig. 8).

The vascular tissue

Each vascular bundle is crescent-shaped and accompanied by the pericycle. The phloem is formed of sieve elements and phloem parenchyma traversed by uniseriate medullary rays consisting of thin walled cells. The phloem parenchyma contains tannin and scattered prisms of calcium oxalate. The xylem elements are radially arranged and consist of lignified spiral and annular vessels traversed by uniseriate medullary rays (Fig. 8).

The powdered petiole

Powdered petiole is light green in color, odorless with an astringent taste and characterized microscopically by the following elements (Fig. 9):

- Fragments of epidermis with isodiametric cells having straight and slightly thick anticlinal walls, and covered with a thin, smooth cuticle.
- Fragments of lignified pericyclic fibres, which are pitted having narrow and wide lumina, and acute apices.
- Fragments of lignified spiral, annular and pitted xylem vessels.
- Numerous clusters of calcium oxalates

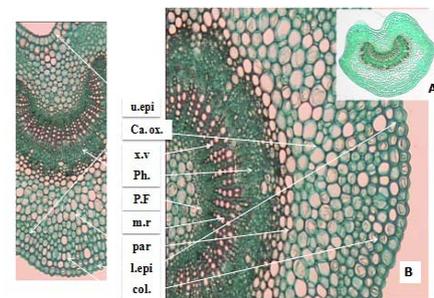


Fig. 8: Photograph of the transverse sector of the petiole of *Prunus amygdalus* Batsch. (A) Low power (x=50); (B) High power (x=200) cl. ca. ox, cluster of calcium oxalate; col., collenchyma; l. epi., lower epidermis; m. r., medullary ray; par., parenchyma; p. f., pericyclic fibres; ph. Phloem; u. epi., upper epidermis; x. v., xylem vessel



Fig. 9: Photographs of the powder of the petiole of *Prunus amygdalus* Batsch (x=200, x=333) clu. ca. ox, cluster of calcium oxalate; epi., epidermis; p. f., pericyclic fibres; x. v., xylem vessel

The stem

A transverse section of the old branch is almost circular in outline (Fig.10). It is formed of an outer cork followed by the cortex (1/4 the diameter of the stem), which is formed of parenchyma containing clusters of calcium oxalate and starch granules. The endodermis is undifferentiated.

The pericycle is formed of groups of fibres that interrupted with parenchyma cells. Sclereids may be scattered between the fibres. The vascular tissue is comparatively wide forming a ring traversed by medullary rays. The central pith is narrow and formed of parenchyma containing starch granules and calcium oxalate prisms (Fig.10 & 11).

The cork

It consists of 5-6 rows of brown radially arranged, tabular, polygonal and tangentially elongated cells, having either thick, suberized or slightly lignified walls (Fig.11).

The cortex

It is formed of 13-17 rows of thin walled parenchyma cells (Fig. 11) containing starch granules; which are simple rounded with neither visible hilum nor striations (Fig. 12). Calcium oxalate clusters are present.

The pericycle

It is formed of patches of lignified fibres interrupted by parenchymatous cells interrupted by some sclerides in groups of 2-4. Sclerides are polygonal with pitted lignified walls containing dark brown content. The fibres are elongated with narrow lumina, straight or undulating lignified walls with acute apices (Fig. 10-12).

The vascular tissue

The phloem

It consists of soft tissue that devoid of fibres and formed mainly of thin walled parenchymatous cells, sieve tubes and companion cells. Some phloem parenchyma contains tannin cells (green colour with ferric chloride) and clusters of calcium oxalate. Starch granules are absent (Fig. 11).

The cambium

It is formed of 5-11 rows of cambiform cells (Fig. 11).

The xylem

It is formed of lignified radially arranged elements. The vessels are formed mostly of pitted, spiral and annular thickenings. Wood fibres are present in groups, fusiform in shape, with wide lumina, acute and slightly tapering apices. The wood parenchyma consists of rectangular elongated cells with pitted lignified walls. The medullary rays are formed of rectangular cells with pitted walls and containing starch granules (Fig. 11).

The pith

It consists of more or less rounded parenchymatous cells with pitted lignified walls containing starch granules and calcium oxalate prisms. Some cells contain tannins (green color with ferric chloride T. S.) (Fig. 11).

The powdered stem

The powder is yellowish brown in color odorless and has an astringent taste. Microscopically it is characterized by (Fig. 12):

1. Fragments of brown, polygonal, suberized or slightly lignified cork cells.
2. Fragments of thin walled parenchymatous cells of the cortex containing clusters of calcium oxalate crystals.
3. Fragments of lignified pericyclic fibers with straight or undulating walls showing narrow lumina, having acute and tapering apices usually accompanied with parenchyma containing clusters of calcium oxalate.

4. Fragments of lignified wood fibers with straight or undulating walls showing wide lumina and having acute tapering or slightly rounded apices.
5. Fragments of wood parenchyma having pitted and lignified walls.
6. Fragments of spiral, pitted and annular lignified xylem vessels.
7. Parenchyma of the cortex containing starch granules.

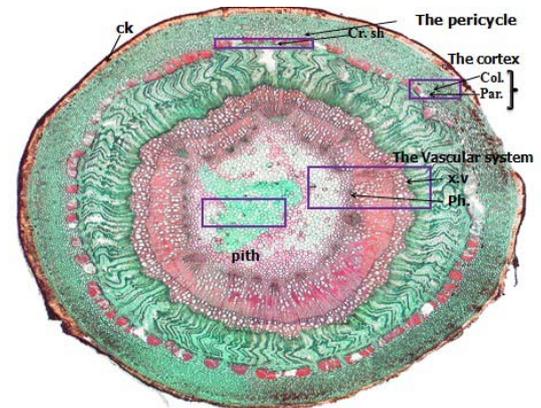


Fig. 10: Photograph of the transverse sector of the stems of *Prunus amygdalus* Batsch ck, cortex; col. collenchyma; cr. sh., crystal sheath; n. g. t., non glandular trichome; par., parenchyma; pi. pith; ph. Phloem; x. v., xylem vessel

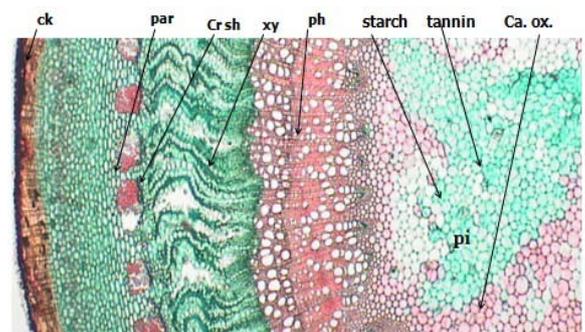


Fig. 11: Photograph of the transverse sector of the stem of *Prunus amygdalus* Batsch (x=200) ck, cortex; cl. ca. ox, cluster of calcium oxalate; cr. sh., crystal sheath; par., parenchyma; pi, pith; ph. Phloem; x. v., xylem vessel

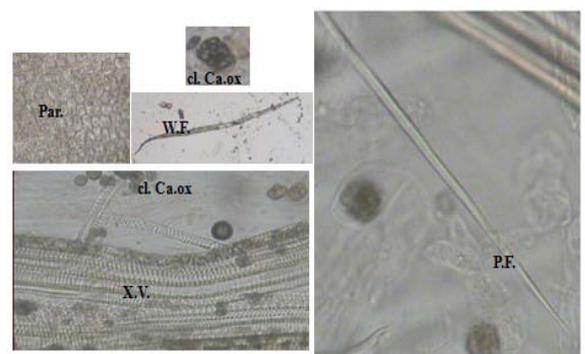


Fig. 12: Photographs of the powder of the stem of *Prunus amygdalus* Batsch high power (x= 333), high power (x= 200), low power (x= 50) cl. ca. ox, cluster of calcium oxalate; par, parenchyma; p. f., pericyclic fibres; w. f, wood fibers; x. v., xylem vessel.

Table 3: Microscopical measurements of the different organs of *Prunus amygdalus* Batsch Umm alfahm cultivar in µm

Item	W	L	D
Leaf			
Crystal sheath		344.8	
Prism of ca oxalate	5.32 7.45 9.41	6.95 16.77 22.5	
Cluster of ca oxalate	9.01 15.13 18.84	3.27 6.55 11.45	
Stomata			23.72 27.03 33.54
Pericyclic fiber		298.93	48.32 16.38 3.28
n. g. t	8.18 12.68 23.13	22.5 11.04 9.41	
Petiole			
Pericyclic fiber		382.7	38.49 23.75 10.65
Xylem vessels		170.55	10.63
parenchyma			13.91 15.95 18.81
Cluster of ca oxalate	14.74 15.56 16.38	22.93 42.59 79.44	
Stem			
Pericyclic fiber	4.91 7.37 13.1	570	
Rosette of ca oxalate	13.09 17.64 21.56	20.86 31.36 33.32	
colenchyma			3.92 9.8 9.8
Wood fiber		807.52	
parenchyma			12 15 20
Xylem vessels	74.48	1468	

D= diameter, L= length, W= width

CONCLUSION

Macro and micro morphological characters, as well as, DNA fingerprinting discussed in this study can be considered as the identifying parameters to authenticate the plant from other closely related cultivars.

CONFLICT OF INTEREST

Declared None

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