Chapter

Genetic Diversity in Almond (*Prunus dulcis*)

Sadia Sana, Naheed Akhter, Fozia Amjum, Samreen Gul Khan and Muhammad Akram

Abstract

Almond (*Prunus dulcis*), a stone fruit belonging to a family *Rosaceae* (rose) is broadly cultivated for ornament and fruit. Within this genus, *the* almond is very much associated with the peach, and these two fruits share the same subgenus the *Amygdalus*. About 430 species are spread all through the northern temperate regions of the world. The Mediterranean climate region of the Middle East like Turkey and Pakistan eastward to Syria is native to the almond and its related species. Almond is one of the ancient fruit trees known to the Asian as well as European regions with the most primitive proof of cultivation dating about 2000 B.C. *Prunus dulcis* (Almond) is a nutrient-loaded nut crop. Almond possesses a great genetic diversity due to the genetically controlled self-incompatibility system which can be estimated by a morphological characteristic including molecular markers and isoenzymes with a wide range of marker techniques. Simple sequence repeats (SSR) involving RFLP or SNP are the most commonly used molecular techniques among the DNA-based molecular symbols. Particular agronomic characters, e.g. kernel bitterness or self-compatibility can also be traced by these molecular markers. The direct association between the level of diversity and the basis of the germplasm cannot be understood by the studies of genetic diversity. Genetic diversity cannot be seriously lost by self-compatibility in almonds. The breeding, conservation, and cultivation of wild-growing almonds may similarly advantageous after the genetic diversity research studies (especially those applying molecular markers).

Keywords: *Prunus dulcis*, Genetics diversity, Simple sequence repeats, Molecular mechanism

1. Introduction

Nuts generally are part of functional foods and their consumption was reported, to protect against cardiovascular disease, certain types of cancer, diabetes, and other disease states, including neurodegenerative conditions. African Black Walnut and Almonds are important but underutilized nuts with inherent medicinal and therapeutic potentials for promoting human health reported that Almonds (*Prunus dulcis*) and Black walnuts (Tetracarpidium conorphorum) help to lower cholesterol levels in the blood, the risk of diseases of heart, control of body weight and control of diabetes. These nuts (Walnut and Almond) are also believed to naturally comprise and polyunsaturated and monounsaturated fatty acids, dietary fiber, and protein, as well as various necessary nutrients including several trace elements and vitamins which contribute significantly to healthy living.

Almonds are prunes, which are small to medium-sized trees of fruit that have their place in the family of rose, which is Rosaceae. They were usually positioned in a Prunoideae (or Amygdaloideae), that is a sub-family, but sometimes, they are positioned in their individual Prunaceae (or Amygdalaceae) family [1]. In recent times, it has become seems that almonds evolved from the Spiraeoideae that is a sub-family [2, 3]. The group Prunus consists of several economically significant fruit trees classes such as apricot, cherry, plum, peach, and almond [4, 5]. In Southcentral Asia, the peach and almonds almost developed from the identical inherited species [6, 7]. 26 classes of Prunus form a distinct taxonomic group on the earth. 21 almond classes and 6 natural hybrids present in Iran [8, 9].

Almonds were cultivated at least by 3000 BC [8, 10]. The almond was spread beside the seashore of southern Europe by Greeks, Egyptians, and Romans, and the Mediterranean in northern Africa [11]. Thus the almond and its associated kinds are native to the Mediterranean environment area of the Middle East indicating Pakistan eastward to Turkey and Syria [12, 13]. In the 1700s, Spanish Padres established the Task at Santa Barbara and carried almonds to California [14, 15].

In the late 1800s, the industry started in California due to the growth of great cultivars on almonds, and importers were forced to defend the industry. From then till about 1960, the industry technologically advanced at a moderate speed [14]. Furthermore, approximately 8% of the total world's almonds are cultivated in California.

Under farming, many fruits trees classes have converted from sexual reproduction into vegetative propagation [1]. Outdated systems of production have continued, where cultivar propagation is established on a diverse reproductive system [16]. For millennia, *Prunus dulcis* has been cultivated by seeds. *Prunus dulcis* grafting continued with little significance until recently [15]. Both clonal and sexual reproductions are used for *Prunus dulcis* propagation [16–18].

2. Botanical description

The *Prunus dulcis* is botanically categorized as a drupe with skin like pubescent exocarp, hell is like a fleshy mesocarp and shell is like a hard endocarp [19]. The embryo is enclosed by a pellicle in the seed, composed of a nucleus, endosperm remnants, and seed coat. *Prunus dulcis* is distinguished from other *Prunus* classes by its leathery and dry mesocarp, at maturity which is dehisced [20–22].

2.1 Plant

Small to the medium that is average-sized tree exist with open canopy with linear or ovate with notched margins leaves sized between 3 to 5 inches, about 3-4 times longer than wide having finely notch margins and sharp tips [21, 23].

2.2 Flowers

Almond tree flowers are sweet-scented with white or light pink and almost identical to peachtree flowers. Almond flowers have a perigynous ovary and many elongate stamens with 5 petals and sepals [22, 23]. Flowers are borne laterally on short lateral branches and spurs, or occasionally on elongated shoots in lateral position [24, 25].

2.3 Pollination

Almonds involve cross-pollination because they are self-incompatible [26]. *Prunus dulcis* is a crop that is a large consumer of fertilizer and water, and it is extremely pollinator-dependent, its production may be dependent on variations in these resources [27]. All pollinators like honey bees are entirely crucial for pollination, particularly since wet and cool weather can arise at the comparatively early blooming period [28, 29]. Moreover in California, almost 8% of the total world's almond fruits are cultivated, where the temperature change is predicted to decrease water accessibility [30].

2.4 Fruit

Almond is a nut fruit. The whole nut fruit includes the hull is a drupe, though the hull dries and splits to reveal the pit of the fruit. Its fruiting starts in 3 to 4 years old trees, and 6-10 years old tree leads with maximal production [26, 31]. On average, an almond tree can produce for more than 50 years. For best fruit cropping high ratio of flowers should be maintained. Almond fruit trees produce flowers in February. Fruits development is considered by an increased cotyledon size and diminishing endosperm and nucleus [23, 32]. The growth of the different fruit tissues in the 4 genotypes presented sequential deviations as has formerly reported in other *Prunus dulcis* cultivars [33]. Therefore, ripening and growth of the different fruit tissues continued in a somewhat shifted mode from one genotype to another. Till April, the tenderly derived fruit tissues as endocarp, mesocarp, exocarp, and tegument are surrounded and protected by cotyledon. The endocarp is soft and green in color that is easy to open [34]. The endosperm and growing embryo with its typical white cotyledons are noticeable in all genotypes and nucleus size has decreased in May [32]. The endocarp has become appear a woody texture and hard to open and is turnoff into brown color. To end, the maturing season ends and the white cotyledons fill the full space inside the tegument. Mesocarp with the exocarp is starting to dry ultimately exposing the endocarp [34].

3. Harvest, postharvest handling techniques

In agronomical processes, irrigation is the most significant aspect affecting almond seed weight, quality, and yield, however, there is no significant impact was observed on the lipid concentration and composition of fatty acid [35]. Practically crop of almonds does not disturb the lipid composition but induces discrepancy to the physical characters of almond grain due to the greater seed moisture concentration. A late harvest of drupes fruits prompts a higher concentration of dry material in the grain. Genetic features, weather and soil conditions, fertilizers usage, and the condition of the plant's maturity can affect at harvest level, and also the concentration of minerals in a plant [36].

3.1 Maturity

At maturity, the hull of the almond splits and physically nuts separate from the tree at this spot. Harvesting of the almond tree started when hulls of almond nut fruit in the inside of the canopy are open [37]. During maturation, the drying of the seed coat proceeds, and the seed coat turns brown. If harvesting is delayed it increases the threat of navel orange worm invasion [38].

3.2 Harvest method

Almond trees are harvested by mechanical or automatic tree shakers. While shaking the young trees may be damaged, therefore in the first few years, the young trees are harvested by hand knocking. Almond nuts are spread on the ground for drying for 1-2 weeks [39].

3.3 Postharvest handling

Immediately after crop harvesting the fruits can be dried and hulled instantly or stocked for fumigation against Navel Orange worm [37]. Fruit nuts are dried under hot air till the moisture content reaches 5 to 7 percent [40]. Then the nuts are dehulled and shelled. If final processing is pending the nuts in the shell can be stored in a container for many weeks or months [41]. Nuts are then shelled and sorted for size and appearance [42]. In the last the nuts are bleached for color development, then salted, roasted, and/or flavored before wrapping. Furthermore, former studies described that storing at low oxygen and low-temperature atmosphere caused less off-flavors development [43].

3.4 Storage

Either in-shell or shelled if dry, almonds may be stored for many months, or frozen for very long periods in years [41]. Commercially, for long-term storage, the nuts are fumigated for navel orange worm and kept at a temperature below 40°F [39, 43].

4. Genetic engineering for the improvement of production yield

The objective of the study was to investigate the genetic diversity of almonds. Genetic engineering contains direct handling of an organism's genome through biotechnology to adapt the genetic makeup of cells, containing the transmission of genes within and across different kind's limits to yield advanced crops [44]. The process can be used to remove, (knock out) or target a specific part of the genome. Genetic engineering techniques have been applied in various fields including medicine, research, industrial biotechnology, and agriculture [45].

There are four main targets in making genetically improved crops. The first aim is to provide defense against environmental pressures, such as pathogens or cold or resistance to herbicides. The second aim is to alter the quality of the crop by raising the nutritious value and providing additional industrially valued qualities and quantities. Thirdly, to construct materials that it does not normally make or to provide the novel model. Forth is to honestly improve yield by accelerating growth, or making a tolerant organism, for example improving salt, cold, or drought tolerance in plants [46, 47]. The basic chromosome number of wild-type almonds is eight and it's DNA substances are small. Almond fruit occupies a very anomalous place between other fruit trees. Almond is considered the main crop and is cultured in diverse climatic areas after tolerance to drought, salinity, and cold [47].

The genotypes of almonds are clustered into 2 main groups, one is wild and the other one is cultivated almonds. The group of cultivated almonds is distributed into four subgroups which are comprised of 2, 3, 44, and 42 genotypes, respectively. The wild group of Prunus almonds has genotypes that had the less average for a maximum of the studied characters, but an average of this group for characters such as kernel color, ease of hulling, shell color, leaf basal shape, sensitivity to *Anarsia Lineatella*, marking of the outer shell, and leaves arrangement was greater than the other cultivated group of almonds. In cultivated almonds average genotypes in the first subgroup for certain main characters such as thickness, kernel weight, and width, double flower in buds, growth tree habit, bearing habit, ease of hulling, flower density, sensitivity to *Anarsia Lineatella,* petiole length, sensitivity to *Myzus persicae* and *Pterochloroides persica* was greater than the other subgroups. The second subgroup of the cultivated almonds had the maximum average for suture opening of the shell, kernel length, leaf length, shriveling of the kernel, leaf width, leaf shape and leaf area, duration of flowering, and sensitivity to *Pseudomonas syringe* [48].

Numerous study of the literature shows that almond tree size and seedling juvenility is the key hurdle to developing the genetic potential of almond fruit and nut breeding stocks. Dwarf trees with usual cultivars revealed that fruit size was not destructively affected by dwarfing whereas the yields considered being high [47]. As in the dwarf tree, the fruit value was reduced it was observed that heritability for fruit firmness, skin color, size, and percentage of soluble solids was in high concentration. This suggested that these characteristics can be improved to meet up the commercial standards within one or two selected cycles. Thus it is recommended that mass selection will be helpful in genetically reducing the juvenile stage [49].

To increase the yield of almond trees different studies were carried to facilitate genetic manipulation, and to increase its production efficiency. The genetically engineered almond tree can be dwarfed (compressed), by manipulating the dwarf (DW) gene of peach [50]. Dwarf almond tree revealed that the heritability of these dwarf traits is high as well as spur density also differs widely. The flower production of dwarf hybrids is copious. While the yield potential of dw/dw dwarf almonds will stay unidentified until fertility is restored. As the international reputation increases, demands are motivating almonds yield to continue to rise across worldwide. Different research studies on varietal chilling necessities involving the particular microclimates in a defined state will provide better assistance in reducing the risks of wasted bloom. Chilling prototypes must also be considered for regional application and accuracy to increase consideration of the causes affecting the timing and the length of the almond bloom and also the association among characteristics of bloom. Continuous changes in climate intensely affect the area where Prunus almonds are grown up. Cultivators have to need to take care to develop different varieties in different climates with sufficient chilling, and also to care for young buds and shoots from chill damage. And also more researches are required on precise climate thresholds and their association to physiological variations during Prunus almond pollination and bloom. Though, the simple training of heat and chill monitoring will permit cultivators to anticipate flowering like to prepare the optimum bee activity in bloom and idea for crop reduction in very warm bloom times [47].

In recent years, molecular markers have been used to study genetic diversity and cultivar identification of almonds. Methods based on knowledge provided by advances in molecular genetics, notably molecular markers, promise faster and more efficient approaches to cultivar improvement. In fact, important tools such as molecular markers, maps, DNA sequences, and quantitative trait loci (QTLs) have been developed and made available to researchers, and applications at the breeding program level have already started. In genetics, a molecular marker is a fragment of DNA that is associated with a certain position within the whole genome [51]. Molecular markers are used to identify a particular sequence of DNA in an unknown DNA pool [52].

For this purpose different types of molecular markers are used for the assessment of the genetic diversity like microsatellite markers, simple sequence repeat (SSR) markers, Informative Markers for morphological traits of almond (*Prunus dulcis*) [53]. Edifying markers are the most suitable and reliable genetic statistics for breeding purposes and are considered as a first fact to examine the genome for the associated characters. In almond Prunus, the practice of breeding faces a distinctive task due to the limited genetic experience of commercial cultivars.

Morphological traits such as tree altitude in cm, leaf length in cm, leaf shape, flowering duration, leaf width in cm, blooming time, petiole length in cm, kernel length in cm, kernel yield in gram, kernel width in cm, kernel thickness in cm, nut weight in g, kernel nut weight in g and kernel percentage are frequently used for cultivar identification in almond. Though, morphological characters are restricted because of their environmental oscillation [54].

The basic worries of present agriculture are the utilization and conservation of priceless genetic resources of different plant crops. The requirement for precise recognition applies to cultivars and sequences, in parallel to their type of maintenance, whether they are preserved in an ex-situ and in-situ gene bank or another in the Vitro gene bank [55]. The tools developed for biodiversity classification may permit explanations of synonyms and improvement in the origin of cultivars and species. At times for cultivars, the description and determination of trees of fruits are hard by using conservative approaches. Although the morphological symbols are prone to uncertain explanations, molecular methods should be applied in the identification and programs of breeding for the cultivars [56]. Molecular markers facilitate distinguishing labeling mistakes and repetitive documentation of cultivars in nurseries etc. Moreover, it reduces the work programs of breeding by speed up the process of breeding by permitting an assortment before the first crop of fruit, by following certain genes or genotypes among offspring of crosses [57]. The use of molecular symbols based on PCR has been the option of plant genetic research studies and in making to impression for many types of fruits. These symbols can be used to regulate the varieties by agreeing a plant to be recognized at any step and vegetative cycle and may undo cases involving plants with undefined sources and names [58].

5. Isozymes detection

Isozymes are various forms of enzymes that catalyzed the conjoint substrate but are mixed based on their physical appearances for example shape, electrical charge, molecular mass, and protein structures [57]. Isozymes can be separated and analyzed due to the difference in their electrophoretic mobility [54].

In-plant genetic and breeding isoenzymes have been used due to their individuality like simple inheritance, lack of gene interactions, co-dominant expression, and polymorphism present in various plant species and lack of environmental effect [59].

Isozymes can be identified in different tissues by different processes. Iso enzyme's variability is the key source of genetic markers which can be used for recognition of hybrids and cultivars, initial selection, recognition of genetic diversity, quantification of genetic associations among populations [55].

In Prunus almond fruit following Isozymes are present these include glutamate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, formate dehydrogenase, and shikimate dehydrogenase [60, 61]. Isozymes can be separated by using the polyacrylamide gel electrophoresis method and these isoenzymes can be used to recognize genetic variability in Prunus almonds [56].

6. Therapeutic applications

Almond numerous active components as dietary fiber [62], proteins like albumin, globulins & amandine, amino acids, certain important essential minerals as magnesium and calcium, vitamins especially B vitamin, and monounsaturated fats [63, 64]. Furthermore, almonds contain phenolic and phytates that constrain the amylase enzyme activity and are supposed to perform synergistically to reduce starch digestibility [65]. The reduced rate of digestion of carbohydrates may describe reported growths in blunted blood glucose response and satiety with consumption of almonds nuts, which describes them as a low glycemic food [66]. Almond flour mixed with honey or sometimes with sugar is often used as a gluten-free food substitute for wheat flour in baking and cooking [67].

These components showed the therapeutic activities. Almond oils also comprise of fatty acids like stearic acid, palmitic acid, palmitoleic acid, oleic acid, eicosanoid acid, linoleic acid, arachidic acid, behenic acid, alpha-linolenic acid, and erucic acids due to these fatty acids almond oil has outstanding emollient properties [68]. The oil can be used for massage therapy to relieve sprains [13].

7. Hypoglycemic action

Almonds nuts, flowers, and seeds lowered the blood glucose level oxidative stress in diabetic patients and also decrease post-prandial glycemia as the almond nuts ingestion is related to a reduction in oxidative damage and blood glucose level [31].

8. Cholesterol-lowering action

Prunus dulcis have a reliable effect of LDL-cholesterol lowering in healthy people and persons with diabetes and high cholesterol [69]. Prunus Almonds are rich in unsaturated fatty acids and low in saturated fatty acids and plant protein, contain fiber, α-tocopherol, phytosterols, magnesium, arginine, manganese, copper, potassium, and calcium [70]. The responsible mechanism for the LDL-cholesterol decline is probably to be linked with the presence of nutrients Prunus almonds, like reduced bile acid and cholesterol absorption, increased excretion of cholesterol and bile acid, and LDL-cholesterol receptor activity is also increased. Prunus Almonds also comprise phytosterols which are accompanying properties of lowering cholesterol [66]. The nutrients present in Prunus almonds control the enzymes involved in the production of bile acid and cholesterol. Almonds also reduced the biomarkers of lipid peroxidation in hyper-lipid emic patients. Regular ingesting of Prunus almonds can be supportive in the regulation of blood pressure as they are low in Sodium and high in potassium [71].

9. Immunostimulant action

Almonds enhanced the immune surveillance of blood mononuclear cells against the infectious virus because the *Prunus dulcis* nuts are associated with high levels of cytokine production including interleukins, interferon-A, (TNF-α) tumor necrosis factor, and INF-gamma. Almonds also induce a considerable decrease and control in the Herpes simplex virus replication [65].

10. Pre-biotic potential

Almond seeds possess prebiotic activity. Prebiotics are non-digestible nutrition elements that stimulate bacterial activity and growth in the system of digestion [13].

In this way, prebiotics is stated to be beneficial to health. Characteristically the prebiotics is carbohydrates (such as oligosaccharides) in nature [71]. As nutritionally soluble fibers are the most common classification of pre-biotic. To a certain level, many forms of dietary fibers reveal some level of prebiotic effects. It has also been shown that Prunes almonds altered the composition of bacteria in the gut by stimulating the Eubacterium rectal and bifid bacteria's growth [68].

11. In amnesia

Almonds have a memory-enhancing activity as they are found to raise the Ach level in the brain and finally improve the brain memory [72]. It may be useful to examine the potential of the almond plant in the management of Alzheimer's. Regular consumption provides power to the brain as they comprise vital nutrients which can essentially help to increase intellectual capabilities [73].

12. Anti-oxidant action

Almonds possess anti-radical and anti-oxidant activities and their phenolic extract may be useful in inhibiting and reducing the process of different oxidative stress linked to disease [72]. The scavenging capacity and reducing the power of the phenolic extracts for hydrogen peroxide, superoxide, and radical nitrite were calculated [74].

13. Hepato protective action

Almond Prunus showed hepatoprotective activity against hepatitis and also improves the biochemical markers to see the hepatic damage like ALP, SGOT, SOD, SGPT, GSH, total bilirubin, catalase, direct bilirubin, and LPO [62].

One study in the almond was tried for its hepatic protecting effect against Paracetamol-induced and CCl_4 hepatitis in rats. The management with the almond fruit extracts carried out the changed levels of the biochemical markers to close to normal levels [72].

14. Conclusion

As the international reputation increases, demands are motivating almonds yield to continue to rise across worldwide. In the end, we concluded that microsatellite indicators can be effectively used to examine the genetic diversity of almonds and to classify useful markers for important traits breeding.

The tools developed for biodiversity classification may permit explanations of synonyms and improvement in the origin of cultivars and species. At times for cultivars, the description and determination of trees of fruits are hard by using conservative approaches. In almond Prunus, the practice of breeding faces a distinctive task due to the limited genetic experience of commercial cultivars. To increase the yield of almond trees different studies were carried to facilitate genetic manipulation, and to increase its production efficiency. And also continuous changes in climate intensely affect the area where Prunus almonds are grownup. Cultivators have to need to take care to develop different varieties in different climates with sufficient chilling, and also to care for young buds and shoots from chill damage.

Author details

Sadia Sana[1], Naheed Akhter[1*], Fozia Amjum[2], Samreen Gul Khan[2] and Muhammad Akram[3]

1 College of Allied Health Professionals, Faculty of Medical Sciences, Government College University, Faisalabad, Pakistan

2 Department of Chemistry, Government College University, Faisalabad, Pakistan

3 Department of Eastern Medicine and Surgery Government College University, Faisalabad, Pakistan

*Address all correspondence to: naheedakhter@gcuf.edu.pk

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