Pomegranate plant material: Genetic resources and breeding, a review

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SUMMARY – The pomegranate (*Punica granatum* L., Punicaceae) is one of the oldest known fruit species. It is considered as a monoecious species developing male and perfect flowers, being self and cross-pollinated. Pomegranate grows wild in the near east, Transcaucasia and in Asia minor. Some parts of the Mediterranean area are also considered as native lands of pomegranate. Many cultivars/varieties are listed and *ex situ* collections are already established in different countries, but interchange of plant material is still not frequent. The description of pomegranate germplasm is based mainly on pomological and agronomic criteria and genetic studies are rare. Modern breeding objectives, systems and techniques are reviewed and discussed. Pomegranate culture is still faced with many problems. Its recent development may generate a genetic erosion. Further prospections and collection have to be carried out. New methods must be developed for cultivar identification and improvement and genetic resources management. Plant material interchange and evaluation should be established at a regional level.

Key words: Pomegranate, *Punica*, genetic resources, floral biology, breeding.

RESUME – *"Matériel végétal du grenadier : Ressources génétiques et amélioration (révision)". Le grenadier (*Punica granatum *L., Punicaceae) est l'une des espèces fruitières le plus anciennement connues. Elle est considérée comme une espèce monoïque qui développe des fleurs mâles et parfaites, étant autopollinisées et à pollinisation croisée. Le grenadier pousse de façon sauvage dans la partie orientale proche, en Transcaucasie et en Asie Mineure. Certaines parties de la région méditerranéenne sont également considérées comme terres d'origine du grenadier. Un grand nombre de cultivars/variétés est inventorié et des collections ex situ sont déjà établies dans différents pays, mais l'échange de matériel végétal n'est pas encore fréquent. La description du germoplasme de grenadier est principalement basée sur des critères pomologiques et agronomiques et les études génétiques sont rares. Les objectifs, les systèmes et les techniques de la sélection moderne sont passés en revue et discutés. La culture du grenadier doit encore affronter plusieurs problèmes. Son développement récent pourrait causer une érosion génétique. Des prospections et collectes restent encore à mener. De nouvelles méthodes doivent être développées pour l'identification et l'amélioration des cultivars et la gestion des ressources génétiques. L'échange et l'évaluation de matériel végétal devraient être établis à l'échelle régionale.*

Mots-clés : *Grenade,* Punica*, ressources génétiques, biologie florale, amélioration.*

Origin and distribution

The pomegranate is one of the oldest known edible fruits. Its history dates to very ancient times. This fruit tree is one of the species mentioned in the Bible and the Koran and is often associated to fertility. It is native to Persia and perhaps some surrounding areas. It was cultivated in ancient Egypt and early in Greece and Italy. The fruit was very popular in Irak. In time it spread into Asia (Turkmenistan, Afghanistan, India, China, etc.), North Africa and Mediterranean Europe. The domestication process took place independently in various regions and not only in the Mediterranean region (Evreinoff, 1949; Zukovskij, 1950; Melgarejo and Martínez, 1992).

In ancient Egypt, the pomegranate received the name "Arhumani". The old Semitic denomination was "Rimmon" from which derived the Hebrew "Ramon" and the Arabic "Rumman" names. The Romans first called this species "malum punicum" (Punic apple or apple of Carthage) that evolved to "punicum granatum" and C. Von Linne, finally, gives the name *Punica granatum*. In the near east (Persia, Turkey, etc.), Central Asia (Afghanistan, etc.) and India, the fruit is commonly named "Anar" (Evreinoff, 1949; Zukovskij, 1950; Diry, 1975).

Pomegranate is considered an excellent tree for growing in arid zones for its resistance to drought conditions. It is now widely cultivated in Mediterranean, in tropical and subtropical areas. It can be encountered as regular plantations in Cyprus, Egypt, Morocco, Spain, Tunisia and Turkey. A high number of scattered trees on the borders or within other fruit orchards are reported in many Mediterranean countries where the fruit is very popular in local markets. It is cultivated in central Asia and to some extent in the USA (California), Russia, China and Japan for fruit production and is also developed as an ornamental tree in east Asia (Mars, 1996; Tous and Ferguson, 1996).

Systematic botany

Botanically, the pomegranate (*Punica granatum* L.) is included in the family of Punicaceae with 2n = 16 or 18. The genus *Punica* is known to include two species*: P. protopunica* and *P. granatum*. *Punica protopunica* is endemic to the Socotra island (Yemen) and is the only relative of the cultivated pomegranate (Zukovskij, 1950; Moriguchi *et al.*, 1987; Guarino *et al.*, 1990). It is presumed that *P. protopunica* played a part in the origin of the cultivated pomegranate. But Zukovskij (1950) affirmed that the Socotran pomegranate has not played any part in the origin of the cultivated one. Some authors classified the ornamental dwarf pomegranate as a distinct species, *Punica nana* (Melgarejo and Martínez, 1992).

Floral biology

Normal flowering of pomegranate varieties occurs, in general, between March-April and July-August. It continues for up to 10-12 weeks or more depending on variety and geographical situation. The period of full bloom lasts about one month, and it was observed that flowering and fruit set occurs in about 3 or 4 distinct waves (Ben Arie *et al.*, 1984; Hussein *et al.*, 1994; El Sese, 1988).

Pomegranate is considered as a monoecious species and develops (on the same tree) two kinds of flowers: (i) male flowers (sterile) with short styles and atrophied ovaries containing few eggs, which are generally, "bell-shaped"; and (ii) hermaphrodite flowers (fertile = perfect) with normal ovary developing to fruit, which are, in general, "vase-shaped" (Shulman *et al.*, 1984; Chaudhari and Desai, 1993). The percentage of male flowers is, in general, very important (more than 60-70%) depending on varieties and season. The male types drop and rarely set fruits leaving the hermaphrodite type to produce the majority of the crop. A positive correlation was found between the bearing capacity and the percentage of perfect flowers (El Sese, 1988; Chaudhari and Desai, 1993).

In some situations, perfect flowers are predominant at the beginning of the season, followed by the development of unfruitful flowers. Almost all of the normal flowers are produced between the onset of flowering and the end of full bloom (Shulman *et al.*, 1984; El Sese, 1988; Hussein *et al.*, 1994; Abou-Aziz *et al.*, 1995). For other cultivars and in other situations, early in the season, male flowers are predominant, while later, flowers are mostly perfect. Male flowers production peaks earlier than for normal flowers (El Kassas *et al.*, 1992). Fruit set varies with flowering period (waves). It is, in general, high during the early flowering, gradually decreasing towards full bloom, then increasing again. Fruit retention (at harvest) increased from about 30% during early flowering to about 80% during full bloom and/or late flowering (El Sese, 1988; Hussein *et al.*, 1994). Flowers produced 4 to 5 weeks after the onset of blooming give the highest fruit set (90%) with the lowest fruit cracking and the best fruit quality.

The pomegranate has been described variously as self-pollinated, self and cross-pollinated, highly cross-pollinated or often cross-pollinated. Recent investigations demonstrated that it is capable of both open and self pollination. The use of a marker gene, governing the pigmentation of bud flower and petiole base, confirmed the fact that pomegranate (cvs 'Ganesh' and 'Kabul Yellow') is self- and, in low proportion (13%), cross-pollinated (Jalikop and Sampath Kumar, 1990). Trials conducted with several cultivars showed that fruit set was 79% and 43.3% for, respectively, intact open and self pollinated flowers or 26.4% and 66.2% for the same after emasculation (Karale *et al.*, 1993).

Germplasm collection and evaluation

Pomegranate genetic resources

Some parts of the Mediterranean area are considered as native lands of pomegranate. Almost all of the varieties in the region are of local type selected by unknown persons and maintained by vegetative propagation. Authors from different countries listed many local cultivars/varieties (Levin, 1995a; Ozguven, 1996; Mars, 1996; Mars and Marrakchi, 1998a). The grown local material may be considered as the pomegranate primary gene pool. *Punica granatum* grows wild in the near east, Transcaucasia, Dagestan and also in Asia minor. In these regions, hybridisation between cultivated and wild forms is, probably, still taking place (Zukovskij, 1950). Wild forms (populations) would be the secondary gene pool. The tertiary pomegranate gene pool would consist of forms of the pomegranate wild relative (*P. protopunica*). Genetic studies are rare or lacking entirely because pomegranate has not been subject of much scientific investigation. Some studies based on morphometric criteria have recently been performed to determine the degree of polymorphism within local material (Mars and Marrakchi, 1998b). Results show a considerable phenotypic (and presumably genetic) diversity among accessions. Cases of cultivar misidentification are detected.

Germplasm collection

For the preservation of local pomegranate germplasm, prospections are realized and collections are already established in several Mediterranean countries (Spain, Morocco, Tunisia, Greece, Turkey, Egypt, etc.) (Mars, 1996). Introduction of foreign materiel is limited. Gulick and Van Sloten (1984) reported that in India there are three principal collections containing, each one, at least 30 accessions and that a collection was established in the USA (Georgia) with 35 accessions. Levin (1995b) reported that 8 countries of the former USSR have collections of pomegranate germplasm. Azerbaijan, The Ukraine, Uzbekistan and Tajikistan have relatively large collections of 200-300 accessions. The collection of the Turkmenistan Experimental Station of Plant Genetic Resources was established in 1934 and is the largest in the world containing 1117 accessions. Samples are from 26 countries on 4 continents, consisting of cultivars received from commercial firms and through interchange with other scientific institutions and material collected from wild populations and landraces (Levin, 1994, 1995a). A core collection was also established and is 10% of the size of the main collection. For the unique wild relative of the pomegranate (*P. protopunica*), an expedition (1989) located the species at 5 sites in the Socotra island (Yemen) and seeds were collected and successfully germinated (Guarino *et al.*, 1990).

Collected material is maintained as living plants. According to Levin (1994) experiments have shown that cryopreservation is suitable for storage of seed and pollen of pomegranate cultivars. Also, natural reserves are used in Transcaucasus and central Asia for *in situ* conservation of wild pomegranate populations.

Interchange of plant material is not frequent. Guidelines for its safe movement are not available. But it seems not to be problematic and it depends on the general policies for genetic resources for each country. Pathological constraints that may impede the movement of germplasm are not reported. Recently the "Hop Stunt Viroid" (HSVd) was detected in pomegranate, which is not previously described as host of this pathogen (Astruc *et al.*, 1996). Nevertheless, Levin (1994) reported that since 1964, material from the collection of the Turkmenistan Experimental Station has been sent to scientific institutes to form working collections.

Germplasm description and evaluation

The description/evaluation of pomegranate germplasm is always performed using *self-devised* list of descriptors with special emphasis on pomological (physico-chemical) characters (Mars and Sayadi, 1992; Melgarejo and Martínez, 1992; Mars and Marrakchi, 1998a) and sometimes on technological properties (Al Kahtani, 1992). Recently, a *Descriptor List for Pomegranate* was prepared in the framework of the "CIHEAM Collaborative Working Group on Underutilized Fruit Crops" (Mars *et al.*, 1997). Melgarejo *et al.* (1995) demonstrated the intervarietal differences in fatty acid composition. These differences appear to correlate well with the sweetness of the juice. The sour varieties contained the highest amounts of myristic and linolenic acid. Results also showed that fatty acid composition of Mediterranean pomegranates can differ from those of Oriental varieties. Ben Nasr *et al.* (1996) classified 8 Tunisian pomegranate cultivars into 4 groups using the proanthocyanidins analysis. However, great attention must be paid while using morphological or physiological characters since some of them may be strongly influenced by the environment such as fruit size, rind and juice colour, ripening date, sugars and acidity in the juice, etc. (Shulman *et al.*, 1984; El Kassas *et al.*, 1992; Ben Nasr *et al.*, 1996).

Varieties are often classified as sweet, sweet-sour and sour, early, mid season and late, juicy and table fruit, soft-seeded and hard-seeded or major and minor. The names originate frequently either from the place of cultivation or from the colour of the fruit (Mars and Marrakchi, 1998a).

Despite the large number of local varieties, in almost all countries, very few are commercially utilised and propagated in nurseries as in the case of Spain (Llacer *et al.*, 1995), Tunisia (Mars, 1995) or Turkey (Aksoy, 1995).

Pomegranate breeding

Almost all pomegranate cultivars being grown throughout the world owe their origins to selection by unknown persons among wild seedling trees and/or volunteer seedlings of cultivated trees. These have been maintained by vegetative propagation and, in course of time, have acquired names. Some breeding programmes have been achieved, recently, and some selections may be found as results of these works. Keskar *et al.* (1993) reported that breeding efforts with pomegranate in India began in 1905. However, concerted efforts have been in place only over the last two decades.

Breeding objectives

Objectives of pomegranate breeding depend on the state of the culture and the planned use of the improved cultivars.

Main objectives related to the tree are:

(i) High productivity: high yielding is desirable. This criterion differs among cultivars being affected by geographical location and orchard management. Some new selections are found to be more productive than traditional cultivars (Shi, 1991; Liu *et al.*, 1997).

(ii) Dwarfing habit: this is a desirable pomegranate tree characteristic for mechanical and easy harvesting in particular growing conditions (Levin, 1990).

(iii) Frost resistance: this is particularly important in zones with very severe winter frost as is the case of Garrygala in Turkmenistan (Levin, 1995a). Frost-hardiness is an integral feature dependent on many factors (age, state of the plant, agro-technical level, time of minimum winter temperature occurring, etc.). Frost sensitivity varies with cultivars. Soft-seeded cultivars are, generally, less frost-hardy than hard-seeded ones. Some selections resulting from breeding programmes are considered frost-hardy (Levin, 1995b).

Main objectives related to fruits are:

(i) Good fruit quality: this includes fruit size and shape, rind and seed colour, juiciness, sugar content and acidity, taste, etc. (Tous and Ferguson, 1996). There is large variability among cultivars for these traits that may be greatly influenced by agro-environment and harvesting date. There is no consistent correlation between either fruit size or skin red coloration extent and internal fruit composition (Mars and Marrakchi, 1998b). It seems to be most promising to aim at producing varieties of moderate fruit size which, generally, show a relatively high juice content.

(ii) Soft-seededness: softness or absence of seeds is a desirable economic trait that improves the consumptive qualities of fruits. Many cultivars and forms of pomegranate are quite heterogeneous for this quality and the complete seedless pomegranate cultivars are restricted to a narrow ecological region (Levin, 1995b). It is also observed that hard seed formation can be induced by cross-pollination of soft-seeded cultivars with hard-seeded ones. Seed hardness/softness is evaluated using scales with several grades from "very soft" to "very hard" or on the basis of seed (aken) weight and dimensions (Mars and Sayadi, 1992). Some new selections are found to be soft-seeded compared to old varieties (Levin, 1990; Shi, 1991).

(iii) Resistance to fruit cracking: this is strongly affected by climate and orchard management, particularly water regime and irrigation scheduling. Some differences are observed between cultivars (Mars and Marrakchi, 1998a).

(iv) Resistance to fruit borers: the "carob moth" (*Ectomyelois ceratoniae* Zeller) and the "anar butterfly" (*Virachola isocrates* / *V. livia* Fabricius) are well known pests of pomegranate and can cause considerable fruit losses (Tous and Ferguson, 1996). It seems that acid fruits are less damaged than sweet ones.

(v) Good post-harvest quality: storage and transport of pomegranate fruits are becoming common in many countries. Keeping post-harvest good quality and resistance to fruit handling are desirable traits. Some studies show intercultivar differences in post-harvest life (Al Kahtani, 1992).

Clonal selection

For improvement purposes, recent works are oriented to clonal selection within local material which is assessed, mainly, for important fruit physico-chemical characteristics (Melgarejo and Martínez, 1992; Ozguven, 1996; Mars and Marrakchi, 1998a,b). Selection is also made in local populations of wild pomegranate as it is the case in the west Kopetdag region of Turkmenistan. The wild forms show a wide range of variation in many characteristics: size of the fruit, sweetness, time of ripening, juiciness and the proportion of seeds to flesh, etc. (Levin, 1995a). Mutation may occur naturally. The new selection '87 - Qing 7' was discovered as a bud-sport of the cultivar 'Qingpitian' in 1987 (Liu *et al.*, 1997). It is early bearing, productive, highly adaptable and produces large, juicy and very sweet yellow fruits.

Seedling selection

Pomegranate seeds can be used for propagation and may be a source of variation (Jalikop and Sampath Kumar, 1990; Mars and Sayadi, 1994). In India, a high degree of variability for fruit characters was observed in the seedling population of the cultivars 'Alandi' (Ramu *et al.*, 1996). It was possible to identify three soft-seeded types. One selection (RCR 1) was highly promising. In China, 'Taishan Dahongshiliu' was discovered as a chance seedling in 1984 in a home garden in Taishan mountains, Shandong province (Shi, 1991). It is very precocious, high yielding, with large, soft-seeded fruits and resistant to cracking.

Hybridization

Some hybridization attempts are reported by authors. However, very few varieties are of hybrid origin. At the Turkmenistan Experimental Station, hybridizations were carried out between 1986 and 1988 with 53 maternal varieties and 13 pollen parents. Hybrids resulting from crossing selected forms are studied for fruit and tree characters (Levin, 1990).

Tissue culture and mutation inducing

Methods for pomegranate *in vitro* culture are developed. Somatic embryogenesis can be induced and plantlets can be regenerated from pomegranate leaf explants, petal explants and shoot tip (Omura *et al.*, 1987), cotyledonary tissues (Bhansali, 1990). Anther culture resulted in regenerated plantlets that were all diploid (2n = 18) (Moriguchi *et al.*, 1987). Suspension cultures were produced from calluses derived from leaf explants but no somaclonal variation was detected among the regenerated plants (Omura *et al.*, 1990).

Treatment of *in vitro* leaf segment culture with gamma rays resulted in regenerated plants including leaf shape variants, dwarfed and upright plants and completely and partially sterile plants. Tetraploids and tetraploid/diploid chimeras were induced by colchicine treatment of leaf segments cultured *in vitro* (Omura *et al.*, 1987).

Artificial mutagenesis was induced (at TES) using gamma rays at 10-20 KR on pomegranate seeds (Levin, 1990). Forms with good fruit and juice quality and good keeping quality were obtained among mutant seedlings. The most promising type produced is the soft-seeded seedling 'Sverkhranii' (= Super early) with fruit ripening in August.

Future prospects

Pomegranate culture is still affronted to many problems that modern breeding programmes must resolve. The future of this fruit depends on the selection of high quality cultivars with soft seeds and fruits resistant to cracking and fruit borers (Tous and Ferguson, 1996). The direct use of existing cultivars and "wild types" may be more effective than new hybridisation. Most of genetic combinations that breeders may look for are probably disseminated in variety-populations and wild ecotypes (Mars, 1996). Since the *ex situ* collection cannot exceed a limited number of accessions, it is difficult to preserve the evolutionary potential of the species. Thus, *in situ/on farm* conservation of genetic resources must be considered. The role of traditional local growers in the preservation of this primary gene pool is valuable (Ghorbel *et al.*, 1996). Nevertheless, further prospecting, collection, evaluation and selection of local material need to be carried out in many countries. A list of descriptors is needed to be adopted on a large scale. New methods (biochemical and molecular techniques, etc.) have to be developed for cultivar identification and genetic studies (Mars and Marrakchi, 1998b). Many limitations are imposed by the tree and fruit characteristics (Jalikop and Sampath Kumar, 1990) and new approaches (*in vitro* techniques, etc.) need to be developed for pomegranate breeding.

On the other hand, plant material interchange should be promoted at regional and international level. Particular guidelines for the safe movement of pomegranate genetic resources have to be elaborated. Adaptation and screening trials should be established with particular emphasis on fruit quality and physiological disorders and pest resistance.

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