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Pomegranate Breeding

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ABSTRACT

Although pomegranate is an ancient fruit plant it has gained commercial significance recently. Nowadays there is increasing worldwide demand for this fruit owing to its superior pharmacological and therapeutic properties. The cultivated species of pomegranate, *Punica granatum*, is reported to contain 2n=16, 18 chromosomes. Inheritance of some fruit and flower types has been studied. Pomegranate, often a cross pollinated crop, is genetically heterozygous and adequate variation for several plant and fruit traits is generated in nature. *Punica* has ornamental 'Double Flower' and 'Nana' types. Some wild forms like 'Daru' possess resistance to pomegranate bacterial blight. Very little breeding work has been carried out in India, Israel, China and Iran. In this review, new varieties that are bred with specific objectives using modern breeding techniques are outlined. Criteria to be considered in developing varieties to suit the needs of growers, retailers and exporters are also presented along with important germplasm sources for various traits. Hybridization, mutation and polyploidy work in pomegranate has recently resulted in some new varieties. Hybridization and development of a hybrid population is relatively easy in pomegranate as the flowers are big, pollen is available in plenty and seeds germinate well; besides, many varieties are easily crossable. The inter-relation and inheritance of some fruit and plant traits is reviewed as these will serve as a guide in making a discreet selection of appropriate progeny from the segregating population. A consortium of pomegranate breeders working in various countries, germplasm exchange, and multi-location testing of varieties and initiating collaborative programmes should help in addressing some regional and international objectives in pomegranate breeding.

Keywords: cytology, genetics, objectives, ornamental *Punica,* seedling selection*,* varietal wealth, wild *Punica*

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INTRODUCTION

Pomegranate (*Punica granatum* L.) fruit has a good consumer preference for its attractive, juicy, sweet acidic and refreshing arils. This ancient fruit has emerged as a commercially important fruit in the recent times. There is a growing demand for good quality fruits both for fresh use and processing into juice, syrup, squash, wine, besides *anardana*, an acidulant product (Pruthi and Saxena 1984). This fruit crop is native to hot dry regions of Afghanistan and Baluchistan (De Candolle 1967), but with time it has diffused and got adapted to a wide range of climatic conditions. It produces fruits in tropical, sub-tropical (Trapaidze and Abuladze 1989), temperate (Purohit 1982; Levin 1995) regions and in hilly areas up to 1800 m altitude (Sharma and Sharma 1990). The unique plasticity of this fruit crop is evident from the threshold limits it exhibits for higher (44°C) and lower (-12°C) temperatures (Westwood 1978). The plant habit, fruiting and flowering physiology are, however, altered with the habitat (Pareek and Godara 1993).

Pomegranate is often-cross pollinated fruit crop (Jalikop and Kumar 1990) and seeds germinate well. As a result a large number of diverse seedling forms have evolved in nature in various regions of the world over the years (Levin 1995). Very few varieties have been developed by systematic breeding programmes despite great opportunities exist

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for developing varieties to meet various objectives. According to Mars (2000) pomegranate culture is still faced with many problems and methods must be developed for cultivar identification and improvement, and genetic resource management. Since there is increasing worldwide demand for this fruit for its superior pharmacological and therapeutic properties, there is a need to initiate and intensify well planned deliberate breeding programmes to meet the demands of local and international consumers, processors, growers and exporters.

CYTOLOGY

Understanding cytology of a crop is useful in planning breeding programs. The cultivated species of pomegranate, *Punica granatum*, is reported to contain 2n=2x=16, 18 chromosomes (Smith 1976). According to Nath and Randhawa (1959) some of the cultivated varieties have 2n=16 while the 'Double Flower' has 2n=18 chromosomes. Recently cytological studies have been reported in the Iranian varieties by Sheidai and colleagues. Sheidai and Noormohammadi (2005) studied chromosome pairing in 19 pomegranate cultivars and noted that all of them possessed 2n=2x=16 chromosomes forming mostly bivalents along with a low number of quadrivalents possibly due to the occurrence of translocations. Sheidai *et al.* (2005) performed meiotic studies on 22 Iranian cultivars and found that all of them possessed 2n=16 (n=8) chromosomes. Further, Sheidai *et al.* (2007) reported the occurrence of 0-5 B-chromosomes in 17 out of 22 cultivars studied. These accessory chromosomes were smaller than the A-chromosomes and did not pair with them or among themselves. In the same study, the B-chromosomes could arrange themselves on the equatorial plane and move to the anaphase poles, although they lagged in some cases. Mamedov (1984) reported a higher frequency of chromosome abnormalities like dicentric bridges, fragments, laggards, micronuclei in pomegranates treated with physical and chemical mutagens than in untreated controls.

GENETICS

Success in breeding for economically important traits depends upon the information available on inheritance like dominance and recessive relationships, number of genes governing the trait, and gene interaction. The breeding program, planning and execution have to be altered accordingly. The pomegranate crop exhibits a relatively long reproductive cycle, heterozygosity and difficulty in raising a massive population; therefore, studies on genetics of various traits are limited (Jalikop 2003). Mars (2000) opined that the description of pomegranate germplasm is based mainly on pomological and agronomic criteria and genetic studies are rare.

Soft-seededness or absence of seeds is a desirable economic trait that improves the consumptive qualities of pomegranate fruit. Many cultivars and forms of pomegranate are quite heterogeneous for this characteristic (Glozer and Ferguson 2008). The trait is under the control of multigenes (Jalikop *et al.* 2005; Lu *et al.* 2006). In order to evolve soft seeded varieties the breeder therefore has to raise a large population when the probability of a recombinant with soft seeds and other traits of interest is greater. According to Jalikop *et al.* (2005), the hard-seeded nature and red and pink aril colour are dominant to soft-seededness and white aril colour. Jalikop (2007) showed that fruit acidity is monogenically controlled and that sourness or higher acidity is dominant over sweet or low acidity, and whether a genotype produces sweet or sour fruit is determined by a major gene (*SS*) while a few modifiers with small effects cause fluctuations in the acidity levels within sour and sweet types. Further, Jalikop observed that the F_1 of 'Kabul Yellow' and 'Ganesh' (sweet × sweet) were highly acidic and attributed it to linked dominant alleles or an epistatic effect of neighboring loci which readily simulate overdominance (pseudo-overdominance). Jalikop and Kumar (1990) reported that a single dominant gene in 'Ganesh' confers red pigmentation in the petiole base, leaf margin, flower bud and fruit rind and its recessive form in 'Kabul Yellow' governs yellow pigmentation in these plant parts. Further, Jalikop (2003) described a genetically induced rosetting occurring at a frequency of 0.12 in the F_2 families of 'Ganesh', and a recessive rosette mutant clone of 'Kabul Yellow'. This was due to a recessive mutant gene of 'Kabul Yellow' and gene symbol *rg* (rosette growth) was assigned to this gene.

'Double Flower' is an ornamental pomegranate type wherein the numerous stamens are modified into petals as a result the flowers are large and attractive, and look like a rose flower. Strebkova (1974) observed that the mottled-pink form, double petals and bright petal colour were dominant. The presence of fruit with cultivated characters in the seed progeny suggest that cultivated plants took part in the formation 'Double Flower' pomegranates. The F_0 fruits produced on 'Double Flower' after pollinating with 'Ganesh', a sweet pomegranate, were highly acidic with hard-seeded arils (Jalikop 2007). Hence a spontaneous dominant gene mutation (*Df*) in an acidic pomegranate should have caused a homeotic conversion of stamens into petals. The heterozygous state (*Dfdf*) of 'Double Flower' however is conserved due to clonal propagation (Jalikop and Kumar 2009).

VARIETAL WEALTH

In different pomegranate growing areas of the world a good number of varieties have been identified by growers and recently by breeders. The Turkmenistan Experimental Station of Genetic Plant Resources, Turkmenistan Academy of Agricultural Sciences, Garrygala, has a large collection, 1157 accessions (Levin 1995, 1996), Iran has about 760 germplasm and China grows 238 cultivars in different provinces (Feng 2006). Though such large diversity is available across the world very little has been exploited in terms of breeding work. A review of varietal status shows that the traditional varieties in different countries are being replaced by modern ones.

Commercial varieties

Hybridization, mutation and polyploidy work in pomegranate have recently resulted in a small number of new genotypes/varieties, which are presented in **Table 1**.

Ornamental varieties

Certain *Punica* forms have ornamental value. There are mainly two ornamental types: 'Double Flower' and 'Dwarf' or 'Nana' types. 'Double Flower', as the name implies, produces double flowers wherein numerous stamens are modified into petals; as a result some types do not set fruits, but because they have large attractive flowers they have ornamental significance. The functionally sterile flowers, however, yield fruits when manually pollinated (Jalikop 2007). Iskenderova (1980) described 4 forms with red, variegated and cream-coloured double flowers. He observed (Iskenderova 1979) that the double forms have advantages like larger juicy fruit with large seeds and a lower acid content, the absence of spines, and weak sucker growth compared with semi-double and simple or normal forms. Pomegranate is widely cultivated as a fruit tree and decorative plant in China (Wang *et al.* 2007). Wang (2003) described a 'Double Flower' variety 'Mudanhua'. This variety bears high quality fruits with pinkish-red, juicy arils and soluble solids content of 17-19% while the flowers are pretty, double, with bright red petals. Another type of pomegranate which has exclusive ornamental value is 'Nana', whose adult plant is a miniature pomegranate like *bonsai.* It has small leaves, grows to a height of 50-70 cm, and bears small flowers and fruits (Nath and Randhawa 1959; Jalikop 2007). Attempts have been made to exploit 'Nana' in breeding for moisture

Table 1 Important commercial pomegranate varieties bred in different countries.

Country	Variety(ies)	Important characteristics	Breeding method	Reference
China	'Hongmanaozi'	Very productive and highly resistant to *Zythia versoniana* and *Cercospora punicae*; seed hardness is 3.56 kg/cm^2.	Mutant of 'Manaozi'	Zhao *et al*. 2007
China	'Taihanghong'	Early maturity and high eating quality, largefruits (625.0 g),pinkish red arils, soluble solids content 15.9%, stores up to 3 months. Fruit yield: 27.5-32 t/ha.	Mutant of 'Mantianhong'	Zhao 2007
China	'Yushiliu 4'	Very good eating quality, high adaptability, deep glossy red rind, pink red large arils, soluble solids content :15.%,sweet flavour.	'Yushiliu 1' X 'Yushiliu 3'	Zhao *et al.* 2006
China	'Hongyushizi'	Fruit weight, 100-grain weight, soluble solid content and juice content higher by 33.4, 16.8, 2.7 and 3.0%, respectively than in cv. Yushizi, seeds soft (3.31 kg/cm^2), highly productive, and highly resistant to diseases caused by *Iythia versoniana* and *Cercospora punicae.*	Mutant of 'Yushizi'	Zhu *et al.* 2005
Israel	'Shani-Yonay'	Early, red appealing skin, softseeds, dark red, sweet arils, fruits less prone to sunburn.	Seedling selection	Holland *et al.* 2007
Azerbaijan	'Nasimi' 'Vurgun'	Cold-resistant	Hybridization	Strebkova 1976
Azerbaijan	'Aleko'	Good quality fruits	Hybridization	Strebkova 1976
China	'Mengliaihong'	Dwarf and compact, 2-3-year-old branches produce fruiting spurs, bright red rind, soluble solids content 15.8%, soft seeds, precocious and productive, yield: 12.9 kg/2 year old tree, no fruit cracking.	Seedling selection	Pu *et al.* 2005
China	'Zaoxuan 018' 'Zaoxuan 027'	Zaoxuan 018- fruit weight 520-680 g, bright red rind, pink-red large arils, soluble solids content of 20.1%; Zaoxuan 027- fruit weight 600-740 g, red blush, bright red large arils, soluble solids content of 20.5%.	'Zaoxuan 018' – seedling selection from 'Dahongpao'; 'Zaoxuan 027'-seedling selection from 'Daqinpi'.	Wang *et al.* 2006
China	'Taishan Dahongshiliou'	Vigorous, very precocious, high yielding, resistant to drought, fruits resistant to cracking, bright red and glossy, soft seeds, arils bright red, acid-sweet, juicy, soluble solids: 17-19%.	Chance seedling	Shi 1991
India	'G-137'	Fruits yellowish-pink, big (289.9 g), juicy (88.7%), less acidic and more sweeter than ' Ganesh'	Clonal selection from 'Ganesh'	Keskar 1990
India	'RCR1'	Fruits yellowish orange to red in colour, with an intense red blush, large size, yield: 267 fruits (58.6 kg/tree).	Seedling selection from 'Alandi'	Ramu *et al.* 1996
China	'Huashu' 'Dahongshiliu'	Produces 3 crops a year; trees are hardy, fruits weigh on an average of 189, 132.5 and 66.3 g in 3 cropping seasons, fruits bright red with pink arils and soluble solids: 15.5-16.5%.	------	Dong 1997
China	'87-Qing 7'	Early bearing, productive and highly adaptable, fruits large (365-650 g), yellowish green with a red blush, arils are large, juicy and very sweet.	A natural mutant of 'Qingpitian'	Liu *et al.* 1997
China	'Linxuan 8' 'Lintong 14'	Linxuan 8 is an early white cultivar; fruits are large (330 g), rind is soluble solids content: 15-16%, soft seeds. Lintong 14 is a late cultivar, fruits are large (370 g), glossy red rind and deep red juicy arils with soluble solids: 16-17%.	------	Zhang 2004
Azerbaijan	'Azerbaijan'	Fruits dark-red, juice contains 15.35% sugar and 1.18% citric acid, weigh 330-400 g, and store 3-4 months or, in cold storage 6 months. Yield 28 kg/tree.	'Purpurovyi' [Purple] X 'Krmyzy-Kabukh'	Sadovodstvo 1974
Soviet Union	'Desertnyi' (Dessert)	Exceeds cv. 'Wonderful' in the yield, sugar content, taste and fruit weight.	('Wonderful' X Soviet Cvs.) X 'Wonderful'	Krestnikov 1974
China	'Baiyushizi'	Large fruits (469 g), glossy white skin, no rust symptoms, good storability.	Bud sport of 'Sanbai'	Zhu 2004
China	'Qingpiruanzi'	Large fruit, thin rind, beautiful appearance, glossy rind covered with red blush, soft seeds, rich in juice, arils pink, soluble solids : 15-16%, titratable acidity: 0.98%.	-----	Diao 2004
China	'Tiepitian'	Large fruits (500 g), yellow-green rind covered in a reddish-brown blush, arils bright red, large and juicy, soluble solids: 14-15.5%. Trees are disease-resistant and adaptable.	-----	Dong 1994

Table 1 (Cont.)

Country	Variety(ies)	Important characteristics	Breeding method	Reference
India	'Mridula'	Evergreen bush , fruits dark red in colour, fruit weight about 250 g, sweet in taste, TSS 16.32%, acidity 0.47%, seed softer than 'Ganesh'.	Selection in F_2 in a cross 'Ganesh' X 'Gul Shah Red'.	http://mpkv.mah. nic.in/Variety
India	'Bhagwa'	Popular Indian variety with thick attractive red glossy skin, semi-hard seeds, dark pink sweet arils.	Selection from segregating hybrid progeny of unknown parents.	http://mpkv.mah. nic.in/Variety
India	'Ruby'	Early maturing, thin skin, red arils with sweet juice and soft seeds.	Selection in the progeny of {['Ganesh' X 'Kabul') X 'Yercaud'] - F_2} X {('Ganesh' X 'Gul Shah Red') - F_2}	Jalikop and Kumar 2000
China	-----	Polyploidy (colchicine)	Tetraploids with short roots, wide short leaves, large flowers	Shao *et al.* 2003

stress tolerant varieties, bacterial blight resistant varieties and to develop yellow miniature pomegranate (Jalikop *et al.* 2003). A tropical F_1 hybrid 'Amlidana' for *anardana* purpose by crossing 'Ganesh' x 'Nana' was reported by Jalikop *et al.* (2000) and Jalikop and Kumar (2000).

Wild forms

Wild *Punica* germplasm is useful in breeding programmes as they carry valuable genes not available sometimes in the cultivated forms. Pomegranate grows wild in Transcaucasia and in Asia Minor. Some parts of the Mediterranean area are also considered as native lands of pomegranate. On the basis of expeditions made during 1964-79, Levin (1981) described the distribution, habitat, reproduction, ecology and biological features of wild material growing in Turkmenistan. He also observed (Levin 1977) that the local populations in Kopetdag carry useful recessive mutations which are promising in breeding. Further, he reported (Levin 1976) that some wild pomegranate collections with narrow petals, friable seeds, fruits resistant to cracking besides weeping forms and forms with a light-blue pericarp. Wild pomegranate grows plentifully in nature in northern India and locally this variety is called 'Daru'. 'Daru' is very common and gregarious in gravel and boulder deposits of dry ravines in the outer Himalayas. These hardy deciduous seedling trees growing over long periods of time possess better climatic adaptability and resistance to pests and diseases (Sharma and Sharma 1990) owing to natural selection. Bist *et al.* (1994) and Sharma and Sharma (1990) studied wild collections, and in the sample collected by the latter, had a mean fruit weight of 82.68 g, rind thickness of 4.25 mm and total titrable acidity of 4.48%. Thus, compared to cultivated types these wild plats produce small size, highly acidic fruits with a thick rind. Dried arils of 'Daru' are used in the preparation of *anardana*, an acidulant product used in souring culinary preparations (Pruthi and Saxena 1984). In order to introgress bacterial blight resistance Jalikop *et al.* (2005, 2006) hybridized cultivated varieties with 'Daru'. When wild non-cultivated types are involved in the breeding work one has to go for repeated backcrosses by making selection in each generation in order to eliminate several undesirable traits of the wild type. Thus, developing pomegranate varieties by backcross breeding is time consuming.

VARIETAL EVALUATION

India

Several varietal evaluation studies have been carried out in various regions of India using different cultivars. Interestingly, most of these studies are confined to the northern part of the country. Evaluation of 38 genotypes under hot arid conditions of Rajasthan by Samadia and Pareek (2006) showed superiority of 'Jalore Seedless', 'G 137' and 'Ganesh' with regard to fruit yield, but 'Mridula' excelled in fruit quality. Similar observations were made by Singh (2004). He noted, based on the performance of fruit and plant characters of 13 cultivars, that 'Jalore Seedless', 'P23', 'G 137', 'Ganesh' and 'Mridula' were the most suitable for cultivation in Rajasthan for the local market. However, in Punjab, Sharma and Dhillon (2002) observed that the juice acid content was lowest in 'G-137'among 30 evergreen cultivars evaluated.

The evaluation of 10 varieties in Kashmir indicated that the highest fruit set, fruit yield and marketable produce were in 'Dholka' (Mir *et al.* 2007). However, the highest total soluble solids (TSS) and total sugars were recorded in 'Kandhari'. Another study in Kashmir by Misra *et al.* (1983) revealed 'Srinagar Special' as the most suitable variety for the region. In Himachal Pradesh, overall performance of 'Kandhari Hansi' was best while 'Chawla' plants were most vigorous (Sharma *et al.* 2005; Sharma and Bist 2005). Kumar (2005) recommended 'Kandhari Kabuli' with pinkish-red fruits and blood red-coloured grains, and a sweet taste with acidic blend, for cultivation in the mid-hill zone of Himachal Pradesh. Studies by Jagtap *et al*. (1992) comprised 29 indigenous and exotic varieties which showed that the most temperate varieties and indigenous 'Jodhapur Red' had a desirable red aril colour. According to Malhotra *et al.* (1983a, 1983b) 'Bedana' and 'Dholka' exhibited the least fruit cracking (40.9 and 46.8%, respectively) and ascorbic acid content in 'Ak Anar' and 'Bedana'. Besides, other studies involving the comparison of a small number of varieties by some Indian workers revealed better performance of 'Jyoti' and 'Bassein Seedless' (Padmavathamma and Prasad 2006), 'Jodhpuri Red' (Pundir and Pathak 1981), 'Mirdhula' (Balamohan *et al.* 2001), 'Ganesh' and 'Ruby' (Tarai and Ghosh 2006).

China

In China, Feng *et al.* (1998) assessed 30 pomegranate cultivars. The superior cultivars noted by them included 'Dabaitian', 'Heyinruanzi', 'Tongpi' and 'Bopi'. 'Honghuachongbai' and 'Baihuachongbai' are double-flowered cultivars with ornamental value and good fruit-eating quality. In another study involving 32 pomegranate varieties 'Heyin Ruanzi' was found to be superior (Feng *et al.* 2003). It produces large bright red arils with soft seeds. The fruits have a rich sweet flavour, and are of very good eating quality. Zhou (2005) described characteristics of 10 cultivars and recommended 'Baishuijing', 'Hongshuijing' and 'Chuanshiliu' for extensive planting in the Baoshan area, Yunnan province. One of them, 'Chuanshiliu', has a large fruit size, weighing 368.5 g on average, maturing in mid-late August; berries are large, pink in colour with a soft seed, containing a soluble solids content of 13.1%, and of very good eating quality.

Other countries

Few pomegranate varietal evaluation progrmmes have been reported from other countries. In Spain, evaluation by Martínez *et al.* (2006) and Amorós *et al.* (2000) of 5 indigenous varieties ('ME14', 'ME15', 'PTO2', 'PTO7' and 'CRO1') revealed that 'ME14' and 'ME 15' were very interesting because of their high production, large fruit size and excellent seed organoleptic characteristics. Comparison of Sicilian pomegranate varieties with Spanish selections indicated that the Sicilian selections were somewhat less inferior to the Spanish ones (Barone *et al.* 2000). Studies in Morocco showed that 'Gjeibi' and 'Dwarf ever Green' were more vigorous (Oukabli *et al.* 2004). In a trial conducted by Onur (1983) involving 72 cultivars, 25 were found to be suitable for the Mediterranean Region.

Such wide differences in the performance of pomegranate varieties could be because of the genotype × environment interaction. The above varietal evaluation studies in different parts of the world imply that a variety found suitable in one agro-climatic region may not do equally well in another. Thus there is a need to locate region specific varieties and breed varieties suitable for various soil and climatic regions. Moreover, for the local market, appropriate varieties need to be identified based on local consumer preferences.

BREEDING OBJECTIVES

Until recently the common objectives in genetic improvement of pomegranate were mainly improving the organoleptic qualities, juice content, seed mellowness, appearance of fruits in addition to fruit yield. Generally fruits having bold, juicy, pink or red arils with small, soft seeds are preferred though likings of consumers are not always uniform. Relatively high juice content may be more desirable than large fruit size. However, improvement for fruit quality differs from region to region (Holland *et al.* 2009) and also whether the fruit is used for fresh or processing purpose. In China pomegranate cultivars have been classified into 4 groups suitable for: fresh consumption, processing, decorative and for consumption, and decorative purposes (Feng *et al.* 2006).

Earlier, new varieties were mostly targeted for indigenous markets and the preferences of the traits were not the same in all regions. Since pomegranate has become an economically important fruit crop and is being exported breeders should consider criteria of international markets like fruit size, fruit shape, skin colour, aril colour, easily separable bold arils, seed size, sugar acid blend and beneficial nutrients in the juice, besides ripening time, shelf life, good post-harvest quality, and handling of fruits during transport. In recent times breeding for physiological disorders like fruit cracking and aril browning or internal break down of arils (Jalikop *et al.* 2005, 2006, 2010) are gaining importance.

Breeding criteria from a cultivation perspective varies as pomegranate is grown on a wide range of climates and soils. As regards plant habit, genotypes having an evenly spreading canopy with strong branches free from thorns are ideal. Sometimes dwarf plant habit is a desirable characteristic for mechanical and easy hand harvesting (Glozer and Ferguson 2008). Region-specific objectives for example, in hot dry regions where water is scarce, high temperature and moisture stress tolerance, and in India where bacterial blight is rampant, blight resistance should receive due importance in breeding programmes (Jalikop *et al.* 2005). In Turkmenistan and Azerbaijan breeding for frost resistance is vital as frost is a common constraint in pomegranate cultivation (Strebkova 1970; Levin 1979). Built-in genetic resistance to pests and diseases makes the cultivation of this fruit crop economical and environmental friendly.

BREEDING STRATEGIES

Pomegranate, often being a cross pollinated crop (Jalikop and Kumar 1990), is genetically heterozygous and adequate variation for several plant and fruit traits is generated in nature as well as upon crossing. Most genetic combinations that breeders may look for are probably disseminated in variety-populations and wild ecotypes (Holland *et al.* 2009). Several cultivars grown today are the result of human selection from naturally occurring intra-species variation. Breeding new varieties may be achieved by conventional approaches like seedling selection, hybridization followed by selection or through mutation. However, mutation breeding has some limitations, as it is only a 'hit and miss' method and needs a large number of plants to be raised to get a desirable type. Attempts made to develop transgenic pomegranate varieties are covered elsewhere in this special issue.

Seedling selection

The presence of numerous seeds in the fruit, good seed germination and sometimes growers preferring cultivation of seedlings has made large pomegranate seedling populations available across the globe. Seed, due to heterozygosity, exhibit variation, which makes the selection of plants for traits of interest possible. Hence, seedling selection assumes great significance in pomegranate breeding (Jalikop and Kumar 1998). Even professional breeders find seedling selection as quite promising in developing new pomegranate varieties. The probability of getting superior recombinants is proportional to the population size used for making the selection. Selection for fruit quality can be accomplished in first year of production, although fruit weight may not be typical of when the plant bears a full crop. Fruits are often larger when production is low in young plants. Based on the phenotype once a desirable plant is located it must be propagated vegetatively (either by air layering or cuttings) and its superiority should be verified in a replicated trial in relation to the original mother or a ruling variety.

Over the years, several varieties have evolved in different pomegranate-growing regions by simple selection by growers. The names were generally assigned either from the place of cultivation or from the colour of the fruit. In Israel, early ripening 'Emek' was selected from seedlings raised from open pollinated seeds. Another selection is 'Kamel', which is akin to 'Wonderful', but for dark red skin colour which appears earlier than in 'Wonderful' (Holland *et al.* 2007). In Tajikstan during 1958-1961 Rozanov (1972) singled out seedling varieties 'Apsheronsk' and 'Melesi' for flavour and yield. Chinese workers Wang *et al.* (2006) selected a chance seedling 'Zaoxuan 018' from 'Dahongpao' and 'Zaoxuan 027' was selected from 'Daqinpi'. Most popular soft-seeded Indian cultivar 'Ganesh' is a selection made from the seedling population of hard-seeded 'Alandi' (Jalikop 2003) and subsequently 'G-137' was located from the open-pollinated progeny of 'Ganesh'. A high degree of variation for fruit characters was observed by Ramu *et al.* (1996) in the seedling population of 'Alandi'. According to Indian workers Wavhal and Choudhari (1985), with the exception of aril colour, the selections from 'Muskat' were more promising than those from 'Ganesh'. Karale *et al.* (1979) identified 2 promising seedlings ('L20P50' and 'L24P42') from 80 seedlings. Seed-propagated plants possess a tap root system that will provide good anchorage and can exploit moisture from deeper layers of the soil. Jalikop and Kumar (1993) therefore attempted to develop an inbred line of 'Ganesh' by repeated selfing over generations so that the plants once attain homozygosity for the traits of interest could be subsequently propagated from seeds. Due to inbreeding depression observed in the S3 generation sibmating was resorted to and the resulting progeny in S4 were found to exhibit normal growth.

Hybridization and selection

Hybridization is a straightforward and conventional approach of combining different traits available in the germplasm. The desirable hybrid progeny is selected in the F_1 or in a later generation, either it can result in a new variety or the selected genotype can serve as a breeding line in future breeding programmes. Hybridization and development of a hybrid population is relatively easy in pomegranate as the flowers are big, pollen is readily available and seed germination is good; besides, many of the varieties are easily crossable (Karale and Desai 1999; Jayesh and Kumar 2004) and produce good percent of F_0 fruits.

Selection of parents: Selection of appropriate parents is critical as success of new varieties developed by hybridization hinges on the parents used in the initial crossing programme. There are several varieties of pomegranate both adapted to tropical and sub-temperate climate offering a wide choice of parental combination in the hybridization programme. A breeder has to be discreet in selection of parents, making the right combination of crosses and raising a nursery by growing a large hybrid population, which requires adequate land, expensive labour and other resources; in addition, evaluation of numerous hybrids is time consuming. Karale and Desai (1999, 2000), in order to locate appropriate parental combinations effected line × tester crosses and derived information on combining ability from data on 9 important fruit characters in 52 pomegranate hybrids derived from a 4 line × 13 tester cross. Strebkova (1970) suggested the use of a pollen mixture from varieties in pomegranate breeding.

Many economically important traits are disseminated in world pomegranate varieties. According to breeding objectives appropriate donor parents can be located from the vast collection. Varieties are often classified as sweet, sweet-sour and sour, early-, mid- and late-season, juicy and table fruit, soft- and hard-seeded (Levin 1995; Jalikop and Kumar 1998; Jalikop 2007). Levin (1995, 1996) grouped a large pomegranate germplasm available at the Turkmenistan Academy of Agricultural Sciences into high yield, high TSS, frost hardy, dwarf types, big fruit size, resistance to cracking and sunburn, good shelf-life, small beak. Holland *et al*. (2009) reviewed in detail several important world pomegranate varieties. Some of the world pomegranate varieties that can serve as useful parents while breeding for: large fruit, very juicy, sweet, pink arils – 'Ganesh', 'Teipitian'; large fruit size – 'Bopi', 'Teipitian'; red skin, pink arils – 'Duanzhihong'; earliness – '87-Qing 7', 'Alack'; sweet-sour, green skin – 'Dabaitian', 'Heyinruanzi'; resistance to fruit splitting – 'Apsheronskii Krasnyi', 'Frantsis', 'Kyrmyz Kabukh'; high juice content – 'Slunar', 'Pirosmani', 'Vedzisuri'; attractive thick skin, late maturing –'Mridula', 'Bhagwa'; thin skin, early maturing – 'Ruby'; 'Muskat', Dholka'; thick red rind, red arils, late ripening – 'Malas-e-saveh', 'Rabab-e-Neyriz', 'Malas-e-yazdi', 'Naderi-e-budrood'; vitamin C – 'Siyah'; yellow skin – 'Lefan', 'Kabul Yellow'; red skin, red arils, hard seeds, sweet sour – 'Janarnar'; red glossy skin, sweet sour – 'Wonderful' ('P.G.101-2'); sweet, pink arils – 'Rosh Hapered', 'Malisi'; sweet fruits with soft seeds, red arils – 'Mollar de Elche' and its selections 'ME1', 'ME5', 'ME6', 'ME14'; red colour, yield – 'Kamel', 'Akko'; high yield – 'ME15', 'ME16', and 'ME17', 'Agria de Blanca', 'Albar de Bianca', 'Borde de Albatera', and its selection 'BA1', 'Borde de Blanka', 'Casta del Reino de Ojos', 'CRO1', 'Mollar de Albatera Mollar de Orichula', 'Lnxuan-8', 'Hicaznar'; resistance to moisture stress – 'Taishan Dahongshiliou', 'Nana'; adaptation to sandy alkaline soils – 'Yushiliu 1', 'Yushiliu 2'; bacterial blight resistance – 'Daru', 'Nana'. Jalikop and Kumar (1998) suggested using soft-seeded cultivars as parents while breeding for high juice cultivars due to their significantly higher content of juice.

Hybridization: Three different flower types are observed in pomegranate: male, intermediate and normal. The male flower (bell shape) is relatively small and the gynoecium is poorly developed while the normal hermaphrodite (vase shape) type is big in size and with a conspicuous gynoecium, and is used for crossing purposes after emasculation. The hermaphrodite flowers have a higher fruit set than the intermediate flowers (Karale *et al.* 1993). The floral biology varies with the variety, location and season. Anthesis in pomegranate began at 8 a.m. and was complete by 4 p.m., with a peak at 2 pm (Josan *et al.* 1979). They also observed that the stigma was receptive one day before anthesis and remained receptive up to 5 days. However, Melgarejo *et al.* (2000) noted that the stigma receptivity period, ranged from 2 days before anthesis to 4 days after anthesis. According to Singh *et al.* (1980) the time and duration of anther dehiscence varied with variety. The detailed pomegranate floral biology is covered in another review of the special issue.

Hybridization was effected by Jalikop (2003) through emasculation of flowers shortly before anthesis using forceps, and pollinating immediately. The crossability studies in pomegranate by Jayesh and Kumar (2004) resulted in good fruit set in all cross combinations in winter possibly due to higher percentage of viable pollen during low temperature and high humidity conditions. However, they noted poor cross and self-compatibility when 'Kabul Yellow' was used in crossing/selfing. In crosses involving this variety Jalikop (2003) also reported extremely meager seed germination.

Progeny selection: Following hybridization, large segregation takes place in the F_1 progeny itself and selection for the desired genotypes can be practiced in this generation. Jalikop *et al.* (2006) observed the occurrence of more recombinants in F_2 than in F_1 hence breeder can look for new recombinants in F_2. Use of molecular markers and morphological markers, if available, significantly reduces the progeny population to be field planted for evaluation. Heterosis for fruit characters was reported by Karale and Desai (2000) over the mid, better and top-parent. The percentage of heterosis over the mid-parental value in the desirable direction was maximum for juice weight (86.44%), followed by aril weight (71.20%), fruit weight (65.1%), rind thickness (-48.92%).

Progeny selection for the traits of interest should be carried out by the breeder with the most care since some of the traits like fruit size, rind and juice colour, ripening date, sugars and acidity in the juice, etc. may be strongly influenced by the environment. Knowledge on the inter-relation and inheritance of various fruit and plant traits is useful in making discreet selection of appropriate genotypes in the segregating population. Few studies have been reported showing direct and indirect relationship between pomegranate fruit and plant traits. Mir *et al.* (2006) noted that the number of fruits/plant, fruit weight, fruit volume and fruit set had a maximum positive direct effect on gross fruit yield. They observed that both the number of fruits/plant and fruit weight could form selection criteria for yield improvement in pomegranate. Studies by Lu *et al.* (2006) showed that seeds in fruits from the north side and inside the tree canopy were softer, while those in fruits from the south and west side of the canopy were harder. They did not find a correlation between seed hardness and aril size. Jalikop and Kumar (1998) opined that soft-seeded genotypes are juicier and Karale and Desai (2000) observed TSS content in the juice was positively and significantly associated with acidity. Jalikop *et al.* (2010) noted with an increase in intensity of aril colour there was a reduction in severity of aril browning while with a rise in TSS, the incidence of aril browning increased, an association not often favorable in selection of desirable genotypes. Jalikop *et al.* (2010) therefore suggested that breeders, while developing varieties free from aril browning, have to strike a balance between aril colour and TSS level.

Glozer and Ferguson (2008) described several aspects that a breeder should consider while making selections. According to them there is no consistent relationship between fruit size or skin color development and internal fruit

quality. Fruit cracking is strongly affected by climate and orchard management, particularly water regime and irrigation scheduling. Acid fruits are less damaged than sweet ones. Frost hardiness is dependent on many factors (age, state of the plant, time of minimum winter temperature occurring). Soft-seeded cultivars are, generally, less frost-hardy than hard-seeded ones. According to Jalikop and Kumar (1998) for many fruit attributes, soft- and semi-soft-seeded pomegranate cultivars share similarities, whereas hard-seeded pomegranates are distinctly different. For example, hard-seeded varieties studied by these authors had significantly higher fruit weight and volume than the semi-soft and soft-seeded ones. The studies of Meena *et al.* (2003) showed significantly higher heritability and genetic advance for fruit weight, weight of 100 arils, juice acidity and weight of 100 seeds; these can be effectively used as selection criteria in breeding programmes.

Once superior selections are made, they have to be multiplied clonally (so that the genetic integrity of the selected progeny is not lost), and tested against the ruling variety in a replicated trail before identifying for release. Multi-location evaluation is preferred before recommending for commercial cultivation. Development of varieties by hybridization and selection has been reported from India, China, Turkmenistan and Azerbaijan. Levin (1990a) studied at the Turkmen Experiment Station hybrids derived from crossing 13 varieties for small-seededness, winter hardiness, dwarf habit, pericarp and seed colour, and seed size and taste. A gene for red aril colour (*RR*) was transferred by Jalikop and Kumar (2000) from a Russian temperate variety and developed a tropical multiple hybrid variety, 'Ruby'. This implies scope for exchange of mutually beneficial genes from tropical and temperate pomegranates. Hybridization between cultivated pomegranate varieties and the wild type, 'Daru' and the ornamental variety, 'Nana' was attempted by Jalikop *et al.* (2005, 2006) in order to transfer resistance of 'Daru' and 'Nana' for bacterial blight disease prevalent in India. When non-cultivated types are involved in breeding, in order to breed out several undesirable traits repeated backcrosses are necessary with the selected progeny in each generation. Hybridization was carried out by Jalikop (2007) between ornamental pomegranate 'Double flower' and commercial single or normal flower types. No inter-specific hybridization has been reported with another species of the *Punica* genus, *P. protopunica*.

Mutation breeding

Mutation breeding, though useful in creating new variation, needs a large population to be raised to locate a desirable type, and the majority of the mutants will be recessive and of little or no use, which makes this technique less attractive. Possibly, this is why limited experiments have been attempted in pomegranate with little success. Akhund-zade *et al.* (1977) gamma (γ)-irradiated seeds and cuttings with 1-40 kR and selected types which exceeded the initial material in fruit yield, size and quality. They also observed varieties with sweet fruit were more sensitive to radiation than those with acid-sweet or acid fruit. Levin (1990b) obtained forms with good fruit and juice quality and good keeping quality in the mutant seedlings developed by treatment with γ rays at 10-20 kR. Promising mutants were also bred by *N*,*N*-dimethyl-*N*-nitrosourea treatment (Levin 1990b). Akhundzade (1981) studied γ irradiation effect on cuttings, seeds and pollen and noted a wide range of variability at 5-10 kR doses. Recently in China some varieties like 'Hongmanaozi', 'Taihanghong' and 'Hongyushizi' were isolated as natural mutants (bud sports) from thee different varieties (**Table 1**).

CONCLUSION

Conventional breeding techniques hold immense prospects in developing desirable varieties of pomegranate using large natural genetic variation accumulated over generations, and numerous diverse varieties spread over in several countries. Some breeding work is reported from India, Israel, China and Iran. Most of the workers have used indigenous varieties as parents. More intensive and comprehensive breeding work with specific objectives is required. It should comprise exhaustive hybridization involving the world varieties or germplasm, evaluation of a large number of progenies, and meticulous selection and testing. New varieties of interest can also be recognized for a given region by simple introduction from foreign countries, or by making direct selection from available natural *Punica* gene pool. Scientists should resort to mutation breeding when trait of interest is not present in the natural gene pool. Better understanding of genetics of pomegranate will guide in designing breeding programmes and systematic exploitation of available variation. At present available knowledge of inheritance of various fruit and plant characteristics is meager.

The requirements of pomegranate fruits especially for international market include good shelf life, easily extractable, vividly coloured bold arils containing small seed or no seed. However more specific needs especially in respect of sugar: acid blend are expected depending on the end market. Additionally fruits should have good eye appeal and nutracitically more valuable. Future research work should focus on breeding for biotic and abiotic problems faced in cultivation of this fruit crop, some of which could be region specific. Pomegranate crop is often cultivated in arid regions where water is scarce therefore, germplasm needs to be exploited in breeding varieties tolerant to moisture stress. Additionally screening of vast *Punica* germplasm should receive due attention for several of the documented medicinal properties of pomegranate as genetic variation is bound to exist for the active chemical compounds. A consortium of pomegranate breeders working in various countries, germplasm exchange, multi-location testing of varieties and initiating collaborative programmes should help in addressing some regional and international objectives in pomegranate breeding.

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