Phenotypic variation and estimation of genetic parameters for plant growth, fruit quality traits and bacterial blight disease resistance in gamma (γ) irradiated seed derived progenies and germplasms of pomegranate (Punica granatum L.)

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Abstract

In order to improve fruit quality traits in a breeding programme, both genetic and environmental effects need to be evaluated. The partitioning of variance and genetic parameters for fruit quality traits and disease resistance were estimated in 475 accessions of pomegranate by considering the effects of genotype, environment and their interactions. The results showed that genotype contributed most to the variance except to leaf thickness, flower length, flower breadth, number of arils per fruit and seed length. Genotype and environment interactions were significant for all traits except for plant spread, leaf thickness, fruit length and rind thickness indicating the influence of environment on the attributes of pomegranate fruit. High heritability, along with genetic advances in days to maturity of fruit, anthocyanin content, phenolic content, rind thickness, volume of juice, plant spread, plant height and acidity suggested that there were significant additive gene effects for such traits. In general, the genotypic co-efficient variances were lower in magnitude than the corresponding phenotypic co-efficient variances for all traits. The estimates of PCV and GCV were high for fruit weight, total aril weight, number of arils per fruit, volume of juice, hundred aril weight, single aril weight, acidity, anthocyanin, phenolic content and bacterial blight on fruits, indicating greater scope for improvement of these characters by simple selection. High heritability and high genetic advance as percent of mean was observed for days to maturity, plant spread, volume of juice, rind thickness, acidity, phenolic content, anthocyanin content bacterial blight on fruit, stem and disease severity.

Keywords: Pomegranate, variability, heritability, genetic advance

Introduction

Any successful breeding programme must have clear goals and make decisions based on clear definitions of those traits to be improved, their genetic control, and the available germplasm (Fehr, 1987)[14]. The evaluation of important traits includes the determination of their genetic variability and its partitioning into components attributable to different causes. Therefore, optimization of the breeding and selection programme requires knowledge of the genetic parameters of the characters to be improved. Optimal selection strategies depend primarily on the heritability of individual characters and the genetic correlations between them (Falconer and McKay, 1996)[11]. Knowledge of the extent of genotype x environment interactions is also needed. However, such interactions can be a limit to the transmission of genetic advance, if the selected strains perform differently according to the site where they have been grown, or within the site where the selection was applied (Falconer, 1989)[12]. Thus, combined multi-year analyses are used to determine genotype x environment interactions and their variance components to allow an estimate of the heritability of characters (Crosa, 1990)[7]. Pomegranate (Punica granatum L.) is one of the most important fruit crops used for fresh consumption and for processing. It is rich in vitamin C and citric acid is the predominant organic acid in pomegranate (Malhotra et al., 1983)[7]. Glucose (5.46%) and fructose (6.14%) are the main sugars with no sucrose in fruits.
Sweet varieties are mildly laxative, whereas sour types are good against inflammation of stomach and heartache. Flower buds are very useful in Ayurveda for managing bronchitis. The stem bark and fruit rind is used to treat diarrhoea and indigestion (Anonymous, 1969) [1] which contains number of alkaloids belonging to pyridine group. The bark is also used in tanning industry (Patil and Karle, 1990) [32]. It is cultivated in the Mediterranean region and all over the World. Due to increasing worldwide demand for the superior nutritional and therapeutical properties of pomegranate, there is a need to initiate well-planned breeding programmes to meet the increasing demands of local and international consumers, processors, growers, and exporters. Pomegranate is genetically heterozygous and sufficient variation for several plant and fruit traits has been generated in nature, as well as by crossing (Mars, 2000). Most of the phenotypic and genetic combinations that breeders require are present in populations of varieties, clones and wild ecotypes (Jalikop, 2010) [19]. At present, crosses are made without any accurate prediction of the performance of the progeny, and breeding is relatively inefficient because the available genetic information is limited. Moreover, the choice of parents is based solely on their phenotypic performance (Mars, 2000).

Before initiating crop improvement program in any crop, breeder should thoroughly evaluate, screen and understand the genetic architecture of the germplasm. Estimation of genetic variability parameters is the foremost step to be adopted in the source population, if the breeding program is aimed at improving economically important traits. The success of a crop improvement program depends on the ability of the breeder to define and assemble the required genetic variability (Mather and Jinks, 1983) [27]. Variability is the key factor for any selection program, which can be generated through various ways. To achieve or create variability, addition of some more diverse genotypes with the available collection is necessary or creation of new variability by other means is very much needed.

Genetic variation in fruit quality traits is generally assumed to be additive. Therefore, combining alleles from both parents will result in genotypes with the desired performance (Hansche, 1983) [16]. Thus, to improve fruit quality, more information on genetic variability and on the inter-relationships among traits is necessary.

The morphological observations recorded in the field usually will be the sum total of genotypic as well as environmental effects. Hence, the diversity obtained from the field data should be verified to ensure that the variability present is at genotypic level. Hence, in the present study a set of 475 pomegranate genotypes were used to study the genetic variability parameters for plant growth, fruit quality traits and bacterial blight disease resistance.

Materials and Methods

Plant material and experiment location

A field experiment was conducted at Horticulture Research and extension Station, Vijayapur (Tidagundi), University of Horticultural Sciences, Bagalkot (Karnataka) during Ambbebaha in the year 2014-15 and 2015-16 to study on genetic diversity, heritability and morphological characterization in pomegranate. The experiment was laid out in a Augmented block design. The climate condition of Vijayapur is tropical with hot dry summer and cold winter and located at 16.59°N latitudes and 75.45°E longitudes. The average rainfall of is about 569 mm. which is mainly distributed from mid June to September. The average maximum and minimum temperature ranges between 42.5°C to 20°C. The soil was medium black (Vertisol) with a pH in the range of 7.0 to 7.5. The experiment comprising four hundred and seventy five γ irradiated seed derived progenies and germplasms of pomegranate (Punica granatum L.) with checks viz., Bhagwa, Ganesh and Ruby. Pomological variability in various fruit and juice characteristics were evaluated over two consecutive seasons (2015 & 2016).

Characteristics of the fruit

Pomegranate trees were selected for their uniformity in fruit yield and canopy appearance. Following the sampling procedure developed by Mars and Marrakchi (1999) [26], fruit (five fruit per tree) were harvested from all sides of each canopy, including the top and inside the canopy, at the full-maturity stage for each clone. Harvest date was determined, mainly, on the basis of fruit size, and external and internal fruit skin colour (Figure 1; Figure 2A). Fruit height (FH; in cm), fruit diameter (FD at the equator; in cm), and rind thickness (RT; in mm) were recorded using calipers. Fruit fresh weight (FFW; in g) and Rind fresh weight (SFW; in g) were determined using a laboratory balance (Sartorius). Total Aril weight (TAW) was measured as a percentage of the edible part of each fruit. The percentage yield of fruit juice (JY) was determined.

Total soluble solids contents (TSSC; in °Brix) were determined using a refractometer (ATAGO, Tokyo, Japan) at 20°C. The pH of the juice was measured using a pH meter (HANNA pH212; Woonsocket, RI USA). Titatable acidity (TA) was determined by titrating 10 ml of juice to pH 8.1 with 0.1 M NaOH according to the AOAC (1984) and was expressed in g malic acid equivalents 1–1.

The total phenolic content was determined with the Folin Ciocalteu reagent (Singleton et al., 1999) [135] and expressed as mg gallic acid 1000ml−1 in automated spectrometer (Effendorf). Total anthocyanin content in juice was evaluated spectrophotometrically using the pH differential method (Giusti and Wrolstad, 2001) [135].

Fig 1: Longitudinal section of a pomegranate fruit showing the different parts (Mars and Marrakchi, 1999). Parameters measured here were: FH, fruit height (mm); FD, fruit diameter (mm); and STH, skin thickness (mm).
Fig 2: Variability in fruit, aril and juice colour in pomegranate genotypes

Statistical analysis
The statistical analysis on the individual characters was carried out on the mean values of each genotype using statistical package GenStat version 13.1 (VSN International, 2010) [36]. The data was subjected to residual maximum likelihood (REML) analysis (Patterson and Thompson, 1971) [33] with season as fixed and accessions as random. The partitioning of variance, analysis of variance (ANOVA), the main effects of variety, environment and the interactions between them were calculated. The analysis of variance for different characters was carried out using the mean data in order to partition the variability due to different sources by following Panse and Sukhatme (1964) [31]. Genetic variability parameter viz., mean, variance (Cochran and Cox, 1957) [5], phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Burton and De Vane, 1953) [4], heritability (h²) (Hanson et al., 1956) [17] and Genetic advance (GA) (Johnson et al., 1955) [20].

Results and Discussion
Analysis of variance revealed that the genotypes recorded highly significant variation for all the characters and it indicated the presence of sufficient variability for these characters (Table 1), thus there is a lot of scope for selection. One of the ways of assessing the variability is through examining the range of variation. The range in the values reflects the extent of phenotypic variability in respect of the character, which includes genotypic, environmental and genotype x environmental interaction components. In the present study the genotypes exhibited considerable amount of variation for all studied characters except for leaf thickness, flower length, flower breadth, number of arils per fruit and seed length (Table 1). Khadivi-Khub et al 2015 recorded higher range for these characters, which was in accordance to the present study. The high range of values indicated the good scope for selection of suitable basic material for breeders for further improvement.

The mean values also play a major role in selecting suitable breeding lines and methods for the improvement of pomegranate. In case of days to physiological maturity lower mean values enabled identification of several short duration genotypes. The lower mean values for these traits were observed in genotypes PG10-50, PK10-64, PG20-16, PK10-74, PK10-89, PK10-227 and PK15-83 and these genotypes can be used in niche areas where early varieties are needed or as parents in hybridization for the development of early duration and high yielding varieties.
variation (PCV) and further to compute heritability and genetic advances for various metric traits. Comparison of variability between two traits is possible with coefficient of variation as it is free of units. As expected, the PCV values were greater than the GCV values for all the characters indicating considerable influence of environment on the expression of these characters under field conditions (Table 2). The difference between PCV and GCV was more for all the studied characters indicating the major role of environment on these characters. Earlier reports on pomegranate by Faten Zaouay and Messaoud Mars (2016) [13] and Munde (2011) [30] are in conformation with these results. In general, the PCV and GCV were quite high for fresh fruit weight, total aril weight, number of arils per fruit, volume of juice, weight of hundred arils, single aril weight, acidity, anthocyanin content, phenolic content and bacterial blight on fruits indicating greater scope for improvement of these characters by simple selection, this means that selection based on phenotype might be useful for fruit quality traits. Several earlier workers also reported high PCV and GCV for Titratable acidity (Faten Zaouay and Messaoud Mars (2014) [13]), fruit weight (Bist et al 1994), number of arils per fruit, rind thickness, maturity index (Munde 2011) [30]. However, days to maturity, plant height, plant spread, leaf thickness, leaf breadth, fruit weight, calyx length, calyx diameter, juice percent, rind thickness, maturity index and bacterial blight incidence on leaves, fruit, stem showed moderate PCV and GCV values, while pH of the juice and seed length exhibited low values. Low PCV and GCV values were also reported for TSS, pH of juice (Faten and Messaouad (2014) [13], leaf breadth, fruit diameter (Munde, 2011) [30], while moderate PCV and GCV values were reported for leaf length, fruit length, TSS, days to maturity and pH of juice (Munde 2011) [30]. The same results were reported by Moraes et al. (2005) on the progenies of yellow passion fruit and by Silva et al. (2007) [34] on custard apple. However, Da Silva et al. (2008) [8] found higher GCV values (= 115%) for some morphoagronomic and fruit quality traits in papaya. Estimates of heritability are useful when studying genetic changes in a breeding population under selection (Falconer, 1989) [12] and to choose the most appropriate breeding procedure (De Souza et al., 1998) [10]. In addition, estimates of heritability are useful to predict the future performance of a phenotype from previous records, as well as to indicate how much can be gained by using repeated measurements (Falconer, 1989) [12]. In pomegranate, the broad-sense heritability varied from low (0%) for Leaf thickness, to very high (97.97%) for Days to maturity. Plant height, Plant spread, Juice percent, volume of juice, Rind thickness, rind percent, acidity, phenolic content, anthocyanin content, seed width, bacterial blight incident on fruit, stem and disease severity also had high broad-sense heritability values (67.93, 70.03, 62.54, 72.54, 87.85, 89.01, 94.52, 85.86, 87.18, 77.48, 86.06 and 81.32%, respectively). These relatively high heritability values indicate considerable genetic variation, indicating that selection for these traits through breeding is feasible. In our case estimates of hereditability were generally similar or rather low to moderate compared to those found for pomegranate (Karale and Desai, 1998) [21], Manohar et al., 1981 [25] and meena et al., 2004) [28], mango (Brown et al., 2009) [3], apple (Dan et al., 2010) [9], apricot (Couranjou, 1995) [6], and peach (De Souza et al., 1998) [10]. However, the genetic control of many quantitatively inherited fruit traits in pomegranate is still unclear. Genetic advance (GA) at a given selection intensity refers to the improvement of characters in the progenies compared to the starting population. The expected GA values for all fruit characters of the pomegranate clones studied here are presented in Table 2. These values are also expressed as a percentage of the general mean value for each character in each clone. The range of expected GA values varied from 0 – 568.35. The highest estimated value of GA was noted for phenolic content. Considering GA as a percentage of the clone mean (GAM), high GAM values were exhibited for anthocyanin, content, phenolic content plant spread, total aril weight, volume of juice, acidity, rind thickness, bacterial blight on fruit, stem, disease severity on tree and low values were observed for leaf thickness, leaf length, leaf breadth, flower length, flower diameter, pistil length, fruit diameter, number of arils per fruit, rind percent, TSS, pH of juice, maturity index and seed length. High heritability along with high GA is an important factor when predicting the effects of selecting the best individuals. Days to maturity, plant height, plant spread, phenolic content, anthocyanin content, volume of juice, rind thickness, acidity, bacterial blight incident on fruit, stem and disease severity had high heritability values accompanied with high GA, suggesting that there was an important additive gene effect for such traits (Johnson et al., 1955) [20]. While, plant height, juice percentage per fruit, rind percentage and seed width had high heritability coupled with moderate GA, leaf thickness, leaf breadth, flower length, flower diameter, number of arils per fruit, juice pH, maturity index, seed length had low heritability coupled with low GA. These results are similar to the findings of Khan et al. (2006) [23] and Ibrahim (2012) [18]. High heritability estimate indicates less influence of environment on respective characters. Hence, direct selection can be followed to improve early maturing genotypes. Low heritability (broad sense) indicates predominance of non-additive gene action indicating the scope for breeding. High estimates of GA coupled with substantial amount of heritability indicate that selection for such characters would result in the improvement of characters in the desired direction as the character is governed by additive genes. High heritability coupled with low genetic advance indicates non-additive gene action. The heritability exhibited due to favorable influence of environment rather than genotypes and selection for such traits may not be rewarding. If, low heritability coupled with low genetic advance indicates such character was highly influenced by environment and selection would be ineffective for those traits.

Table 1: Analysis of Variance (ANOVA) for plant growth, fruit quality traits and bacterial blight disease resistance in pomegranate genotypes

| Sl. No | Characters                     | Sources of variation |
|-------|-------------------------------|---------------------|
|       |                               | Genotypes | Environment | Gen. x Env. | Error |
| 1.    | Days to maturity of fruit     | 731.23**   | 0.001      | 26.48**     | 7.46   |
| 2.    | Plant height (cm)             | 326.27**   | 602.93     | 293.68**    | 27.95  |
| 3.    | Plant spread (cm)             | 202.69***  | 207.09     | 54.82       | 230.94 |
| 4.    | Leaf thickness (mm)           | 0.00001    | 0.006      | 0.004       | 0.011  |
| 5.    | Leaf length (cm)              | 0.054**    | 0.094      | 0.12**      | 0.143  |
| Sl. No. | Characters                                      | Mean          | Range            | $\sigma_1^2$       | $\sigma_2^2$       | PC.V. | GC.V. | Heritability (%) | GA | GAM (%)     |
|--------|-----------------------------------------------|---------------|------------------|--------------------|--------------------|-------|-------|------------------|----|-------------|
| 1      | Days to maturity of fruit                     | 139.13        | 124.12-209.37    | 746.39             | 731.23             | 19.64 | 19.45 | 97.97            | 55.14 | 39.63       |
| 2      | Plant height (cm)                             | 157.64        | 63.02-226.17     | 480.29             | 326.27             | 13.90 | 11.46 | 67.93            | 30.67 | 19.45       |
| 3      | Plant spread (cm)                             | 122.52        | 54.29-161.32     | 289.45             | 202.69             | 13.89 | 11.62 | 70.03            | 24.54 | 20.03       |
| 4      | Leaf thickness (mm)                           | 0.51          | 0.40-0.58        | 0.00               | 0.00               | 13.25 | 0.02  | 0.00             | 0.00  | 0.00        |
| 5      | Leaf length (cm)                              | 4.85          | 2.88-5.77        | 0.15               | 0.05               | 8.07  | 4.35  | 30.45            | 0.28  | 5.88        |
| 6      | Leaf breadth (cm)                             | 1.63          | 0.82-3.33        | 0.04               | 0.01               | 11.93 | 5.60  | 22.07            | 0.09  | 5.46        |
| 7      | Flower length (cm)                            | 3.62          | 2.85-5.71        | 0.06               | 0.01               | 6.85  | 2.26  | 10.89            | 0.06  | 1.55        |
| 8      | Flower breadth (cm)                           | 1.47          | 0.9-3.04         | 0.02               | 0.00               | 9.33  | 3.88  | 17.30            | 0.05  | 3.33        |
| 9      | Pistil length (cm)                            | 1.26          | 0.85-2.55        | 0.01               | 0.01               | 9.55  | 5.84  | 37.43            | 0.09  | 7.38        |
| 10     | Fruit length (cm)                             | 5.62          | 4.35-6.62        | 0.27               | 0.14               | 9.28  | 6.75  | 52.88            | 0.57  | 10.13       |
| 11     | Fruit diameter (cm)                           | 5.57          | 3.91-7.01        | 0.35               | 0.15               | 9.97  | 8.66  | 47.33            | 0.54  | 9.73        |
| 12     | Fresh fruit weight (g)                        | 107.62        | 23.19-235.86     | 1285.07            | 434.27             | 33.31 | 19.36 | 33.79            | 24.96 | 23.19       |
| 13     | Calyx length (cm)                             | 1.74          | 1.04-2.92        | 0.07               | 0.04               | 16.06 | 10.90 | 48.46            | 0.27  | 15.63       |
| 14     | Calyx diameter (cm)                           | 1.64          | 0.86-2.29        | 0.04               | 0.12               | 12.97 | 9.23  | 53.92            | 0.23  | 13.90       |
| 15     | Total aril weight (g)                         | 55.53         | 20.13-104.64     | 312.32             | 134.75             | 31.82 | 28.47 | 72.54            | 40.33 | 29.14       |
| 16     | Number of arils per fruit                     | 297.22        | 115.53-584.62    | 6926.67            | 9003.87            | 676.20 | 76.70 | 62.54            | 0.36  | 15.38       |
| 17     | Weight of 100 arils (g)                       | 18.86         | 8.17-34.03       | 17.73              | 7.08               | 22.82 | 11.41 | 15.76            | 25.62 | 8.62        |
| 18     | Single aril weight (g)                        | 0.19          | 0.09-0.33        | 0.00               | 0.00               | 21.84 | 13.44 | 39.91            | 0.37  | 18.38       |
| 19     | Aril percentage per fruit (%)                 | 45.83         | 34.32-54.33      | 12.89              | 5.20               | 7.83  | 4.98  | 40.33            | 2.98  | 6.51        |
| 20     | Juice percentage per fruit (%)                | 42.00         | 27.29-70.99      | 25.14              | 15.72               | 11.94 | 9.44  | 62.54            | 5.64  | 15.38       |
| 21     | Volume of juice (ml)                          | 27.42         | 16.3-57.41       | 39.24              | 28.47               | 22.85 | 19.46 | 72.54            | 3.96  | 34.14       |
| 22     | Total soluble solids (B)                      | 14.16         | 11.03-17.61      | 1.26               | 0.40               | 7.92  | 4.45  | 31.50            | 0.73  | 5.14        |
| 23     | pH of juice                                   | 3.32          | 2.86-3.83        | 0.03               | 0.01               | 5.26  | 2.86  | 29.56            | 0.11  | 3.19        |
| 24     | Acidity (%)                                   | 0.49          | 0.34-4.22        | 0.04               | 0.03               | 38.04 | 35.89 | 89.01            | 0.34  | 70.00       |
| 25     | Maturity Index (TSS/Acidity)                  | 30.31         | 11.9-43.76       | 20.60              | 5.67               | 14.98 | 7.85  | 27.50            | 2.57  | 8.49        |
| 26     | Phenolic content (mg/1000 ml gallic acid)     | 277.78        | 18.75-1938.59    | 85210.98           | 908537.59          | 105.09 | 102.17 | 94.52            | 568.35 | 204.61     |
| 27     | Anthocyanins (mg/100 ml)                      | 71.67         | 0.17-679.97      | 8102.83            | 6957.49            | 125.59 | 116.38 | 85.86            | 159.22 | 222.16     |
| 28     | Rind thickness (mm)                           | 3.30          | 1.63-4.94        | 0.32               | 0.28               | 17.07 | 16.00 | 87.85            | 1.02  | 30.88       |
| 29     | Rind weight (g)                               | 53.56         | 10.06-158.03     | 495.98             | 151.21             | 41.58 | 22.96 | 30.49            | 13.99 | 26.11       |
| 30     | Rind percentage (%)                           | 44.73         | 33.08-56.98      | 16.90              | 11.53              | 9.19  | 7.59  | 68.21            | 5.78  | 12.92       |

* **significant at 5% and 1% levels respectively.

| Table 2: The estimates of variability and genetic parameters for plant growth, fruit quality traits and bacterial blight disease resistance in γ-ray irradiated seed derived progenies and germplasm of pomegranate |
Conclusions
Our results showed large variability in fruit and juice traits among pomegranate clones, which indicates a high potential for effective improvement through breeding programmes. The evaluation of pomegranate fruit quality parameters and bacterial blight disease resistance over several years demonstrates that fruit characteristics were influenced more by clone and environmental conditions. Phenotypic coefficients of variation (PCV) values were shown to be higher than GCV values for all the studied traits. This means that improvements in pomegranate fruit quality could be achieved even if selection was based on phenotype. Most of the traits studied showed significant variations of heritability and GA. This indicates the potential for further pomegranate improvement for most traits of interest. The high heritability and GA values observed for days to maturity, plant spread, rind thickness, acidity, phenolic content, anthocyanin content, bacterial blight on fruit, stem and disease severity suggest important additive gene effects for such traits. Thus, there are ample opportunities to select improved pomegranate clones adapted to the climatic conditions.

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