Impact Of Hybridity On The Flavonoid Spectrum Of Pomegranate

Rakesh Bhargava[1*]

[1*]RNB Global University, Sri-ganganagar Road, Khara, Bikaner 334601, Rajasthan, India

***Corresponding Author:** Rakesh Bhargava
[*]RNB Global University, Sri-ganganagar Road, Khara, Bikaner 334601, Rajasthan, India

Abstract:
An attempt was made to assess the impact of hybridity on the flavonoid composition of the pomegranate plants. A cross was made between pomegranate cultivar Jalore Seedless and Mirdula and 13 F1 offspring were grown in the farm. An in-depth study was made of the flavonoid spectrum of parents and offspring. The data revealed that both parents have typical spots which aids in their identification. The comparison of the flavonoid spectrum of parents with F1 hybrids demonstrated that the spectrum of the parents contains the typical spots of either one or both parents. In addition to this, some offspring have novel spots. It was concluded that occurrence of novel spots may be an out come of gene interaction which are expressed in the hybrids.

Introduction:
Polyploidy and hybridity has played an important role in plant genetic diversity. In a number of plant species, polyploidy has introduced the morphological variations. Bhargava (1983) has demonstrated that polyploidy has induces morphological variations in *Narcissus*. In nature, a huge genetic diversity has been introduced in plant species on account of natural hybridization of plant species. This help in improving the genetic makeup of the plants and are fit to survive in natural environment and produce better (Sharma, 1989; King, 1977; Mierziak et al., 2014).
Although, enormous work has been done on the morphological divergence due to hybridity, yet very few work has been done to assess as to how the hybridity changes the metabolite composition of the plants. Some studies have been conducted to assess the changes in flavonoid composition of naturally occurring hybrids (Bhargava, 1983; Crius et al., 1988; Pacheco et al., 1991; Bhargava et al., 2005).

Among the metabolites flavonoids qualifies to be an ideal parameter for study since it is stable and does not change with environmental fluctuations. Therefore, in the present study an attempts was made to assess the changes that are introduced in the flavonoid spectrum of F1 plants by making deliberate hybrids using pomegranate varieties Jalore Seedless and Mirdula. The results thus obtain constitutes the text of the present communication.

Material and Methods:
Crossing was performed using the plants of Jalore Seedless and Mirdula and seeds thus produced were collected and grown in net house and subsequently transferred in the field. The plants of 2 years of age were used for the study purpose. A total of 15 lines (2 patents and 13 F1 hybrids) were used in the present study. Freshly mature leaves were used for the extraction of flavonoids.

Extraction of flavonoids:
Two gram mature leaf sample (three replications of each) were fixed in 10 ml of methanol containing 1% HCL. The samples were stored in dark for 3 days at room temperature. Before analysis, the samples were transferred in mortar and pestle and macerated. The whole smash was filtered and the filtrate was subjected to centrifugation at 10000rpm at room temperature for 10 minutes. The clear supernatant was taken and evaporated to dryness at 60^{o}C. Before analysis the sample was taken in 1 ml methanol for plotting on TLC plates.

Analysis of flavonoids:
The procedure followed was same as described by Bhargava et al., 2005. The flavonoids were separated on TLC plates coated with 0.6mm thick layer of Cellulose. An aliquot of 10 µl was applied on one corner of TLC plate. The TLC plates were developed first with 2% formic acid and after drying. The plates were rotated by 90^{o} and again subjected to development in solvent system consisting of amyl alcohol: acetic acid: water in ratio of 10:6:5.
The plates after drying were viewed under i) without spray; ii) after exposure to ammonia vapours under UV; iii) after spray with 1% methanolic $AlCl_3$ under UV and iv) after spray with 1% methanolic NaOH under UV. Individual chromatogram was developed by treatments as given and spots visible were marked. All chromatograms of one line was developed by overlapping the chromatograms developed under each treatment. Finally, a pooled chromatogram was developed by overlapping the master chromatograms of each line. The spots were numbered and are presented in Table 1.

Results and Discussion:

Perusal of the master pooled chromatogram show a total of 30 flavonoid spots (Table 1). Of these a total of 21 spots were found the profiles of both the parents. Comparison of chromatographic profile of parents shows that spot nos. 4, 5, 11, 13, 14, 15, 17, 23, 25 and 30 were common between the parents. However, each parent can be typified by specific spots. For instance Jalore Seedless can be typified by spots Nos. 6, 8 and 22 whereas Mirdula by spots 1, 2, 7, 12, 16, 18, 20 and 27. Our results are in line with those reported by Bhargava et. al., 1988 in Argemone; Sharma et al., 1989 in Plantago. Perusal of profiles of every hybrid revels that the typical spots of both the parents can be traced in their profiles. For instance, Spot No.2, 7, 16 and 20 which are typical of Cv. Mirdula were present in majority of the hybrid lines. Similarly, spot Nos. 6, 8 and 22 which were typical of cv. Jalore seedless were traced in most of the hybrids. This shows that the phylogeny of the hybrids can be traced by using flavonoid spectrum.

Perusal of data in Table 1 further reveals that the flavonoid spectrum of hybrid lines also varies from each other. For instance, spot nos 2, 7, 12, 16, 20 and 28 are present in most of the hybrid lines. Showing thereby that they have either contributed by one of the parents or has universal genes for coding them.

On the contrary, some spots which have limited occurrence. For instance, spot No. 3 is found in only 4 hybrid lines and absent from both the parents. Similarly, spot nos. 21 was found in only one hybrid, spot nos. 24 in 2 hybrids, spot no. 26 in 3 hybrids, spot nos. 10 and 29 in 5 hybrids and spot no. 19 in 8 hybrids. Such variation was caused due to expression of these genes due to complimentary gene interaction. (Crius, et al., 1988; Pacheco et al., 1991; Orians, 2000; Lim et al., 1999; Bhargava, 2005).

In addition to the above, the hybrids does not show the spots present in the parents. For instance, spot nos. 6,8 and 22 which were present in Jalore Seedless were not visible in most of the hybrids. Similarly spots nos. 18 present in cv. Mirdula was not seen majority of hybrids. The spot Nos 30 which was present in both the parents was present in only four hybrid lines. The disappearance of some spots in the hybrids can be explained on the basis of epistatic phenomenon i.e. masking effect of one gene over other.

References:

1. Bhargava, R. (1983). Metabolites as indicators of polyploidy and hybridity in some bulbous angiosperms. Ph.D. thesis, University of Jammu, Jammu.
2. Bhargava, R., Jasrotia, R.S., Sharma, M.C. (1988). Studies on Argemone II. Impact of polyploidy and hybridity on some plant metabolites. Cytologia 53: 697-701.
3. Bhargava, R., Shukla, A.K., Chauhan, N., Vashishtha, B.B. and Dhandar, D.G. (2005). Impact of hybridity on flavonoid spectrum of ber (*Ziziphus mauritiana* Lamk.). Environmental and experimental Botany, 53:135-138.
4. Crius, W.J. , Bohm, B. A. and Carr, G.D. (1988). Flavonoids as indicators of hybridization in a mixed population of Lava-colonizing Hawaiian Tarweeds (Asteraceace: Heliantheae: Madiinae). Systematic Botany, 13(4): 567-571.
5. King, B.L. (1977). Flavonoid analysis of hybridization in Rhododendron section Pentanthera (Ericacceae). Systematic Botany 2(1): 14-27.
6. Lim, C.E., Park, J.H. and Park, C.W. (1999). Flavonoid variation of the Aconitum jaluense comples (Rannunculaceae) in Korea. Plant systematics & Evolution, 218: 125-131.
7. Lopez-Caamal. A. and Tovar-Sanchez, E. (2014). Genetic, morphological and chemical patterns of plant hybridization. Revista Chilena de Historia Natural, 87: 1-16.
8. Mierziak, J. Kostyn, K. and Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. Molecules 19(10): 16240-16265.
9. Orians, C. M. (2000). The effect of hybridization in plants on secondary chemistry: Implications for the ecology and evolution of plants- Herbivore interactions. Am. J. Botany, 87(12): 1749-1756.
10. Pacheco, P., Stuessy, T.F. and Crawford, D.J. (1999). Natural inter-specific hybridization in Gunnera (Gunneraceae) of the Juan Fernandez Island, Chile. Pacific Science 45: 389-399.
11. Sharma, P.K., Bhargava, R., Koul, A.K. (1989). Studies on the flavonoid spectrum of disomic. Cytologia, 54: 197-202.

Table 1: Flavonoid spectrum of pomegranate progenies

Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Jalore Seed				+	+	+		+			+		+	+	+		+					+	+		+					+
Mirdula	+	+		+	+		+				+	+	+	+	+	+	+	+		+			+		+		+			+
P1S1	+	+	+	+		+	+		+		+		+		+	+			+	+	+		+		+			+		
P1S2		+	+	+	+		+	+	+			+		+	+	+	+			+				+				+	+	+
P1S3		+	+			+	+		+		+	+	+		+		+		+	+					+			+	+	+
P1S4	+	+		+	+		+						+			+			+							+		+		
P1S5	+	+				+	+			+			+	+		+				+							+	+	+	+

P1S6		+		+			+				+	+					+				+							+	+	+	
P1S7		+		+			+			+		+			+		+				+						+					
P1S8		+		+			+								+				+				+						+				+
P1S9		+		+			+			+		+					+		+										+		+		
P1S10		+		+		+	+	+	+	+				+			+				+								+		+		
P1S11		+					+							+																			+	
P1S12				+					+	+		+			+																		
P1S13	+			+			+					+			+		+																