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CHARACTERIZATION OF POMEGRANATE (*PUNICA GRANATUM* L.) CULTIVARS USING FOLIAR FLAVONOID BASED SPECTRUM

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KEYWORDS

Flavonoid spectrum
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ABSTRACT

An experiment was conducted to develop the flavonoids patterns of ten pomegranate cultivars at Plant Physiology Laboratory of Central Institute for Arid Horticulture, (C.I.A.H) Bikaner. A pooled chromatogram possessed a total of 25 flavonoid spots. The distribution of these spots among the taxa was highly variable. Flavonoid patterns varied from 10 to 16 in different genotypes, Spot no. 8 and spot no. 20 showed highest representation in 80 per cent genotypes, spot nos. 9 and 16 showed restricted distribution in 10 per cent genotypes. Spot no. 9 and 16 were found exclusively in Ganesh, spot no. 11 in Jalore Seedless, Spot no. 15 in Khog, spot no. 21 in Bassein Seedless, spot no. 24 for Phule Arakta, hence they proved to be marker spots for the respective cultivars, these could used as an aid in varietal identification indicating the absence and presence of particular spots. Clustering using NTSYS-PC 2.02 depicted a dendrogram which grouped cultivars into two major groups. Hence, flavonoid serves as an ideal parameter, as well as an excellent marker tools for varietal identification, characterization to understand the phylogenetic kinship among the cultivars.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) commonly known as *Anar* belongs to family *Punicaceae* is extensively cultivated in tropical, subtropical and arid regions of the world.The scientific name of pomegranate (*Punica granatum* L.), is derived from words *Pomum* (apple) and *granatus* (grainy) or seeded apple. Pomegranate is one of the oldest known edible fruit to be cultivated, even mentioned in the Bible and the Koran (Chandra *et al*. 2010). Hardy nature, high yield potential, good keeping quality and demand in market as well its ability to withstand the extreme climatic condition reflects its wide geographical distribution. The genus *Punica* consists of two species, *Punica granatum* L. and *P. protopunica* Balf. However, *P.nana* L. is an ornamental dwarf pomegranate and has been considered as a distinct species by some authors (Melgarejo and Martinez, 1992). It is believed to be originated in Central Asia especially the Transcaucasia-Caspian region in Iran, from where it spread to the rest of the world (Harlan, 1992; Smith, 1976; Levin, 1994 and Verma *et al*., 2010). It was cultivated in ancient Egypt and early in Greece, Italy and Iraq. Later, it spread to Asian countries like Turkmenistan, Afghanistan, Iran, India, China, North Africa and Mediterranean Europe (Melgarejo and Martinez, 1992) and to some extent in the drier parts of South East Asia, Malaya, the East Indies and Africa, USA, China, Japan and Russia (Mars, 1994).

Genetically, crop is heterozygous, a wide genetic diversity has been found in nature and majority of this has been stabilized, since it has vegetative method for propagation. The classification of these cultivars rests mostly on floral and fruit characters, at times becomes overlapping and hence renders the identification difficult. These parameters becomes insufficient to clearly distinguish the cultivars. Role of flavonoids in biosystematics at species or sub-species level has been documented and discussed from years, flavonoid since then have been used for cultivar identification as well as for assessing phylogenetic relationship among taxa, demonstrated by Bhargava *et al*. (2010) in ber, Bhargava *et al*. (2011) in pomegranate, Dass *et al*. (1977) in Citrus *spp*, Garcia and Oieda (2004) in *Morusalba,* Gopi *et al*. (2013) in *Rauvolfia spp*, Kharazian *et al*. (2013), Koul *et al*. (1985), Russak *et al*. (2005) in *Iris* spp., Sanchez-Yelano (2004) in *Erucastris* and *Brassica* and Sozinov *et al*. (2004) in *Filipendula ulmaria* (Rosaceae). The present study is a recourse to the modern methods which rests upon the use of flavonoids spectrums in varietal identification to resolve taxanomic riddles. Secondary metabolites such as flavonoids have gained reputation as an excellent taxanomic markers.

MATERIALS AND METHODS

Thin layer chromatographic analysis (TLC) was conducted at Plant Physiology laboratory of C.I.A.H, Bikaner, Rajasthan during year 2015. The method of extraction of Flavonoids for TLC was in light by earlier by several workers (Bhargava *et al*. (2010) in ber, Bhargava*et al*. (2011) in pomegranate, Dass, *et al*.(1977) in Citrus spp , Garcia and Oieda (2004) in *Morus alba* and Koul*et al*. (1985), V. A. Edalli and C. M. Kamannavali (2010) in mushroom. The leaf sample constituted the leaves of ten pomegranate genotypes viz., Jalore Seedless, Ganesh, G-137, Khog, Mridula,

Bassein Seedless, Bhagwa, Phule Arakta, GKVK-1 and Dholka; collected from pomegranate germplasm repository, C.I.A.H, Bikaner. 2g of leaf sample fixed in methanol containing (1%) HCl and supernatant collected was dried and final sample was dissolved in 1ml methanol. An aliquot of 50 μL of the test extract was loaded at predetermined point at the corner of the cellulose coated TLC plates (20x20cm), through a micropipette. The extract was dried with the help of hot air blower, so as to keep the loaded spot as small as possible. Loaded plates were developed at room temperature in two solvent systems A. 2% formic acid B. amyl alcohol, acetic acid and water (10:6:5) in ascending direction. Development was carried out in all glass chambers where the loaded TLC plates were so inserted into the chamber that they remained upright with their loaded edge just touching the first solvent. The whole set up was left undisturbed till the solvent front ascended to a predetermined level (16 cms from the point of application of extract) on the plates. Thereafter, they were removed, air dried, rotated through 90º angle at the loaded spot and reintroduced into the chamber now containing the second solvent system. Thereafter, the plates were removed and air dried. The metabolites were analyzed by UV light or by spray enhancing chemicals like 1% methanolic Sodium hydroxide or 1% methanolic AlCl3 solution.

RESULTS AND DISCUSSION

Foliar flavonoids patterns have been worked out for ten pomegranate (*Punica granatum* L.) cultivarsand TLC plates after air drying were viewed for flavonoid spots based of their respective fluorescence properties and positioned on the composite chromatograms. Fluorescence was checked under UV-light before and after spraying enhancing chemicals.- without any spray after spray with 1% methanolic $AlCl_3$ under UV and after spray with 1% methanolic NaOH under UV. A total of 25 flavonoid spots were observed in pooled master chromatogram (Fig.1).

A wide variation was observed for their distribution among the genotypes investigated (Table1). For instance, spot no. 8 and spot no. 20 had highest representation; it was observed in 80 per cent of the genotypes. Spot nos. 1, 2, 3, 12 and 13 showed representation in 70 per cent genotypes. Similarly, spot nos. 4, 7, 14, 18, 19 and 22 were represented in 60 per cent genotypes. Spot nos. 10 and 15 showed representation in 50 per cent genotypes .On the contrary, few spots showed restricted distributions as illustrated by spot nos. 9 and 16, these were found only in 10 per cent genotypes, spot nos. 11, 21, 24 and 25 showed representation in 20 per cent genotypes, spot no 17 showed representation in 30 per cent genotype, spot nos. 5, 6 and 23 were observed in 40 per cent genotypes. The number of spots varied from 10 to 16 in different genotypes, maximum (16) in Ganesh and minimum (10) in Jalore Seedless, G-137 and Dholka. Jalore Seedless, G-137 and Dholka showed 10 spots, Mridula and GKVK-1 showed 11 spots, Bhagwa and Khog showed total 13 spots and Bassein Seedless, Phule Arakta and Ganesh showed 14, 15 and 16 spots respectively. Apart from the number of spots in the respective profile, the cultivars also differed with respect to the type of spots in flavonoids profile. Spot no. 9 and 16 were found exclusively in Ganesh, spot no. 11 in Jalore Seedless, Spot no. 15 in Khog, spot no. 21 in Bassein Seedless, spot no. 24 for Phule Arakta, since these spots were only found in respective genotypes, hence these could be used as aid in varietal identification.

Table 1: Spotting pattern for flavonoids in different pomegranate cultivars

Spot Number	Jalore Seedless	Ganesh	G-137	Khog	Mridula	Bassein Seedless	Bhagwa	PhuleArakta	GKVK-1	Dholka
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	-	-	-	-	-	-	-	+	-	+

*'+' for presence of spot and '–'for absence of spot

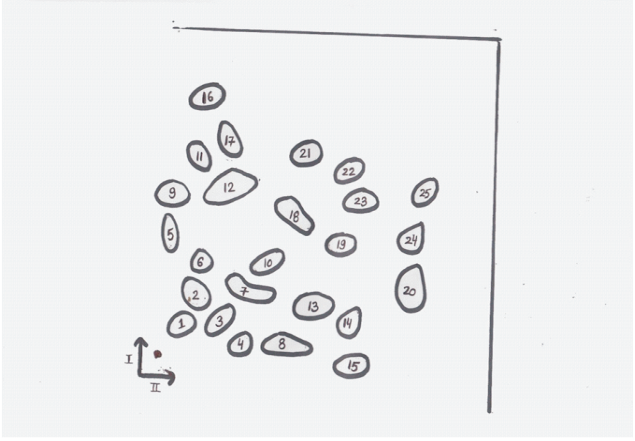

Figure 1: Pooled Master Chromatogram for flavonoids; *(Chromatogram showed the presence of 25 flavonoids spots on two phased mobile solvents systems (I. 2% Formic acid II.Amyl alcohol, Acetic acid and Water (10:6:5) under visible UV radiation based on fluorescence)

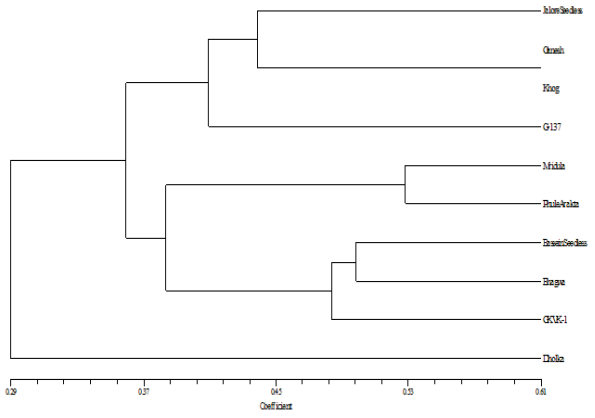

Figure 2: Dendrogram for flavonoids of 10 pomegranate genotypes

Various studies markers biochemical as well as molecular studies (Kathuria *et al*., 2015) have been attempted to assess the genetic diversity and systematics of pomegranate species. The present results are in line with those reported earlier by Bhargava *et al*. (1983) in angiosperms, Bhargava *et al*. (2010) in ber, Bhargava *et al*. (2011) in pomegranate, Dass *et al*.(1977) in Citrus spp, Garcia and Oieda (2004) in *Morus alba,* Koul *et al*. (1985), Sanchez-yelamo (2004) in *Erucastris* and *Brassica*, Sozinov *et al*. (2004) in *Filipendula ulmaria* (Rosaceae) and Russak *et al*. (2005) in *Iris* spp. The distribution of flavonoid spots or spot combination revealed specific spots for each cultivar which could be used not only for varietal identification but also for assessing the phylogenetic kinship among pomegranate cultivars. The distribution of flavonoid spots or spot combination revealed specific spots for each cultivar which could be used not only for varietal identification but also for assessing the phylogenetic kinship among pomegranate cultivars. To assess the phylogenetic relationship among the cultivars, the data was subjected to cluster analysis using NTSYS-PC-2.02e software (Rohlf, 1998). The dendrogram classified the pomegranate cultivars into two major groups. Group I consisted of cultivar Dholka which was clearly distinct and showed less close relationship with other cultivars. Group II is further subdivided into two groups – II A consisted of four cultivars (Jalore Seedless, Ganesh, Khog, and G-137) and group II B consisted of five cultivars. (Mridula, PhuleArakta, Bassein Seedless, Bhagwa, GKVK-1) The group II A further reveals that cv. G-137 is distantly related among other cultivars of this group and cv. Ganesh and Khog are more closely related. Among the cultivars in Group II B, Mridula and Phule Arakta forms one separate cluster whereas the remaining cultivars showed closer affinities.

Thus, from results, it is evident that foliar flavonoid spectrum can be used as an ideal biochemical marker tool for cultivar identification as well as for assessing phylogenetic relationship among taxa in pomegranate.

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