

PHENOLIC COMPOUNDS AND ELEMENTS OF LEAVES AS AN AID FOR THE TAXONOMIC DELIMITATION OF THE GENUS *CLEOME* L. (CLEOMACEAE) FROM PAKISTAN

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ABSTRACT

Phenolic compounds and elements in the leaves of 7 species of the genus *Cleome* L. from Pakistan were studied. A total of 44 phenolic compounds and 15 elements were detected in leaves of the genus and found to be significant enough to strengthen taxonomic delimitation of the genus. The genus *Cleome* was characterized by the presence of quercetin, carbon, oxygen, potassium, calcium, magnesium, boron, chlorine, copper and sodium. On the other hand, genus *Cleome* can be bifurcated on the basis of presence or absence of kaempferol. Similarly, the data obtained by the chemical analysis of leaves can also be well correlated with morphological and palynological characters of the species within the genus *Cleome*.

Key words: *Cleome*, elements, leaves, Pakistan, Phenolic compounds, Taxonomic delimitation.

INTRODUCTION

Utilization of chemical data in systematics can be traced back by the work of Petiver (1699) and amongst all of the chemicals, phenolic compounds have received more attention for systematic delimitation (Harborne, 1973; Smith, 1976; Crawford, 1978) and to trace out the hybridization (Harborne, 1973). While, amongst the phenolic compounds, flavonoidal data have considerable potentialities as a taxonomic marker (Fang *et al.*, 2002).

Regarding to the use of phenolic compounds there are number of reports available such as, Harborne and Williams (1973) studied phenolic compounds for 344 species of Ericaceae and 37 species of related families where they concluded that the data supports the taxonomic delimitation. Similarly, Harborne and Green (1980) analyzed the flavonoids for 97 taxa of the family Oleaceae and data was found significant for classificatory purpose as well as for tracing evolutionary advancement.

Similarly, Haron (1992) studied the distribution and taxonomic significance of leaf flavonoids in 17 species of *Eugenia* L. (Myrtaceae). Blatt *et al.* (1998) detected the flavonoids of Bignoniaceae and data was used to delimit the various tribes within this family. While, Fang *et al.* (2002) studied *Eugenia* species and a distinct flavonoid pattern in this genus was observed where they concluded that chemotaxonomy provide major source of characters for classification. Similarly, Abid and Qaiser (2003) studied 21 species of *Inula* L. and its allied genera for their phenolic compounds and data was used for supporting the taxonomic delimitation. While, Marzouk *et al.* (2010) isolated 38 compounds (flavonols and their derivatives) from 9 species of different tribes of the family Brassicaceae and the data was correlated with gross morphology and anatomy for taxonomic purposes.

Regarding to the chemical constituents for the genus *Cleome* L. various workers have made attempts but no one gave the attention neither to all Pakistani species nor the utilization of data for taxonomic purposes. Such as, Wollenweber *et al* (2007) studied 7 species of *Cleome* and isolated flavonoids including two novel compounds i.e., 5,3',4'-triOH-3,6,7,5'-tetraOMe-flavone from *C. felina* L.f. and 5,3'-diOH-3,7,8,4',5'-pentaOMe-flavone from *C. viscosa* L. Similarly, Sharaf *et al* (1997) also studied flavonoids of four *Cleome* species and 13 different flavonoid glycosides were identified. Aboushoer *et al.* (2010) isolated four sesquiterpene derivatives from *C. droserifolia* (Forssk.) Delile. Moreover, Kapoor and Mishra (2013) isolated quercetin and kaempferol from *C. viscosa*. While, Kasem and Fathy (2016) detected quercetin, kaempferol and their derivatives in 5 species of the genus *Cleome*.

From the above mentioned reports it is evident that the genus *Cleome* has been studied for their phenolic compounds but the data was not used for taxonomic delimitation. Similarly, no reports were found on the leaf elements. The present study is first of its kind to correlate the data of phenolic compounds and leaf elements for strengthening the specific delimitation of the genus *Cleome* from Pakistan.

MATERIALS AND METHODS

Phenolic compounds:

Leaves of 7 species of the genus *Cleome* were analyzed for their phenolic compounds (Appendix-I). For extraction, 1 gm dried leaves from fresh material or herbarium specimens were soaked in 70% ethanol for 24 hours at room temperature. Extracts were filtered and concentrated and chromatographed two dimensionally on thin layer chromatographic (TLC) plates using BAW (n-butanol : acetic acid : water, 4 : 1: 5) versus 15% acetic acid, following standard procedure of Harborne (1973).

Phenolic compounds were identified by comparing with the Rf values and their colour in ultra-violet light before and after ammonia fumigation (Tables 1-4).

Energy-dispersive X-ray spectroscopy (EDS):

Mature and healthy leaves of 7 species of the genus *Cleome* were studied for the leaf elements (Appendix-I). The mature dried leaves were directly mounted on metallic stub, using double adhesive tape, then gold plated in sputtering chamber for a period of 6 minutes and observed under scanning electron microscope for the detection of elements (Table 5) (Fig. 1 and 2). Leaf elements were detected by energy-dispersive X-ray spectroscopy (EDS) using EDS detector (EX-54175jMU) attached to scanning electron microscope (JSM-6380 A). Quantitative analysis was performed by ZAF method standardless quantitative analysis.

RESULTS AND DISCUSSION

Key to the species of the genus *Cleome* based on leaf phenolic compounds and elements

1 + Kaempferol present.....	2
- Kaempferol absent.....	4
2 + p- coumaric acid and sinapic acid present.....	3
- p- coumaric acid and sinapic acid absent.....	<i>C. dolichostyla</i>
3+ Isovitexin, isoliquiritigenin, aureusidin and naringin present, Sulphur and Manganese absent	<i>C. brachycarpa</i>
- Isovitexin, isoliquiritigenin, aureusidin and naringin absent, Sulphur and Manganese present	<i>C. viscosa</i>
4 + Aesculin and hesperidin present.....	<i>C. rupicola</i>
- Aesculin and hesperidin absent.....	5
5+ Quercetin, gossypetin, apigenin and kayaflavone present, Iron absent.....	<i>C. karachiensis</i>
- Quercetin, gossypetin, apigenin and kayaflavone absent, Iron present.....	6
6 + Quercetin 3-glucoside, quercetin 7-glucoside and aureusidin 6-glucoside present, Aluminium and Silicon absent	<i>C. ariana</i>
- Quercetin 3-glucoside, quercetin 7-glucoside and aureusidin 6-glucoside absent, Aluminium and Silicon present.....	<i>C. scaposa</i>

Table 1. Phenolic acids and flavonols in *Cleome* L.

S. No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	<i>Cleome ariana</i>	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	-
2	<i>C. brachycarpa</i>	+	+	-	-	+	-	+	-	+	-	+	-	+	+	+	-	-
3	<i>C. dolichostyla</i>	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	-	-
4	<i>C. karachiensis</i>	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+
5	<i>C. rupicola</i>	-	-	-	+	-	+	-	+	-	+	-	-	-	+	-	-	-
6	<i>C. scaposa</i>	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-
7	<i>C. viscosa</i>	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-

Key: + = Present, - = Absent

1= p-coumaric acid, 2= sinapic acid, 3= 3, 4, 5'-trimethoxy cinnamic acid, 4= aesculin, 5 = Kaempferol, 6 = Quercetin, 7 = Quercetin 3- Arabinoside, 8 = Quercetin 3- xyloside, 9 = Quercetin 3- glucoside, 10 = Quercetin 3- galactoside, 11 = Quercetin 3- Rhamnoside, 12 = Quercetin 3- Glucuronide, 13 = Quercetin 3- Rutinoside, 14 = Quercetin 7- Glucoside, 15 = Quercetin 4' Glucoside, 16 = Azaleatin, 17 = Gossypetin.

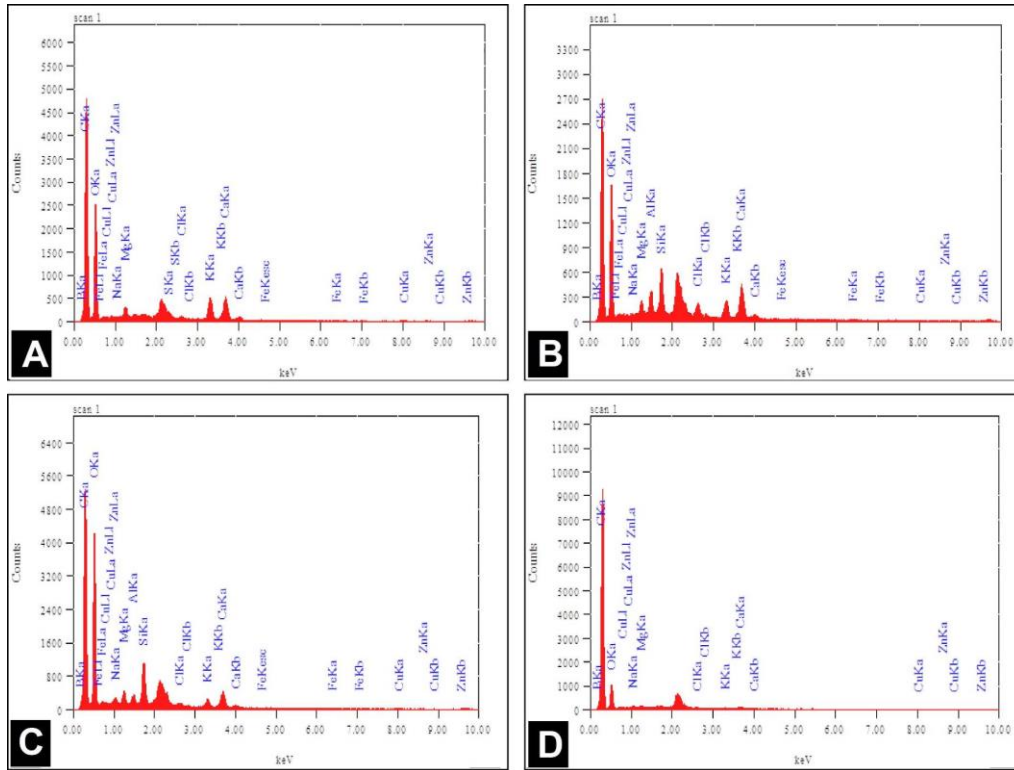


Fig. 1. Energy-dispersive X-ray spectroscopy (EDS) graphs showing leaf elemental composition. **A**, *Cleome ariana*; **B**, *C. brachycarpa*; **C**, *C. dolichostyla*; **D**, *C. karachiensis*.

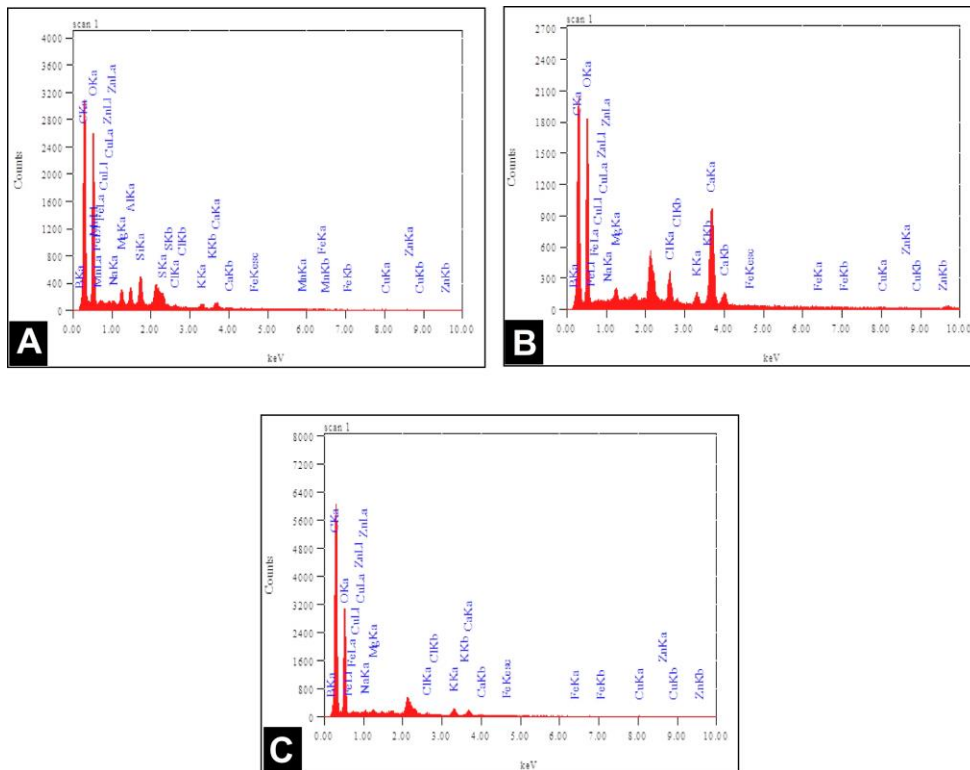


Fig. 2. Energy dispersive X-ray spectroscopy (EDS) graphs showing leaf elemental composition. **A**, *C. rupicola*; **B**, *C. scaposa*; **C**, *C. viscosa*.

Thin layer chromatography of aqueous ethanolic extracts of 7 species of genus *Cleome* resulted in the isolation of 44 phenolic compounds including 13 unknown compounds (Tables 1-5). While, EDS revealed 15 elements found in leaves including 1 heavy metal i.e. Aluminium.

The results revealed that quercetin and its derivatives are the most widespread compounds and C, O, K, Ca, Mg, B, Cl, Cu and Na are the most abundant elements within the genus *Cleome*. While, genus *Cleome* can be divided into two groups on the basis of presence or absence of kaempferol. The group of species with kaempferol comprises *C. brachycarpa*, *C. dolichostyla* and *C. viscosa* and the grouping of these species may also be supported by comparatively larger pollen grains (24.8 - 29.4 μm). The other group of species, which lacks kaempferol includes *C. rupicola*, *C. karachiensis*, *C. ariana* and *C. scaposa* having smaller pollen grains (12.8 – 16.5 μm) (Sana, 2018). Amongst the species of group I, *C. dolichostyla* can be distinguished from other two species by the presence of quercetin and diadzein along with simple, suborbicular and cordate leaves (Jafri, 1973). While, the remaining two species *C. viscosa* and *C. brachycarpa* are closely related with each other by having palmately compound leaves and elliptic petals (Riaz *et al.*, 2019). Furthermore, these two species remain distinct by the exclusive morphological and chemical data such as; semi erect habit, 6 stamens, oblong capsule, prolate pollen grains (Riaz *et al.*, 2019), isovitexin, isoliquiritigenin, aureusidin, naringin, Aluminium and Silicon in *C. brachycarpa* and *C. viscosa* is characterized by erect habit, 10-20 stamens, linear capsule, subprolate pollen grains (Riaz *et al.*, 2019), luteolin, chrysoeriol, 3,4,5'-trimethoxycinnamic acid and aureusidin 4- glucoside.

In group II, *C. rupicola* remains distinct from other three species by the presence of aesculin, hesperidin linear sepals and prolate pollen with rugulate-striate tectum (Sana, 2018) as well as absence of Zn. Similarly, *C. karachiensis* can be differentiated from *C. ariana* and *C. scaposa* by the presence of apigenin, gossypetin and kayaflavone with unbranched stem (Riaz *et al.*, 2019) and lack of Fe. On the other hand, *C. ariana* and *C. scaposa* can be differentiated from each other by having quercetin 3-glucoside, quercetin 7-glucoside, aureusidin 6-glucoside and S in *C. ariana*. While, *C. scaposa* is characterized due to the presence of quercetin 3- arabinoside, rutin, Aluminium and Silicon. Similarly, they have distinct compound and simple leaves respectively (Riaz and Abid, 2021)..

Thus, from the ongoing discussion, it is evident that the data obtained from leaf phenolic compounds and elements can be utilized to strengthen the taxonomic delimitation of the genus *Cleome* from Pakistan.

Table 2. Flavones, glycosyl flavones, chalcones, aurones, flavonones and isoflavonones in *Cleome* L.

S. No.	Species	Flavones		Glycosyl flavones	Biflavonyl	Chalcones		Aurones			Flavones			Isoflavone
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	<i>Cleome ariana</i>	-	-	-	-	-	-	-	-	+	-	-	-	-
2	<i>C. brachycarpa</i>	-	-	-	+	-	+	+	-	+	+	-	+	-
3	<i>C. dolichostyla</i>	-	-	-	-	-	-	-	-	-	-	-	-	+
4	<i>C. karachiensis</i>	+	-	-	-	+	-	-	-	-	-	-	-	-
5	<i>C. rupicola</i>	-	-	+	-	-	-	-	-	-	-	+	-	-
6	<i>C. scaposa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
7	<i>C. viscosa</i>	-	+	+	-	-	-	+	-	+	-	-	-	-

Key: + = Present, - = Absent

1 = Apigenin, 2 = Luteolin, 3 = Chrysoeriol, 4 = Isovitexin, 5 = kayaflavone, 6 = Isoliquiritigenin, 7 = Isoliquiritigenin 4' glucoside, 8 = Aureosidin, 9 = Aureosidin 4- glucoside, 10 = Aureosidin 6- glucoside, 11 = Hesperitin, 12 = Hesperidin, 13 = Naringin, 14 = Daidzein.

Table 3. Frequency of occurrence of different phenolic compounds in *Cleome* L.

S. No.	Compounds	No. of species	Percentage of species
Phenolic acids			
1	p- coumaric acid	2	28.57 %
2	Sinapic acid	2	28.57 %
3	3,4,5 Trimethoxycinnamic acid	1	14.28 %
4	Aesculin	1	14.28 %
Flavonols			
5	Kaempferol	3	42.85 %
6	Quercetin (Including glycosides)	7	100.00 %
7	Azaleatin	3	42.85 %
8	Gossypetin	1	14.28 %
Flavones			
9	Apigenin	1	14.28 %
10	Luteolin	1	14.28 %
11	Chrysoeriol	2	28.57 %
Glycosylflavones			
12	Isovitexin	1	14.28 %
Biflavonyl			
13	Kayaflavone	1	14.28 %
Chalcones			
14	Isoliquiritigenin (including glucoside)	2	28.57 %
Aurones			
15	Aureusidin (including glucosides)	3	42.85 %
Flavanones			
16	Hesperitin	2	28.57 %
17	Hesperidin	1	14.28 %
18	Naringin	1	14.28 %
Isoflavones			
19	Daidzein	1	14.28

Table 4. Unknown compounds in *Cleome* L.

S. No.	Species	Rf Values		Colour in UV	
		BAW	15 % HoAc	With Amonia	Without Amonia
1	<i>Cleome ariana</i>	34.50	45.50	Fl. Blu.	Blu.
		21.17	85.51	Br.	Br.
2	<i>C. brachycarpa</i>	29.34	55.19	Br.	Br.
		46.16	66.47	Fl. Blu.	Fl. Blu.
3	<i>C. dolichostyla</i>	35.22	55.19	Fl. Gr.	Fl. Gr.
		25.78	42.40	Gr.	Gr.
4	<i>C. karachiensis</i>	44.38	91.97	Fl. Blu.	Blu.
		55.05	91.97	Fl. Blu.	Blu.
		69.10	91.97	Fl. Blu.	Blu.
5	<i>C. rupicola</i>	25.1	3.33	Fl. Gr.	Gr.
		23.89	16.11	Fl. Blu.	Blu.
6	<i>C. scaposa</i>	36.93	58.04	Blu.	Blu.
7	<i>C. viscosa</i>	37.27	61.62	Blu.	Blu.

Key: Fl. = Florescent; Gr. Green; Blu. = Blue; Br. = Brown.

Table 5. Elemental composition of leaves determined using energy-dispersive X-ray spectroscopy (EDS) and the associated analytical program EDS Analysis Station.

Percentage of Elements in Leaves																
S. No.	Name of species	C	O	K	Ca	Mg	S	B	Cl	Mn	Zn	Fe	Cu	Na	Al	Si
1	<i>Cleome ariana</i>	31.32	33.47	3.24	3.21	0.61	0.09	26.54	0.20	-	0.38	0.27	0.61	0.06	-	-
2	<i>C. brachycarpa</i>	29.84	31.77	1.71	4.49	0.81	-	26.29	1.22	-	0.07	0.51	0.25	0.08	0.84	2.13
3	<i>C. dolichostyla</i>	25.80	30.98	2.10	3.60	1.12	0.97	27.32	0.45	0.05	0.20	0.72	0.69	0.51	1.75	3.76
4	<i>C. karachiensis</i>	41.98	16.21	0.47	1.02	0.10	-	33.99	0.20	0.14	0.30	-	0.94	0.07	-	4.58
5	<i>C. rupicola</i>	32.00	33.95	0.49	0.88	0.90	0.36	27.87	0.22	0.09	-	0.21	0.06	0.22	1.00	1.76
6	<i>C. scaposa</i>	20.89	34.83	0.99	12.47	0.79	-	22.76	1.99	-	0.87	0.41	0.68	0.08	0.47	2.79
7	<i>C. viscosa</i>	37.03	31.43	1.12	1.10	0.16	0.06	28.13	0.31	0.11	0.18	0.01	0.10	0.26	-	-

Key: C = carbon, O = oxygen, K = Potassium, Ca = calcium, Mg = Magnesium, S = sulphur, B = boron, Cl = chlorine, Mn = manganese, Zn = zinc, Fe = iron, Cu = copper, Na = sodium, Al = aluminium, Si = silicon.

Appendix-I List of voucher specimens for phenolic compounds.

S. no.	Species	Collector, number and herbarium
1	<i>Cleome ariana</i>	G.R. Sarwar & S. Omer 3019 (KUH); Haider Ali 6358 (KUH); Muqqarab Shah & Dilawar 1717 (ISL); WaliurRehman & Subhan 299 (ISL); Sultan-ul-Abedin 8003 (KUH); Kamal Akhter Malik & S. Nazimuddin 1473 (KUH); Hakim Khan 209 (RAW); M. Qaiser & A. Ghafoor 6592 (KUH); Haider Ali 1370, 1464 (KUH).
2	<i>C. brachycarpa</i>	Siddiqui & Hassan ud din 2671 (RAW); M.Tanvir & Dilawar 1007 (ISL); M.A. Siddiqui, Akram & Lal Khan 53 (ISL); Tahir Ali & G.R. Sarwar 2548 (KUH); Dr. Rubina Akhter & Dr. M. Reidl 25 (RAW); A. Ghafoor & S. Omer 1681 (KUH); M. Qaiser, K.H. Rechanger, Jennifer Lamond & Tahir Ali 8196 (KUH); M. Qaiser, S. Omer & T. Ahmed 8133 (KUH); Rizwan yousuf 9 (KUH).
3	<i>C.dolichostyla</i>	S.I. Ali, S.A. Farooqi & Sultan-ul-Abedin 1840 (KUH); A. Ghafoor & S. Omer 1804A (RAW); M. Qaiser, Asad Raza & Abrar Hussain 922, 1043 (KUH); A. Ghafoor & S. Omer 1863 (KUH).
4	<i>C. karachiensis</i>	Sana &RubinaAbid 65
5	<i>C. rupicola</i>	S. Nazimuddin, S. Abedin & Hameedullah 557 (KUH); Sultan-ul-Abedin & Abrar Hussain 6801,6802, 6804, 6805, 6943 (KUH); S.M.H. Jafri 2689 (KUH); S.I. Ali, S.A. Farooqi, S. Abedin 1033 (KUH).
6	<i>C.scaposa</i>	A. Ghafoor & Tahir Ali 3910 (KUH); Sultan-ul-Abedin & M. Qaiser 9328 (KUH); S.A. Farooqi & M. Qaiser 2843 (KUH); Anjum Perveen & Ishtiaq Hussain 107 (KUH); Kamal A. Malik, M. Qaiser, Saood Omer & Gohar Khan 2123 (KUH); Kamal Akhter & S. Nazimuddin 938 (KUH); Dr. Rubina Akhter 101 (RAW); Ali 454 (KUH); G.R. Sarwar, Qurban & Zamarrud 1288 (KUH).
7	<i>C. viscosa</i>	A.R. Beg 1451 (RAW); Sultan-ul-Abedin 3902, 7989 (KUH); M. Rashid Awan & Jamshed Saqib 1076 (PMNH); Farrukh Hussain 7085 (RAW); Sultan-ul-Abedin & M. Qaiser 9079 (KUH); Ayaz & Dilawar 23 (ISL); Anjum Perveen 136 (KUH); Rubina Akhter & Tariq Khan 086 (RAW); Sultan-ul-Abedin & Abrar Hussain 9461 (KUH).

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