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Research Article

Systematics of medicinally important weeds of genus Convolvulus: Convolvulaceae

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INFORMATION ARTICLE

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The authors declare that there is no conflict of interest regarding the publication of this manuscript.

HIGHLIGHTS

- To determine the medicinal uses of the two important weeds
- To determine the Pollen grains morphology through (SEM)
- Foliar anatomical characters (LM) of the weed plants for correct systematic identification.

ABSTRACT

Background: Palyno-morphological and foliar epidermal studies plays a very important role in the correct identification and differentiation of two weeds of genus convolvulus.
Objective: The aim of the study is to determine the medicinal uses of the two important weeds for correct systematic identification.
Methods: Both the qualitative and quantitative features were measured with the help of Light microscopy (LM) and scanning electron microscope (SEM).
Results: Variations were observed in both the weeds, i.e *Convolvulus arvensis* (bindweeds) and *Convolvulus prostatus* (soft bindweed) in epidermal cells, stomatal size and number, guard cell shape and size, subsidiary cell and diversity of trichomes. Paracytic type of stomata was found in both the weed species. Stomata shape in *Convolvulus arvensis* is elliptical and oval to oval oblong in *Convolvulus prostratus*. Weed species have a difference in size, shape, polarity and exine ornamentation. Tricolporate pollen type was observed. The shape of pollen grains is Prolate, Perforate. By using these Palyno-morphological characteristics a taxonomic key is prepared for the identification of these weed plants.
Conclusions: High fertility rate i.e Convolvulus arvensis 96% and in *Convolvulus prostatus* is 90 % shows the weed species are well adapted in the area. Systematics studies of the weed play a very important role not only incorrect identification but also differentiation with other weed plants and subsequently for the conservation purposes.

1 INTRODUCTION

Convolvulaceae is one of the attractive eurpalynous family, consisting of herbs, shrubs, and vines. Family Convolvulaceae extending globally most abundantly in America and Asia. (Saensouk, 2007). According to Austin (1975), the family contains about almost 50 genera and 2000 or more species. There are about thirteen genera exist in Pakistan that includes both natives and cultivated (Ali et al., 1979).

Convolvulaceae is divided into two groups Psiloconiae and Echinoconieae (Sengupta, 1972). Genus convolvulus was placed in Psiloconiace, *Convolvulus arvensis* L. bindweeds and *Convolvulus* prostatus (soft bindweed) are the most important medicinal weeds (Jaradat, 2005). *Convolvulus arvensis* is a perennial vine, persistent, spread by rhizome and seeds, having a cylindrical and branched stem. Leaves are variable in shape; triangular to ovate (Weaver and Riley, 1982). Species is native to Eurasia, but commonly distributed world wild and ranked as noxious weeds of the world (Austin, 2000; Holm et al., 1977).

Convolvulus arvensis was traditionally applied to treat epilepsy in north Pakistan (Ashfaq et al., 2019a; Bahadur et al., 2018a; Murad et al., 2011). *Convolvulus prostatus* is one of the important medicinal weed that is the main source of Shankhpushi, considered as the best brain tonics (Sethiya et al., 2009). Leaves powder of *Convolvulus arvensis* is used to treat boils and inflammation. Whole plant juice of *Convolvulus prostratus* is used as a Nerve tonic and the treatment of fever, jaundice (Qureshi et al., 2010; Waheed et al., 2020). First palynological survey on Convolvulaceae was made by Hallier in 1893.

Pollen grain morphology is very important for the identification of plants. According to Arora and Modi (2008), the Palynological evidence provides valuable information and conformation about genera and species that are closely related. Scanning electron microscope significantly improved the pollen surface (Ridgway and Skyvarla, 1969). Both light microscopy (LM) and scanning electron microscopy (SEM) are distinct interest for researchers to observe different taxonomic features. (Qureshi et al.,2019; Khan et al., 2017). Foliar anatomical characters are very important for correct identification. Specific kind of trichomes can be used frequently to delineate genera, species and family (Khan et al., 2013). Type of trichome is one of the main characters used in identification. The taxonomic value of the trichomes and their inference in systematic is well known (Cantino, 1990; Khokhar, 2009).

The main objective of the present research is to determine the medicinal uses of the two important weeds along with pollen grains morphology through scanning electron microscope (SEM) and foliar anatomical characters (Light microscopy) of the weed plants for correct systematic identification.

2 Materials and Methods

2.1 Plant collection and identification

Both the weeds of genus Convolvulus i.e *Convolvulus arvensis* and *Convolvulus prostratus* were collected during the period of March 2016 - May 2017. Five to six specimens were collected from a different locality. For correct taxonomic identification, the International Plant Name Index (IPNI) was used. Plant specimens were submitted in the Herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad, Pakistan (Table 1).

2.2 Foliar epidermal anatomy

Subrahmanyam technique was used for light microscopy, small pieces of the leaf are boiled in

Table 1 - Morphological and ethnobotanical uses and distribution of weeds

Taxonomic name/voucher specimen no	Common name	English name	Flowering	Morphology	Distribution in world	Ethnobotanical uses
Convolvulus arvensis L./ SA-400	Prewatay, Lily, Hiranpadi	Bindweed	Through out the year	Climbing and prostrate annual herbs, Stems 42-100 cm long, glabrous. Leaves glabrous to pubescent, 23-47 x 3-30 mm, ovate to broadly oblong or linear-oblong, sagittate to hastate, acute to obtuse, petiole 8-20 mm long. Flowers campanulate, bracteate, axillary, pale pink to white with dark purple bands, pedicellate, pedicel 8-16 mm long, bracteoles linear, 3 mm long. Sepals scarious, 4-5 mm long, broadly oblong, obtuse, retuse or apiculate, sparsely pubescent to glabrous. Corolla 18 mm long. Filaments unequal, 5-8 mm long, anthers 2 mm long. Ovary glabrous, on a disc. Stigma 4 mm long, filiform. Capsule globose, 5 mm in diameter. Seeds 4, 4 mm long, dark brown, tuberculate	Afghanistan, Armenia, China, India, Pakistan, Iran, Iraq, Israel, throughout the tropical and temperate region of the world except for Australia.	It is useful for piles and skin wound. Roots are Purgative. It is used as fodder and is a weed of wheat crop.
Convolvulus prostratus Forssk/SA-89	Sireen, Dodak.	Soft bindweed	Jan-April	Perennial herb, 10-25 cm high, woody base, branching basally, branches ascending or decumbent, rufous-fulvous tomentose, varying in density, patent or/and appressed. Leaves sessile, 15-30 x 3-7 mm, linear to oblong, oblanceolate to lanceolate, subacute to obtuse, villous, and mid-vein prominent on the lower surface, leaf sometimes slightly mucronate. Flowers 1-3, sessile to subsessile in axillary heads, peduncles up to 25 mm long, decreasing in length upwards, finally becoming subsessile. Bracts linear to oblanceolate, acute. Bracteoles filiform. Sepals 4-8 mm long, ovate-lanceolate, acuminate, villous, proximally convex and colorless, distally green, the outer sepals longer, the inner short. Corolla 10-12 mm long. Style slender, glabrous, stigma 2-4 times the length of the style, filiform. Capsule glabrous, somewhat scarious, bilocular, subglobose, 3 mm in diameter. Seeds 2-5, brown-black, 2 mm long, sparsely to densely white pubescent	India, Pakistan, and Egypt.	The leaves are the major constituent of a herbal drug Shankha Pushpi. The drug is used as antiepileptic. It is used alone or is administered along with modern antiepileptic drugs.

4 mL of nitric acid and 0.2 g of potassium chloride in a test tube (Subrahmanyam, 1996). The epidermis was separated in the form of the thin pellicle. Both the abaxial and abaxial epidermal surface was separated and washed with ethanol so that extra stain was removed easily. Putting 1 to 2 drops of lactic acid on the epidermal strip covered with a coverslip. By using light microscope both the qualitative and quantitative characters were observed, and photographs were taken with the help of Nikon (FX-35) camera fitted on the Leica Light microscope (Model: DM 1000).

2.3 Pollen morphology

Pollen grains were prepared by using the technique of Erdtman (1953) for scanning electron microscopy (SEM). Pollen grains that are acetolyzed were suspended in 90% ethanol, mounted on stubs. Specimens were sputter-coated with gold and with the help of a scanning electron microscope (Model JEOL JSM-5910), photographs were taken (Figures 1 and 2).

3 Results and Discussion

Foliar epidermal morphological characters were observed with the help of a light microscope. Light microscopy plays a key role in the investigation of foliar epidermal characteristics. Previous records also show the importance in the delineation of other groups of plants taxa (Ullah et al., 2011; Munir et al., 2011; Riaz et al., 2010). A significant variation was observed in both the qualitative and quantitative characteristics. A taxonomic key was prepared for both weed plants i.e *Convolvulus arvensis* and *Convolvulus prostratus.* Different researchers also used these characters for the preparation of taxonomic key (Khan et al., 2017b).

Variation was observed in stomatal number, length width and stomatal index in both adaxial and abaxial surface. In *Convolvulus arvensis* the epidermal cell was found to be Polygonal on adaxial surface and both polygonal and irregular on abaxial surface*.* Mostly paracytic type of stomata is present which shows similar to the previous work of (Tayade and Patil, 2011). Paracytic type of stomata was found in both the weed species. Shah. (1967) also reported three type of stomata i.e Paracytic, Anisocytic and Anomocytic during stomatal development in *Convolvulus arvensis.* The shape of stomata in

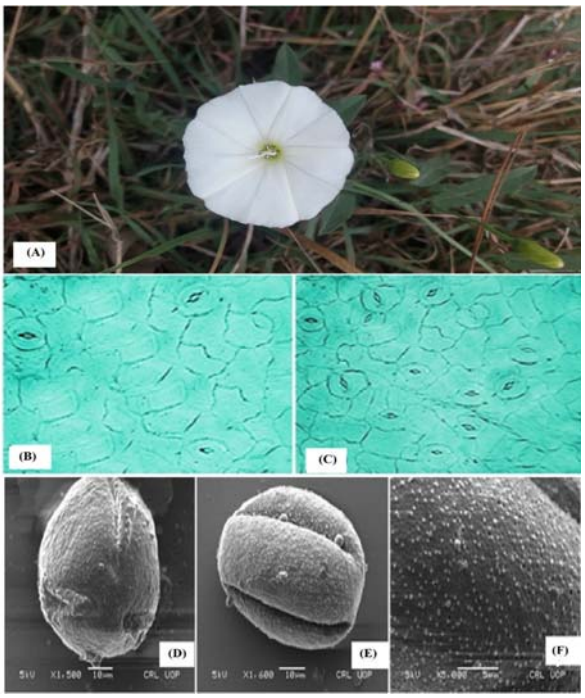

Figure 1 - Plants species of genus Convolvulus (A) *Convolvulus arvensis*. (B) Adaxial surface showing paracytic stomata (C) Abaxial surface (D) *Convolvulus arvensis* (Polar view), (E) *Convolvulus arvensis* (equatorial view), (F) *Convolvulus arvensis* (exine sculpture).

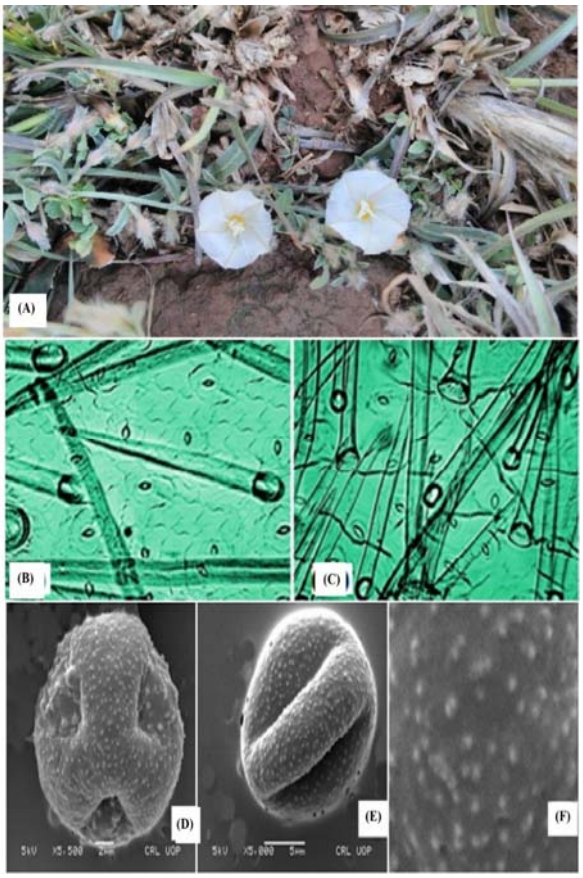

Figure 2 - Plants species of genus Convolvulus (A) *Convolvulus prostratus*. (B) Adaxial surface showing Wavy and irregular epidermal cells and paracytic stomata (C) Abaxial surface with Unicellular non-glandular and un branched with pointed tips trichomes. (D) *Convolvulus prostratus* (polar view), (E) *Convolvulus prostratus* (equatorial view), (F) *Convolvulus prostratus* (exine sculpture).

Convolvulus arvensis is elliptical and oval to oval oblong in case of *Convolvulus prostratus* (Table 2). Glandular sub-sessile capitate trichomes are present on *Convolvulus arvensis* at the adaxial surface while trichomes are completely absent on abaxial surface showed similar features to glandular trichome of species of Lippia (Combrinck et al., 2007). Non-glandular unicellular, unbranched, cylindrical shape trichomes are present on the adaxial surface of *Convolvulus prostrates.* Glandular trichomes are the main site for the synthesis of plants natural products and buildup for the protection against insects (Aziz et al., 2005). Stomatal cell length is (33.25±0.63µm) in *Convolvulus arvensis* on adaxial surface and (29.75±0.72 *µ*m) in the abaxial surface. Stomatal index at the adaxial surface is 24.118 and in *Convolvulus prostratus* is 55.22 at the abaxial surface. Variation was also observed in epidermal cell size, shape, length and width. Variation was found in epidermal cell length width size and shape (Tables 3, 4 and 5).

Palynological characters of both the weeds were randomly measured. Both the quantitative and qualitative characteristics were shown in Tables 6 and 7. In the examined weed species Tricolporate pollen type was observed. The shape of pollen grains is Prolate, Perforate in both the species. Skvarla et al. (1976) and Wodehouse (1930) were also reported similar results. Perforate is the basis sculpturing in genus Convolvulus that is a primitive characteristic but the echinate sculpturing is one of the advance features observed in genus Ipomoea. According to Telleria and Daners (2003), a highly diverse pollen morphology of family Convolvulaceae having a unique taxonomic importance. A wide range of variation in size and sculpture was noted. P/E ratio is 1.37 in *Convolvulus prostrates.* Exine sculpturing is

Table 2 - Qualitative analysis of adaxial and abaxial surfaces

Name of species	Adaxial/abaxial surface	Shape of epidermal cell	Type of stomata	Shape of stomata	Non-glandular trichomes	Glandular trichomes
C. arvensis	Adaxial	Polygonal	Paracytic	Elliptical	-	Sub-sessile capitate
	Abaxial	Polygonal and irregular	Paracytic	Elliptical	-	-
C. prostratus	Adaxial	Irregular	Paracytic	Oval	Unicellular, unbranched, cylindrical shape having pointed tips and bulbous base	-
	Abaxial	Irregular	Paracytic	Oval-oblong	Unicellular, unbranched, rarely cylindrical shape	-

Table 3 - Quantitative analysis of epidermal cells and trichomes

Name of species	Surface	Epidermal cells min (mean ± S.E) max		Trichomes min (mean ± S.E) max		Trichome index (TI)
		Length (µm)	Width (µm)	Length (µm)	Width (µm)	
C. arvensis	Adaxial	50(52.75±1.07)56.25	38.75(40.25±0.72)42.50	-	-	-
	Abaxial	51.25(53.50±0.72)55	23.75(26.00±0.72)27.50	-	-	-
C. prostratus	Adaxial	85(88±1.01)91.25	35(39.25±1.22)42.50	262.50(2.77±6.12)300	15(16±0.612)17.50	25.64
	Abaxial	85(91.75±3.20)101.25	36.25(37.75±0.612)40	275(2.97±13.91)350	15(17.75±0.82)20	42.30

Table 4 - Quantitative analysis of stomata and stomata pore

Name of species	Adaxial/abaxial surfaces	No. of stomata (Avg)	Stomata length and width (µm) min (mean ± S.E) max		Stomatal pore length and width (µm) min (mean ± S.E) max	
			Length	Width	Length	Width
C. arvensis	Adaxial	15.0	31.25(33.25±0.63)35	21.25(22±0.30)22.50	12.50(12.85±0.18)13.50	2.50(2.75±0.11)3
	Abaxial	23.8	27.50(29.75±0.72)31.25	17.50(20.75±0.84)22.50	15(15.95±0.46)17.50	5(5.75±0.56)7.50
C. prostratus	Adaxial	7.4	22.50(25±0.79)27.50	11.25(12.75±0.61)15	10(11.50±0.61)12.50	5(5.50±0.50)7.50
	Abaxial	22.2	25(27.25±1.00)30	13.75(14.80±0.50)16.50	12.50(13.25±0.50)15	5(6±0.46)7.50

Table 5 - Quantitative analysis of guard cell, subsidiary cell, and stomatal index

Name of species	Adaxial/ abaxial surfaces	Guard cells length and width (µm) min (mean ± S.E) max		Subsidiary cell length and width (µm) min (mean ± S.E) max		Stomatal index
		L	W	L	W	S.I
C. arvensis	Adaxial	26.25(28±0.63)30	5.50(7.15±0.41)7.75	42.50(44±0.46)45	13.75(15±0.34)15	24.9
	Abaxial	22.50(23.75±0.55)25	5(6.40±0.49)7.50	47.50(49±0.46)50	12.50(14.50±0.63)16.25	34.5
C. prostratus	Adaxial	25(25.50±0.50)27.50	7.50(8±0.50)10	110(1.11±0.50)112.50	38.75(40.50±0.63)42.50	24.18
	Abaxial	25(26.50±0.72)28.75	8.75(10.45±0.62)12.50	110(1.13±1.08)116.25	42.50(43.50±0.46)45	55.22

Table 6 - Quantitative data of pollen morphology of weed species

Name of species	P/E ratio	Polar axis	Equatorial diameter	No. of colpi	Colpi		Exine thickness
					Length (µm)	Width (µm)	
C. arvensis	1.33	45(47.2±1.0)50	50(52±0.93)55	3	2.5(3.25±0.3)3.75	3.75(4.5±0.3)5	2.5-3.75
C. prostratus	1.37	45(48.7±1.11)51	47(52.5±1.53)56	3	2.5(4±0.4)5	7.5(8±0.30)8.75	1.25-3.75

Table 7 - Qualitative features of pollen morphology of weed species

Name of species	Size of the pollen	Type of pollen	Shape of the pollen	Exine	Description of pollen
C. arvensis	Medium	Tricolporate	Prolate	Perforate	Colpi are long and narrow while colpus membrane perforated
C. prostratus	Medium	Tricolporate	Prolate	Perforate	Colpi are long and broad, prominent pits are present on colpus membrane

perforate and scabrate in *Convolvulus arvensis*. the total exine thickness is (2.5-3.7 mm), whereas the exine sculpturing is scabrate and psilate in *Convolvulus prostatus*. *Convolvulus arvensis* show 96% pollen fertility, while *convolvulus prostrates* show 90% fertility percentage. The highest rate of fertility illustrations of a good adaptation of the weed species in the area. According to present research, pollen type is tricolporate. According to Doyle (1969) and Muller (1969), the tricolporate pollen type is the basis and primitive. Palynological characteristics are very useful. Some previous studies also show the use of scanning electron microscope for the identification of different groups of plants (Ahmad et al., 2018; Ahmed et al., 2019; Ashfaq et al., 2018; Ashfaq et al., 2019b; Amina et al., 2020; Bahadur et al., 2018b; Bahadur et al., 2019a; Bahadur et al., 2019b; Gul et al., 2019a; Gul et al., 2019b; Gul et al., 2019c; Sufyan et al., 2018; Bano et al., 2019; Qureshi et al., 2019; Arshad et al., 2019; Rashid et al., 2018).

Morphology of pollen is a very important taxonomic tool in the identification of higher plants. The present findings also encourage taxonomists to use the Light microscope (LM) and Scanning electron microscope (SEM) for the identification and complete phylogeny of medicinally important weeds.

3.1 Pollen key based on Pollen micromorphology

1. Prolate, tricolporate, Medium size pollen grain with narrow colpi

2. Sparsely perforated Colpus membrane …. *Convolvulus arvensis*

1'. Prolate, Medium size, tricolporate with broad colpus

2'. Prominent pits on Colpus membrane …. *Convolvulus prostatus*

3.2 Taxonomic key based foliar epidermal characters

1. + Epidermal cells polygonal and irregular …. *Convolvulus arvensis*

   - Epidermal cells irregular, U-shaped rectangular, V-shaped elongated …. 2

2. + Epidermal cells irregular, simply undulate to little sinuous anticlinal wall…. *Convolvulus prostratus*

   - Epidermal cells rarely polygonal, sinuate anticlinal wall….3

4 Conclusion

The study plays a very important role in the correct identification and differentiation of two weeds of genus convolvulus. Palyno-morphological and foliar epidermal studies of weeds were determined. Both unicellular and multicellular (glandular and Non-glandular) are found in these weeds species. Capitate trichomes have been reported to secrete a small number of essential oil and flavonoids. Pollen micromorphological features play a vital tool in the systematics of these weed plant, afterward conservation of these weed species in the area.

5 Contributions

All authors contributed to the research work. SA, and MN: performed experimental work. ANK, and SS: did sample collection and paper write up. MA, and MZ: supervised the work.

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