**INTRODUCTION**

The genus *Tetrapogon* Desf. belongs to subfamily Chloridoideae. The species in this genus are distributed across Africa, around the Mediterranean sea, and through the Middle East, as far east as India [1]. According to *Flora of Pakistan*, this genus is represented by two species: *T. tenellus* (Koen. ex Roxb.) Chiov. and *T. villosus* Desf.

The genus *Parapholis* C.E.Hubb. belongs to subfamily Pooideae (Poaceae) and is represented by one species in Pakistan, *P. incurva* (L.) C.E. Hubb. [1]. In the present study, two new species are recorded for Pakistan: *T. cenchriiformis* (A.Rich.) Clayton and *P. strigosa* (Dum.) C.E.Hubb collected in the salt range of Pakistan.

**MATERIALS AND METHODS**

Specimens of *T. cenchriiformis* and *P. strigosa* were collected in Kala Bagh and Kallar Kahar of the Salt Range of Pakistan, respectively. Vouchers were deposited in the herbarium of Pakistan (QAU Islamabad). Morphological, palynological and anatomical studies were carried out and identification of species was confirmed by *Flora of Saudi Arabia*, a guide to grasses by Hubbard and *Tropical grasses* by Skerman and Riveros [2-4].

Detailed morphological study was carried out under dissecting binocular. Different morphological (vegetative and floral) characters were observed and confirmed by *Flora of Pakistan* [5]. Light microscopy (LM) and scanning electron microscopy (SEM) was used to study pollen morphology. For light microscopy florets were dissected and anthers were placed on the slide with the help of forceps, added a drop of 45% acetic acid and crushed with iron rod. Pollen were acetolysed according to modified method of Ahmad et al. who followed Erdtman. For scanning electron microscopy (SEM) anthers were crushed in 45% acetic acid and one to two drops of material containing acetolysed pollen were mounted on metallic stubs with a fine pipette, and coated with gold in vacuum coater and examined with, a Jeol microscope (JSM 1200) [5,6]. For leaf epidermal anatomy leaf samples were prepared according to the modified method of Cotton who followed Clark technique [7,8]. The fresh or dried leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath for about 50-60 minutes.

The abaxial and adaxial epidermis was removed, along with the mesophyll cells by using scalpel blade, until only the abaxial epidermis of the leaf remained on the tile. The epidermis was placed on the slide and mounted in clean 88% lactic acid. The micro photographs of the mounted materials were taken by using a camera mounted on Leica light microscope.

**ABSTRACT**

*Tetrapogon cenchriiformis* and *Parapholis strigosa* (Poaceae) are reported for the first time in Pakistan. *Tetrapogon cenchriiformis* is characterized by spatheolate inflorescence, oval elliptic seeds and large stomatal complex, while *Parapholis strigosa* has long anthers and straight spikes. Anthers are larger in *P. strigosa* than in *Parapholis incurva* (L.) C.E. Hubb. These two new reported species showed marked difference from the other species of Parapholis and Tetrapogon treated in *flora of Pakistan*.
RESULTS

Tetrapogon cenchtriformis (A.Rich.) Clayton

An annual or short lived perennial, tufted grass, up to 40 cm long. Leaf blades 5-16 cm long, 1.9-2.0 mm wide, narrow pointed, flat or folded; Ligule a lacerate membranous, 0.5 mm long; Sheath whitish at the margins, sheath of basal leaves strongly keeled (flabellate) shiny, glabrous; Inflorescence spatheolate, (Figure 1D) two spikes merged together, and appeared as one spike, rachis persistent, spikes 4-7.5 cm long, villous, spikelet 3 mm long having 5 awns, in the two rows on the rachis, spikelets having 5 florets, 2 fertile and 3 sterile; Glumes persistent, upper glume 3.6 mm long, 0.9 mm wide, 1 nerved, hyaline, blunt (obtuse) with a short awn point, about 0.3-0.4 mm long, lower glume 2.8-2.9 mm long, 0.5-0.6 mm wide, 1 nerved, lanceolate, hyaline; The lemmas of the lateral or lower florets, 2.5-3.0 mm long, with subapical awn, 11-11.5 mm long, dense hairy on the back, coriaceous, 3 nerved, nerves greenish, obtuse, blunt at the tip; Palea 2.2-2.6 mm long, little shorter than or almost equal to lemma, soft hairy at margins; The lemmas of the upper or middle florets, 1-1.6 mm long, clavate when unopened, 3 nerved, a few cilia on the back of 3rd floret, the top floret in the mid, short and their lemmas glabrous at the back; The lower or lateral florets fertile; Anthers sagitate, 0.5 mm long, pale whitish, filament 0.5-0.6 mm long; Caryopsis 1.3 mm long, 0.6 mm wide, light brown, oval elliptic.

Distribution in salt range and Pakistan: Khewra, Kalar Kahar, Kala Bagh (not recorded from other localities of Pakistan).

Distribution in world: Tropical Africa, east wards to India and south wards to Rhodesia and Angola [4].

Occurrence and habitat: Rare and only present on mountains, sandy clay soil.

Flowering: March-September

Palynology (LM and SEM)

Pollen were circular in polar view and prolate spheroidal in equatorial view. Polar diameter is 24.37 μm (20-30 μm) and equatorial diameter is 23.5 μm (20-27.5 μm). P/E ratio is 1.03. Pollen were ectoporate and monoporate. Pore diameter were 2.7 μm (2.5-3.5 μm) and exine thickness, 0.95 μm (0.75-1 μm). Pollen fertility was 89.18%. Sculpturing was verrucate and verrucae were narrowly spaced (Figure 1F).

Leaf Epidermal Anatomy

Abaxial intercostal zone: Abaxial intercostal long cells with irregular sinuous walls and 31.5-55 μm long and 10-12.5 μm wide. Number of rows of long cells between two costal zones is 4-8. Number of stomatal rows between two costal zones is 2. Stomatal complex is 20-27.5 μm long and 20-22.5 μm wide, guard cells were dumb bell shaped, 5 μm wide, subsidiary cells high dome shaped and 6.25 μm wide. Microhairs, macrohairs and hooks were not seen.

Costal zone: Silica bodies saddle shaped and dumbbell shaped. Saddle shaped silica bodies, 9-10 μm wide horizontally and vertical diameter 10-11.25 μm wide. Dumb bell shaped silica bodies, 15-17.5 μm long and 7.5-8.75 μm wide. Long cells with sinuous walls, present over the veins with silica bodies, 36.25-37.5 μm long and 8.75-9.37 μm wide. Angular prickles present at the margins, 32-35 μm long and 8-10 μm wide (Figure 1E).

Parapholis strigosa (Dumort.) C.E.Hubb.

Annuals, up to 76 cm long, rooting at the lower nodes; Leaf blades, 6.5-13.0 cm long, 3 mm wide, glabrous on the surface and scabrid on the margins. Ligule a short ciliated rim, 0.2-0.3 mm long. Sheath whitish, glaucous and glabrous, hyaline at the margins. Inflorescence 2-5.5 cm long, spikelets arranged alternatively on the axis; (Figure 1A). Spikelets sessile with one floret, not fully exerted from the subtending sheath. Glumes almost equal, both glumes laterally keeled, broadly lanceolate. Upper glume 4.5 mm long, 1 mm wide and obtuse at the tip, upper glume hardened, coriaceous, 7-nerved. Lower glume closely adhering to axis, thin and pointed, and almost equal to upper glume. Both lemma and palea thin membranous; Palea equal in length to lemma; Florets bisexual; Stigma 2, 1.0 mm long; Anthers 3, 1.5-1.8 mm long.

Distribution in salt range and Pakistan: Kallar Kahar (Peshawar Distt, Rawalpindi Distt, Quetta Distt, Makran Distt).

Distribution in world: Middle east and the Mediterranean region, coast of west Europe, introduced in North and South America and Australia [4].

Occurrence and habitat: Rare in fields, near Kallar Kahar garden (not recorded from other areas), wet clay soil.

Flowering: June-August

Palynology (LM and SEM)

Pollen were circular in polar view and sub prolate in equatorial view. Polar diameter is 30.55 μm (22.5-45 μm) and equatorial diameter 25.60 μm (20-30 μm). P/E ratio is 1.19. Pollen are monoporate and ectoporate. Pore diameter was 2.87 μm (2.5-4.0 μm) and exine thickness is 1.15 μm (1-1.5 μm) and pollen fertility was 93.66%. Sculpturing was scabrate (Figure 1C).
Leaf Epidermal Anatomy

**Abaxial intercostal zone:** Abaxial intercostal long cells with thin sinuous walls, 140-225.5 μm long and 16.5-22.25 μm wide. Number of rows of long cells between two costal zones, 5-10. Number of stomatal rows between two costal zones was 1-3. Stomatal complex, 35-40.5 μm long and 16-18.25 μm wide, subsidiary cells low dome shaped. Microhairs and macrohairs were not observed.

**Coastal zone:** Silica bodies mostly rounded to elliptical, 6-8.5 μm long. Short cells frequently present between the silica bodies, 8-12.5 μm long and 3-5 μm wide. Angular prickles at the leaf margins, 40-52 μm long and 5-11 μm wide. Adaxial surface similar to the abaxial surface (Figure 1B).

**Figure 1 (A-C).** *Parapholis strigosa*, (A) inflorescence,(B) Leaf abaxial epidermis (C) Pollen sculpturing (D-F) *Tetrapogon cenchriformis*, (D) spatheolate inflorescence(E) Leaf abaxial epidermis(F) Pollen sculpturing.

**DISCUSSION**

*Tetrapogon cenchriformis* has spatheolate inflorescence (Figure 1D). That makes it different from *T. villosus*. This species is not mentioned in Flora of Pakistan and it is the new report from the area. Anthers were sagitate and 0.4-0.5 mm long and this species also differs in the shape of caryopsis as it is trigonous and light brown in *T. villosus*, while oval elliptic in *T. cenchriformis*. However no difference was observed in the size of caryopsis that was almost same in both species.

Pollen in both species of *Tetrapogon* differed in equatorial view, as oblate spheroidal in *T. villosus* and prolate spheroidal in *T. cenchriformis*. Pollen was large in polar view (24.16 m) in *T. cenchriformis* but smaller in equatorial view (23.5 m) than *T. villosus*. Other quantitative characters such as P/E ratio, pore diameter and exine thickness were recorded more in *T. cenchriformis*.

The stomatal complex was large in *Tetrapogon cenchriformis*, 20-27.5 μm long and 20-22.5 μm wide while in *T. villosus*, stomatal complex was 17.5-18.75 μm long and 15 μm-17.5 μm wide. In both species of Tetrapogon, macrohairs were present on the adaxial side only but in *T. cenchriformis*, macrohairs were deeply sunken into the mesophyll (Figure 1E).

The morphological studies of *Parapholis strigosa* showed that spikelets were arranged alternatively on the axis, spikelets were sessile having one floret. One side of the spikelet was completely embedded in the cavity of the axis and is the distinguishing character of this tribe. Glumes were laterally keeled and broad lanceolate. Lower glume was thin and pointed and closely adhering to axis, while upper glume was hardened, coriaceous and 7 nerved (Figure 1A).

*Parapholis strigosa* is very similar to *P. incurva*, but according to Hubbard, *P. incurva* is usually smaller (2.0-20 cm long), and has more rigid shorter curved spikes. Another character which is used to differentiate these species is the length of anthers. In *P. incurva* anthers were very small, ranging in length from 0.5-1 mm, while in *P. strigosa* they ranged in length from 1.5-4.0 mm.

These characters helped in the identification of species. *Parapholis strigosa* was collected from moist and shady places of Kallar Kahar garden, in the salt range of Pakistan. Its height was recorded up to 76 cm while in *P. incurva* maximum height was 25 cm and anthers in this species were 1.5-1.8 mm long while in *P. incurva*, anthers were very small, 0.5-1 mm long. So the plant height, straight spikes and length of anthers are the characters which justify it as *P. strigosa*.

**REFERENCES**