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BIOCHEMICAL CHARACTERIZATION AND ALLERGENIC POTENTIAL OF *CENCHRUS PENNISETIFORMIS* HOCHST. & STEUD. EX STEUD. POLLEN GRAINS

SHAISTA ZEB, ANJUM PERVEEN* AND MUNEEBA KHAN

Centre for Plant Conservation, University of Karach, Karachi-75270, Pakistan
**Correspondence author's email: anjum_tahir@hotmail.com*

Abstract

Pollen of grasses have been recognized as major aeroallergens. It is reported that grass pollen may trigger respiratory allergies particularly allergic rhinitis and asthma in approximately 40% of allergy patients worldwide. In most of the aerobiological surveys, Grasses pollen were observed in highest concentration in various parts of the world. The present study was designed to analyze the biochemical properties of *Cenchrus pennisetiformis* Hochst. & Steud. ex Steud. pollen grains. The pollen were subjected to quantitative estimation of proteins, carbohydrates, amino acids, phenols and reducing sugars. Protein qualitative analysis was also done by SDS-PAGE in order to identify the allergenic properties of *Cenchrus pennisetiformis* pollen. The study results showed that *Cenchrus pennisetiformis* pollen contained 19.66mg/g proteins, 71.29mg/g carbohydrates, amino acid 12.23 mg/g, phenols 1.47 mg/g and reducing sugars 8.29 mg/g. SDS-PAGE analysis showed 13 protein bands with a range of molecular weight 28-110kDa. The pollen grains of this grass contained low molecular weight protein bands which might cause allergic rhinitis or elicit other allergy symptoms on the exposure to this grass. Thus *Cenchrus pennisetiformis* can be characterized as pollen allergy causing grass.

Key words: Pollen allergy, Grass pollen, Allergic rhinitis; Pollen biochemical characterization.

Introduction

Plant pollen grains are one of the most common cause of seasonal allergic rhinitis worldwide. During flowering season pollen grains are discharged in the atmosphere by anthers. As a result of inhaling pollen aeroallergens released from weeds, grasses and trees people suffer from hypersensitivity, allergic rhinitis, and asthma. Pollen allergens are generally proteins and glycoproteins and these have the ability to evoke an IgE antibody-mediated allergic reaction within no time. . These allergenic particles are usually present in pollen walls and cytoplasm. Various studies reported that the allergenic proteins with low molecular weights ranging from 10–70 kDa. In grass pollen it is also observed that the allergenic pollens may remain suspended in the atmosphere for a long period or travel to distant areas (Behrendt *et al*., 1997; Behrendt & Becker, 2001; Taylor *et al*., 2007).

Grasses (Poacae) are found in almost every type of habitat. Poaceae flowers are wind pollinated and thus the pollen are produced in huge amounts. Throughout the world study aerobiological studies reported grasses pollen as the most dominant airborne pollen type (Kazmi *et al*., 1984; Chakraborty *et al*., 1998; Garcia-Mozo *et al.*, 2006; Alwadie, 2008; Perveen *et al*., 2014). Due to several reports on grass pollen allergy, airborne pollen concentration has been predicted on daily basis and pollen calendars are prepared throughout the world (Smith & Emberlin, 2005, 2006; Cassagne *et al*., 2007; Garcıia-Mozo *et al*., 2009; Voukantsis *et al*., 2010; Kizilpinar *et al*., 2011; Piotrowska, 2012; Tassan-Mazzocco *et al*., 2015).

The Poaceae family is one of the largest vascular plant families and comprising about 620 genera and 10,000 species throughout the world. According to Flora of Pakistan, Poaceae family represented 158 genera and 492 species (Cope, 1982). Plants belonging to this family are also of paramount importance as they provide major food crops including Wheat, Rice, Maize and Barley for human and provide fodder for animals as well as grasses cover 25-45% of the world's flora ecologically (Stanley *et al*., 1999; Damialis *et al*., 2011).

There are reports that grass pollen allergy affect 25 % people living in temperate zones on the world (Knox & Suphioglu, 1996; Mohapatra *et al*., 2005; Garcı´a-Mozo *et al*., 2006; Nitiu, 2006; Murray *et al*., 2007; Bilisik *et al*., 2008; Abreu *et al*., 2008; Kiotseridis *et al*., 2013; Nayak *et al*., 2013).

This study was designed to perform biochemical investigation of *Cenchrus pennisetiformis* Hochst. & Steud. ex Steud. pollen. In Pakistan, this grass is distributed in Sindh and Baluchistan and it is fairly common. It is 10-40 cm in height and the leaf is 2-20 cm long while leaf blade is 2-5 mm wide. Its panicle/floral head is 2-6 cm long. This grass has two flowering periods i.e. February-April and August-October. This grass is a valuable fodder as it remains green in hot weather conditions (Cope, 1982). This grass is common in our area and the pollen allergy has not been investigated yet.

Materials and Methods

Chemical Characterization of Pollen

Pollen collection: Polliniferous material of *Cenchrus pennisetiformis* was collected floral spike. Dried anthers were crushed to obtain pollen. Pollen grains purity was further confirmed by light microscopy.

Quantitative Estimation

Total Carbohydrate: 0.2gm of pollen grains was homogenized in ice chilled Tris-HCl Buffer then mixture was centrifuged. After centrifugation upper layer of solution was used to determine the amount of sugars and reducing sugars (Hassid & Abraham, 1957).

Phenols: 0.1 gm pollen grains sample were homogenized in ethanol. The mixture was then centrifuged at 1000 rpm for 15 minutes. After centrifugation total phenols were quantified (Chandini *et al*., 2008).

Amino acids: For estimation of total amino acids 0.5 g defatted pollen grains were crushed and homogenized in ethanol and centrifuged. Amino acid were quantified by the procedure of Sadasivam & Manickam (1992).

Protein: For extraction of proteins, pollen powder was homogenized in Phosphate Buffer Saline solution (PBS). The solution was centrifuged and proteins were quantified. Estimation of proteins was done by Bradford assay (Bradford, 1976).

Qualitative Estimation

SDS-PAGE analysis of Pollen Protein: Pollen **p**rotein were analyzed by SDS PAGE technique. Stacking and resolving gels were prepared according to the method described by Laemmli (1970). 20 µl of protein sample and 10 µl of standard protein ladder were used. The gel was run for 2 hours. After fixation Silver stain was used to stain resolving gel. After that washing of gel was done by acetic and ethanol solution.

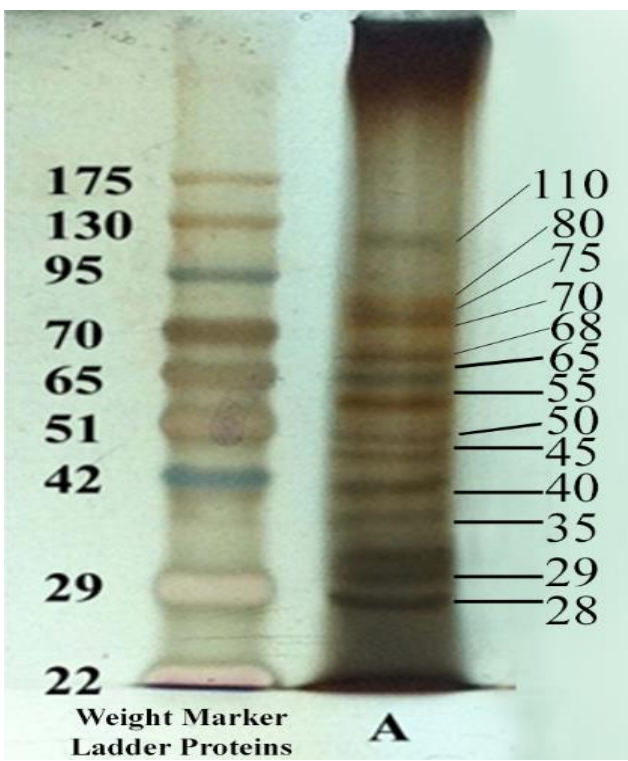

Fig. 1. Protein profiling of pollen grains by SDS-PAGE. A-*Cenchrus pennisetiformis*

Results

Biochemical analysis of *Cenchrus pennisetiformis* pollen was done in which pollen proteins, carbohydrates, amino acids etc. were analyzed.

Our results showed that pollen extract of *Cenchrus pennisetiformis* showed protein concertation i.e. 19.66 mg/g of pollen; a carbohydrate concentration 71.29 mg/g; amino acid concentration of 12.23 mg/g; 1.47 mg/g of phenols; and reducing sugar.8.29 mg/g of pollen

SDS-PAGE analysis: The protein extract of *Cenchrus pennisetiformis* pollen showed 13 protein bands with molecular weight ranging from 28-110kDa. The protein bands were of the molecular weight viz. 28, 29, 35, 40, 45, 50, 55, 65, 68, 70, 75, 80 and 110 kDa (Fig. 1).

Discussion

Pollen grains cytoplasm constitute proteins, sugars and amino acids etc. (Singh & Mathur, 2012). Pollen allergens may cause allergy after interaction with human bronchial system (Knox & Heslop-Harrison, 1970; Singh *et al*., 1991; Arnon & Van Regenmortel, 1992; Vrtala *et al*., 1993; Yli-Panula & Rantio-Lehtimaki, 1995). Amino acids play a vital role in different physiological functions of the plants. Pollen fertility is also affected by these amino acids (Kim *et al*., 1987; Jianjun *et al*., 1995; Rashed *et al*., 1995; Mondal *et al*., 1998). Amino acids are building blocks of proteins. Proteins and glycoproteins are major cause of bronchial allergies in susceptible individuals (Vik *et al*., 1987; Karmakar & Chatterjee, 1992; Mondal *et al*., 1998).

Pollen of grasses have been recognized as potent aeroallergens (Freidhoff *et al*., 1986, Celenk & Bicakci, 2005; Recio *et al*., 2006; Duffort *et al*., 2008; Hrabina, 2008; Mahram *et al*., 2013). The protein profiling of the pollen grains extracts through SDS-PAGE electrophoresis indicated that protein bands having molecular weight between 10-70kDa were responsible for triggering allergy in patients sensitive to pollen.

Results of SDS-PAGE investigation in of pollen grains of *C. pennisetiformis* showed low molecular weight proteins bands viz. 28, 29, 35, 40, 45, 50, 55, 65, 68, 70, 75, 80 and 110kDa in which 09 protein bands were 28, 29, 35, 40, 45, 50, 55, 65 and 68 less than 70kDa bands. Various grasses have been reported to cause airways allergies viz., *Cynodon dactylon*, *Poa* sp., *Dactylis glomeratus*, *Phleum* sp. etc. (Weber, 2003). *Cenchrus ciliaris* pollen were characterized as allergy causing agents as the protein extracts were tested against allergy patients sera and positive results were obtained (Sridhara *et al*., 2000). Ford *et al*., (1985) showed allergenic proteins ranging from 30-36 kDa from *Dactylis glomerata* (Orchard grass). In *Lolium* sp. Ford & Baldo, (1986) showed the presence of allergenic protein bands ranged 28–60 kDa. *Cannabis sativa* was reported as aeroallergen pollen producing plant (Tanaka *et al*., 1998). *Imperata cylindrica* pollen extract low molecular weight proteins were detected as allergenic (Kumar *et al*., 1998).

The present study showed low molecular weight protein bands in *Cenchrus pennisetiformis* pollen, which have been reported for the first time from this grass. Due to the presence of these low molecular weight protein bands, the *Cenchrus pennisetiformis* pollen could be considered as the causative agent of allergy and asthma in susceptible individuals.

Conclusion

In the present study low molecular weights allergenic proteins were identified by chemical characterizations of pollen grains of *Cenchrus pennisetiformis*. Grasses pollen allergy is frequently reported in aerobiological investigations. This *Cenchrus pennisetiformis* pollen are likely to cause of pollen allergy. Further confirmatory allergy testing is required to check its pollen allergenic potential.

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