IJNLS
INTERNATIONAL JOURNAL OF NATURE AND LIFE SCIENCES
Research article

International Journal of Nature and Life Sciences (IJNLS)
https://www.journalnatureandlifesci.com
e-ISSN: 2602-2397; ISSN: 2602-2478
Vol. 1(2), December 2017, pp. 83-88

Comparison of pollen protein patterns and pollen morphology of *Taxus baccata* L. and *Cupressus arizonica* Greene

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Received: 5 December 2017, Revised: 16 December 2017, Published Online: 30 December 2017

Abstract
Cupressaceae and Taxaceae pollen grains are similar due to their pollen morphological properties and they are both released abundant to the air. Cupressaceae pollen allergy is very common in Mediterranean countries include Turkey. *Taxus baccata* L. is the only species of *Taxus* L. to be naturally distributed as an adaptive fence plant for landscaping in Turkey. However it was not reported any allergic potency for *Taxus baccata* pollen. In this study it was aimed that to compare the pollen morphological and protein profile characteristics of *Cupressus arizonica* Greene. and *Taxus baccata* which release abundant pollen to air in our country. The pollen structure were examined by Light microscopy (LM). Pollen protein content was investigated by Bradford protein assay. SDS-PAGE analysis were carried out for determining protein profiles. Both of the species were found very similar in terms of pollen morphology however intine thickness was barely different. In addition, it was not observed any prominent protein that corresponding to Cup a 1 in *T. baccata*, that remind us this pollen could not be allergic as *C. arizonica* but, still other unidentified protein bands, whether they are allergic or not, should be demonstrated by further immunological assays.

Key words: *Cupressus arizonica*, pollen, protein, SDS PAGE, *Taxus baccata*.

1. Introduction

Taxus L. genus belongs to the Taxaceae family (Davis and Cullen, 1965) and is widely distributed in Europe, North America, Eastern Asia and Asia Minor (Persico et al., 2011). It is represented by ten species throughout the world (Erdemoğlu et al., 1998). Among these *Taxus baccata* L. (yew tree) which is distributed throughout the temperate zones of Northern hemisphere is the only species of Taxus to be naturally distributed in Turkey. *T. baccata* is an evergreen conifer that is widely grown as a fence plant in parks and gardens due to its decorative appearance. In addition, *T. baccata* plants have been used for medicinal purposes about high blood pressure, cancer and bone fracture (Phondoni et al., 2010). Cupressaceae family is mostly distributed in Mediterranean Regions, in the southern states of the USA and in Japan. Members of Cupressaceae are notable potential to adapt to different climatic conditions and widely used in landscape in our country. Also, Cupressaceae members have been used to make spoon and the cones were used for cough treatment (Deniz et al., 2010; Polat et al., 2016). Cupressaceae pollen has commonly been reported to be an important aeroallergen and causal factor of spring, autumn and winter pollinosis in many countries. However, there has not been purified pollen allergen from *T. baccata* pollen so far. The level of allergenicity of the different species largely depends on the quantity of allergen or allergens that the pollen releases 5–10 minutes after coming into contact with the respiratory tract. But, there are Cupressaceae species with a poor allergenic capacity, but cases have been reported of cross

reactivity with some Pinaceae (Pettyjohn and Levetin, 1997) and Taxodiaceae species (Crosta et al., 1996).

It is well known from aeropalinological studies that *T. baccata* pollen showed morphological similarity with Cupressaceae pollen. The Cupressaceae family produces spheroidal, pseudoporate, anemophilous pollen grains that are small in size. Taxaceae and Taxodiaceae species, which are similar from a pollen morphology point of view, have also been included in the Cupressaceae pollen type in aerobiological studies (Acar et al., 2015; 2017a; Díaz de la Guardia et al., 2006; Nicoleta, 2009). Although pollen of Cupressaceae and Taxaceae are monitored almost whole year in studies done in Turkey, commercial skin prick test serums of these families have not been included in test panel of many allergy centers.

In this study it was aimed that to compare the pollen morphological and protein profile characteristics of *C. arizonica* and *T. baccata* which release abundant pollen to air.

2. Material and Methods

2.1. Collection of pollen samples and preparation for analysis

Pollen from *T. baccata* and *C. arizonica* were directly collected from mature male cones of the trees planted in Ankara University Tandoğan campus during the pollen season. The pollen samples were sieved through a sieve with 100 µm pore size. The sieved pollen samples stored at -20 °C until use. Purity of pollen samples were checked by a light microscope through 1000 particles prepared by Wodehouse Method (1935). The samples with a 98% purity threshold were used for the analysis.

2.2 Protein extraction and quantification

Firstly, the dry *C. arizonica* and *T. baccata* pollen treated with 0.01 M phosphate buffered saline (PBS) buffer (pH 8). 100 mg pollen of each taxon 1:10 (w/v) were mixed with PBS buffer. These samples were centrifuged at 14000 g for 30 minutes at 4 ºC and the supernatant was used. The amount of total protein was determined by Bradford method (1975).

2.2. SDS PAGE analysis

After extraction of pollen proteins, the crude extracts were separated by 4-10% SDS-polyacrylamide gel electrophoresis (Laemmli et al., 1970) and the gels were stained with Coomassie Brilliant Blue. Molecular weight of proteins were estimated using proteins of known Mw.

3. Results and Discussion

3.1. Pollen morphology

The pollen grains of *C. arizonica* were apolar and spheroidal with polar and equatorial axes of 25.18-32.5 µm. *Taxus baccata* pollen grains were apolar, spheroidal, small or rather small (20-28 µm). The pollen grains of the both species were inaperturate. They have a distinctive pseudopore and a 10 x 15 µm size. The exine was a weakened 0.75 µm thick. Intine was 3.70 µm thick on average in *C. arizonica* and the thickness varying by 3-5 µm in *T. baccata*. The percentages of intine thickness in relation to pollen grains diameter were calculated. The intine covered a greater percentage of 38.9% in *C. arizonica and* 31% in *T. baccata*. The exine was rugulate-granulate with microechinate orbicules appear tenuously attached to the surface in both species (Figure 1).

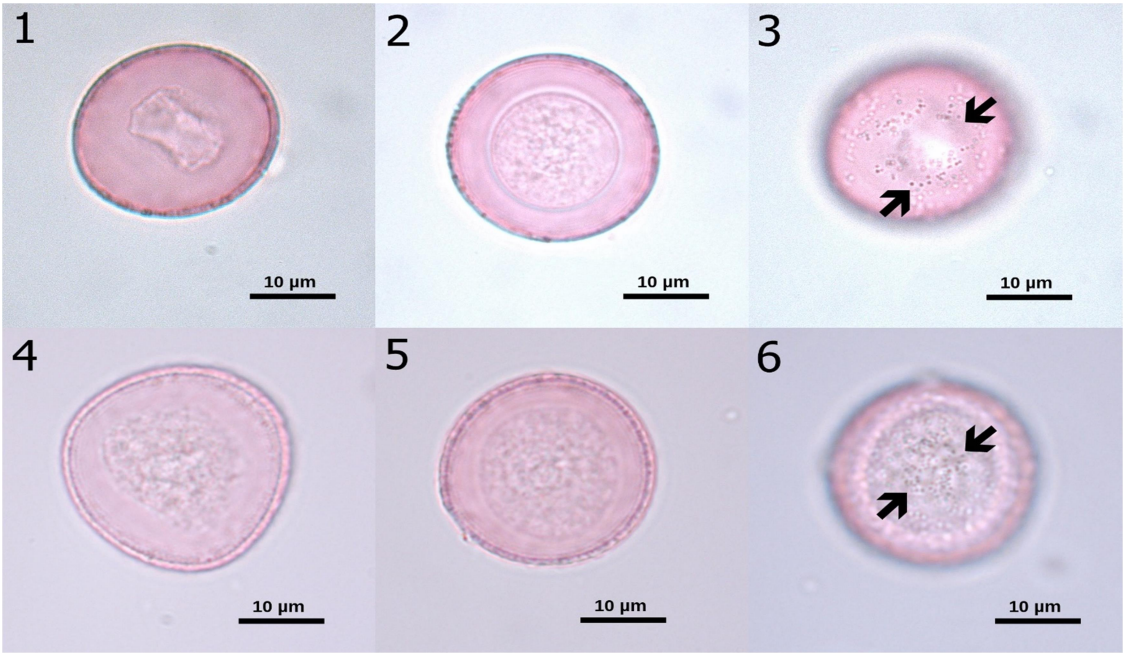

Figure 1. LM microphotographs of *C arizonica* (1-3) and *T. baccata* (4-6), orbicules are shown by arrows.

3.2. SDS Page analysis

It was observed 7 distinct protein bands from pollen of *C. arizonica*, whereas 10 different protein bands were obtained from *T. baccata* polen in the result of SDS-PAGE analysis (Figure 2). Molecular weight of these bands were given in Table 1.

Table 1. Estimated molecular weight (kDa) of protein bands from separation of *C. arizonica* and *T. baccata* pollen by SDS-PAGE.

C. arizonica (0.223mg/ml)	***T. baccata*** (0.448mg/ml)
106	95
95	87
87	76
81	69
51	57
40	50
37	42
	34
	31
	25

Bradford protein assay revealed that, total protein amount was found higher in *T. baccata* (0.448 mg/ml) than in *C. arizonica* (0.223 mg/ml) (Table 1). Although protein band observed in the range of 40 kDa which is possible corresponding to major allergen from *C. arizonica* (42 kDa), expression level of it was higher in *C. arizonica* than in *T. baccata*. Three of seven bands obtained from *C. arizonica* were similar in *T. baccata*. Pollen allergens are generally smaller than 50 kDa. While it was determined 5 protein bands which is below this threshold value in *T. baccata,* three protein bands were found in *C. arizonica*. It is known that carbohydrate content of Cupressaceae and Taxaceae is high. Thus smearing of band is very common in SDS-PAGE gels of these families. But according to our results, proportion of smearing was higher in *T. baccata* pollen protein profiles.

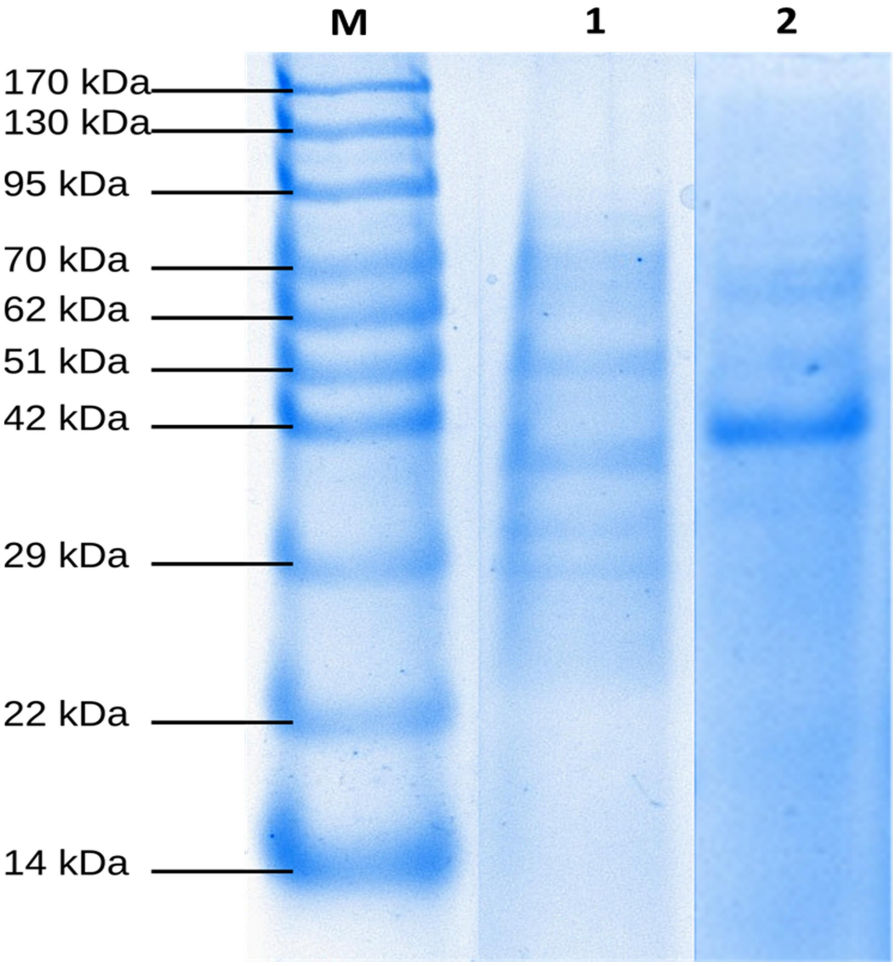

Figure 2. Comparison of protein profile of *T. baccata* (1) and *C. arizonica* (2) pollen, (M: marker).

Despite of levels of carbohydrate did not measured in this study, it could be easily said that carbohydrate content of *T. baccata* was higher than *C. arizonica* based on smear effect in the gel.

Analysis of protein profiles of *C. arizonica* and *T. baccata* pollen extract has been revealed that the Cup a 1 like protein (40 kDa) is expressed in *T. baccata*. On the other hand, molecular weight of Cup a 1 like band in *T. baccata* was lower than that of *C. arizonica*. This could be indicator of different allergenicity. But it is important point is that expression level of this protein is lower in *T. baccata*. Therefore, *T. baccata* could be ideal shrub for landscaping. However, the number of protein bands that lower than 50 kDa in *T. baccata* brings to mind that *T. baccata* could be express different allergen protein(s) that hasn't identified so far. So it is necessary to do a study on allergenicity of these bands using

sera of sensitive patients. Considering that possibility of discover new major allergen is low, anyway using *T. baccata* in landscaping will be wisely choice.

An epidemiological survey was conducted for allergy to Cupressaceae and Taxodiaceae pollen in Italy. The diagnostic panel consisted of seven Cupressaceae and Taxodiaceae genus (*Cupressus sempervirens, Cupressus arizonica, Cryptomeria japonica, Chamaecyparis obtusa, Thuja orientalis, Taxus baccata*, and *Juniperus oxycedrus)* While the more frequent allergens at prick tests are Cupressus sempervirens (90%) and Cupressus arizonica (88.9%), the frequency of Taxus baccata was found 27% (Ariano et al., 2002). Another study which supports lower allergenicity of *Taxus* has been done in Japan. They emphasised that *T. cuspidata* could be minor allergen that causes nasal allergy in early spring in Hokkaido (Maguchi and Fukuda, 2001).

The possible explanation of higher expression of Cup a 1 containing band could be different isoforms of Cup a 1. A few studies confirmed that Cup a 1 and Cup s 1 protein which show higher cross reactivity with Cup a 1 protein have different isoforms (Rea et al., 2004; Shahali et al., 2012; Acar et al., 2017b).

4. Conclusion

Selection of less-allergenic plant in landscaping is becoming important due to increasing in allergy prevalence. Many fence plants are known to be allergic such as privet. Therefore, determining of allergenic potency of *T. baccata* which is commonly used as fence plant in landscaping, it will help selection of suitable plants for landscaping. *C. arizonica* plants should be avoided using in landscape due to their pollen are so allergic.

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