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Research Article

Isolation and identification of allergens and biogenic amines of *Prosopis juliflora* genotypes

Abdulrahman A. Al-Soqeer [a], Qasi D. Alsubaie [a], Mohamed I. Motawei [a,*], Hassan M. Mousa [b], Ahmed M. Abdel-Salam [c]

[a] Plant Production and Protection Department, College of Agriculture & Veterinary Medicine, Qassim University, Saudi Arabia
[b] Food Science and Human Nutrition Department, College of Agriculture & Veterinary Medicine, Qassim University, Saudi Arabia
[c] Dairy Science Department, Food Science and Nutrition Division, National Research Centre, Dokki, Cairo, Egypt

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ABSTRACT

Background: Prosopis, or mesquite (*Prosopis juliflora* (Sw.) DC.), was introduced in Saudi Arabia several decades ago and is heavily used in street, roadside, and park plantations. It shows great adaptation to the prevailing climatic conditions such as high temperature, severe drought, and salinity and spreads naturally in many parts of the Kingdom. This research was conducted to isolate allergen proteins and biogenic amines from the pollen grains of *P. juliflora* genotypes in Saudi Arabia from two regions, namely Al-Qassim and Eastern regions.
Results: The results showed that 18 different allergen proteins were detected in *P. juliflora* genotypes, with molecular weight ranging from 14 to 97 kDa. Moreover, *P. juliflora* genotypes from the two studied regions contained eight biogenic amines, namely histamine, tyramine, tryptamine, β-phenylethylamine, butricine, codapherine, spermidine, and spermine. All genotypes from the Al-Qassim region were found to contain all eight amines, while in the Eastern region, histamine was absent in three genotypes, spermine was absent in six genotypes, and spermidine was absent in three genotypes. Genotypes B23, E20, and E21 had the lowest biogenic amine quantity.
Conclusions: All identified proteins from mesquite trees from both regions (Eastern and Al-Qassim) cause allergies in patients who are sensitive to pollen grains. Bioamines, except histamine and tyramine, were recorded at varying concentrations in different genotypes.

1. Introduction

The genus *Prosopis* (family *Fabaceae*) occurs worldwide in arid and semi-arid regions. It includes 44 species that are trees and shrubs and are found in the Near East, North and Central Africa, North and South America, and the Caribbean. *Prosopis* spp. vary widely in their productivity and their use and utilization by humans [1]. They constitute a very important natural resource for dry zones because of their multi-purpose nature, with the potential to provide a wide range of products, and their ability to grow on the poorest of soils where few other useful species can survive. *Prosopis juliflora* trees are harvested for pods, fuel or timber wood, and many other products, such as medicinal extracts or foliage for animal fodder. In addition, they stabilize the soil and prevent erosion, and through biological nitrogen fixation, they increase the fertility of soils. The usefulness of *P. juliflora* has long been recognized [2,3,4]. It is considered a valuable tree species of the desert ecosystem. Its multiple use possibilities have attracted growing interest in this species, especially in arid zones. *P. juliflora* trees have a tremendous potential for pod production. Vimal and Tyagi [5] have reported that the pods contain protein (16.5%), fat (4.2%), carbohydrate (57%), fiber (16.8%), ash (5.4%), calcium (0.33%), and phosphorus (0.44%). Because of the high carbohydrate content and good amount of protein, the spongy walls of ripe pods are highly nutritive and traditionally used in making meals for humans (pinole) and alcoholic products [6]. The husk of the pods is used for dying; they contain tannin (1.9%). Studies on palatability and the nutritive value of pods and their source as livestock feed and milk production, particularly goats, sheep, and camels, have been conducted in many regions worldwide. Moreover, *P. juliflora* is an important source of natural products with biological properties [7]. Evaluation of the in vitro antioxidant activity of ethanolic extracts of pollen of *P. juliflora* suggest that the pollen possesses high free radical scavenging activity that is related to its phenolic composition [8].

Pollen of *P. juliflora* is an important cause of respiratory allergy in various countries [9]. *P. juliflora* is a major cause of allergic diseases in Saudi Arabia and South Africa [10]. Assarehzadegan et al. [9] indicated

* Corresponding author.
E-mail address: rumotawei@hotmail.com (M.I. Motawei).
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that proteins with a molecular weight of 10 to 85 kDa are the major allergens in *P. juliflora* pollen extract. *P. juliflora* is a legume with several variations that have been used for the reclamation of desert lands and as a wood resource. It is easily dispersed and has far-traveling pollen, which is a significant source of allergens. In addition to pollen exposure, the burning of mesquite wood and its resulting smoke may be another source of exposure to some of these allergens [11]. Samples of *P. juliflora* collected from different areas may have incipient races due to geographical distances and morphological differences.

In Saudi Arabia, large saline desert areas and severely degraded land of limited use for traditional crops are present. Moreover, inadequate availability of good quality water is one of the major limitations in irrigated agriculture. Furthermore, there is a great concern regarding water resources and their use in agriculture in Saudi Arabia. Thus, *P. juliflora* may have potential for cultivation as it can grow and flourish with a very limited supply of water and tolerate soil and water salinity. However, for successful and profitable production of *P. juliflora* as a multipurpose tree and to consider it as a reliable natural resource in Saudi Arabia, it is very important to identify and quantify the allergen proteins and biogenic amines in different genotypes, which were collected from two regions in Saudi Arabia in this study.

2. Materials and Methods

2.1. Plant Materials

Fifty genotypes of the mesquite plant, *P. juliflora*, were used in this study. Because mesquite tree is cross-pollinated, each individual tree is considered a genotype. Twenty-five genotypes (trees) were collected randomly from each of Al-Qassim and Eastern regions, Saudi Arabia, to study the most important allergen proteins, such as the amines histamine and tyramine. The genotypes were labeled B1 to B25 for the Al-Qassim region and E1 to E25 for the Eastern region. Voucher specimens were collected from Buraidah and Dammam Municipalities, Saudi Arabia, and were registered (ENV#1064-09) at King Abdulaziz City for Science and Technology (KSA).

2.2. Separation of different protein fractions in P. juliflora

2.2.1. Pollen handling and storage

The inflorescences of *P. juliflora* plant were collected when almost mature during February–April, 2014, from each tree (genotype) in the two regions and stored immediately at -20°C. The flowers were transferred to trays over sheets of butter paper. As the pollen were shed (assisted by manual rubbing), they fell onto the paper and were allowed to remain there until they were air dries and then stored at -20°C.

2.2.2. Preparation of extracts

Plant materials were defatted using repeated changes of diethyl ether. The extracts were prepared in ammonium bicarbonate buffer (50 mM NH_4HCO_3, 1 mM phenylmethanesulfonyl fluoride and 2 mM ethylenediaminetetraacetic acid), pH 8.0, by continuous stirring for 4 h at 4°C. The supernatant was then separated by centrifugation at 10,000 g for 30 min, filtered through a 0.22-μm membrane under sterile conditions, and lyophilized. Protein concentration was determined by conventional proximate analysis techniques [12].

2.2.3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the protein ratio of *P. juliflora* pollen extract were performed according to the method described by Laemmli [13] and conventional proximate analysis techniques [12]. Pollen extract samples containing 100 μg of protein were separated and diluted with 3x SDS-PAGE gel-loading buffer (0.6 M Tris-HCl, 10% SDS, 20% glycerol, and 0.01% bromophenol blue and β-mercaptoethanol, pH 6.80) and denatured at 95°C for 5 min. The SDS-PAGE apparatus included glass plates, combs, spacer, casting device, gel chamber, and power supply unit (LKB-Pharmacia, Sweden). The formulation of SDS-PAGE resolving gel is shown in Table 1.

Table 1
Formulation of SDS-PAGE for preparing resolving gels.

Solution component (30 ml)	10%	Spacer
Water (ml)	11.9	6
1.5 M Tris-Cl, pH 8.80 (ml)	7.5	–
0.5 M Tris-Cl, pH 6.80 (ml)	–	2.5
30%Acrylamide solution (ml)	10	1.3
10% SDS (μl)	350	50
10% APS (μl)	200	50
TEMED (μl)	50	10
Total volume (ml)	30	10

Proteins were fixed in the gel by immersion for 1 h in 40% methanol (v/v) and 10% trichloroacetic acid (TCA) solution and stained with Coomassie Brilliant Blue R-250 (Fisher Biotechnology, Inc., Fair Lawn, NY). A 10% T resolving gel was used for separating pollen protein extract with molecular weight standards ranging from 10 to 250 kDa. Dual Color Precision Plus Protein Standards expressing calibration points of 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa (Bio-Rad) were included in the electrophoretic separation. Different protein bands were identified and compared.

2.3. Biogenic amine determination

Tryptamine, β-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, and spermine were extracted and their quantities determined according to Mietz and Karmas [14], Ayesh et al. [15], and Sultan and Marrez [16], with some modifications. The data recorded of each genotype were the means of three samples.

2.3.1. Reagents

a) Dansyl chloride solution: 500 mg of dansyl chloride (5-{Dimethylamino} naphtalene-1-sulfonyl chloride) dissolved in 100 ml acetone.
b) Standard solutions: Stock standard solutions of the tested amines: 25 mg of each standard dissolved in 25 ml distilled water individually.

2.3.2. Extraction

Twenty-five grams of different homogenized samples of *P. juliflora* pollen extract were blended with 125 ml of 5% TCA for 3 min using a Waring blender. Whatman No. 1 filter paper was used for filtration. Ten milliliters of the extracts were transferred into a culture tube containing 4 g NaCl and 1 ml of 50% NaOH. The tubes were shaken; extraction was performed three times using 5 ml n-butanol/chloroform (1:1 v/v) in stoppered tubes that were shaken vigorously

Table 2
Gradient program of HPLC.

Time (min)	Flow rate (ml/min)	Solvents		
		Methanol	Acetonitrile	0.02 M acetic acid
0	1	20	20	60
10	1	40	40	20
15	1	35	50	15
20	1	55	40	5
25	1	30	40	30
30	1	20	20	60
35	1	20	20	60

Table 3
Molecular weight (kDa) of allergen proteins *in Prosopis juliflora* genotypes from the Al-Qassim region.

Genotypes	No. of Proteins	Molecular weight of protein (kDa)								
B1	4	42	29	19	16					
B2	6	42	29	18	17	16	15			
B3	7	45	42	29	21	19	18	16		
B4	6	45	42	29	19	18	17			
B5	5	42	29	21	19	15				
B6	5	45	42	20	18	15				
B7	5	97	45	42	29	18				
B8	6	97	45	42	29	19	18			
B9	7	97	45	42	29	20	18	15		
B10	4	97	20	18	15					
B11	5	97	20	19	18	15				
B12	7	20	19	18	17	16	15	14		
B13	6	19	18	17	16	15	14			
B14	5	42	29	21	18	15				
B15	5	45	42	21	19	15				
B16	5	97	45	42	19	15				
B17	9	97	45	42	29	21	20	18	15	14
B18	6	97	29	21	19	18	15			
B19	6	97	29	21	19	18	15			
B20	7	97	42	29	21	19	15	14		
B21	5	97	42	29	21	15				
B22	6	97	29	21	18	15				
B23	6	97	42	29	21	19	15			
B24	5	97	29	21	18	15				
B25	5	97	20	18	17	15				

for 3 min. Centrifugation was performed for 5 min at 3000 rpm, and the upper layer was transferred to a 50-ml separating funnel using a disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 ml of n-heptane was added and extracted three times using 1.0 ml portions of 0.2 N HC1. The HCl layers were collected in a glass stoppered tube. The solution was evaporated just to dryness in a water bath at 95°C, with the aid of a gentle current of air. Twenty milliliters of 5% TCA was added to 1 g of ground plant materials in 50 ml centrifuge tubes. Samples were mixed and sonicated for 5 min (Diagger ultrasonic processor, 750 watts). The tubes were centrifuged at 4500 rpm for 5 min. Ten milliliters of the supernatant was transferred into a culture tube containing 4 g NaCl and 1 ml of 50% NaOH. The tubes were shaken; extraction was performed three times using 5 ml n-butanol/chloroform (1:1 v/v) in stoppered tubes that were shaken vigorously for 3 min. Centrifugation was performed for 5 min at 4500 rpm, and the upper layer was transferred to a 50-ml separating funnel using a disposable Pasteur pipette. To the combined organic extracts (upper layer), 15 ml of n-heptane was added and extracted three times using 1.0 ml portions of 0.2 N HC1. The HCl layers were collected in a glass stoppered tube. The solution was evaporated just to dryness in water bath at 95°C, with the aid of a gentle current of air.

2.3.3. Formation of dansylamines

One hundred microliters of each stock standard solution was transferred to a 50-ml vial and dried. About 0.5 ml of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard). The tube was stoppered and carefully mixed to prevent loss from spattering. Carefully, 1.0 ml of dansyl chloride solution was added and mixed thoroughly using a vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water was added to the reaction mixture, and the tube was stoppered and shaken vigorously using a vortex mixer. The extraction of dansylated biogenic amines was performed using 5.0 ml portions of diethylether in stoppered tubes that were shaken carefully for 1 min. This procedure was repeated three times, and the ether layers were collected in culture tubes using disposable Pasteur pipettes. The combined ether extracts were carefully evaporated at 35°C in a dry bath with the aid of current air. The obtained dry film was dissolved in 1 ml methanol, and then 10 μl was injected in a high-performance liquid chromatography (HPLC) column using a gradient program as shown in Table 2.

2.3.4. Apparatus

The HPLC system for dansylamine determination was an Agilent 1100 system equipped with quaternary pump model G1311A, UV

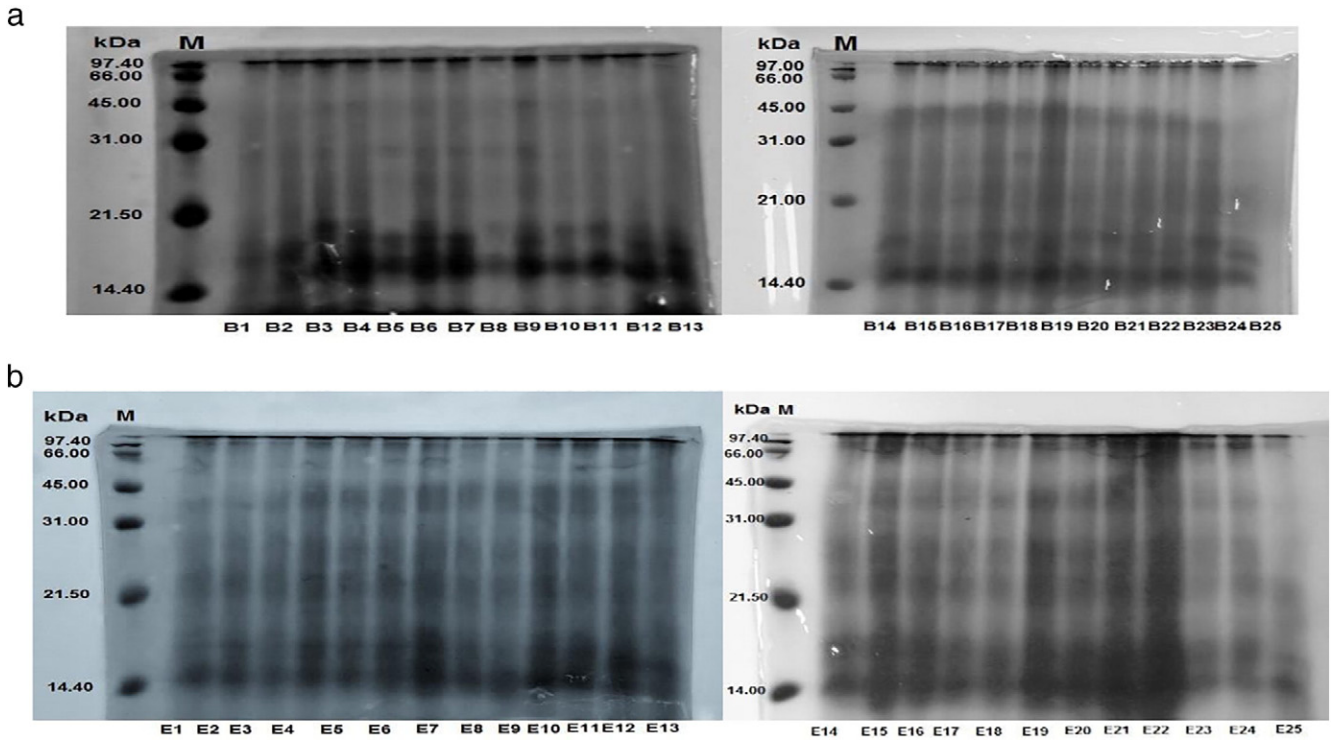

Fig. 1. Allergen proteins in *Prosopis juliflora* genotypes. (a) Electrophoresis gel lane of allergen proteins from *P. juliflora* genotypes from the Al-Qassim region (B1-B25) and (b) the Eastern region (E1-E25). M lane: molecular weight marker (kDa).

Table 4
Molecular weight (kDa) of allergen proteins in *Prosopis juliflora* genotypes from the Eastern region

Genotypes	No. of Proteins	Molecular weight of protein (kDa)									
E1	5	97	29	19	16	15					
E2	6	97	59	36	23	18	16	15			
E3	5	97	59	36	18	15					
E4	6	97	59	36	23	18	15				
E5	6	97	42	36	23	19	15				
E6	7	97	65	42	33	21	18	15			
E7	10	97	65	59	42	36	23	18	16	15	14
E8	3	97	66	15							
E9	3	97	19	15							
E10	9	97	59	42	36	33	23	18	16	15	
E11	7	97	59	42	33	18	16	15			
E12	8	97	59	42	33	23	18	16	15		
E13	5	97	42	33	18	15					
E14	5	97	66	16	15	14					
E15	9	97	66	36	23	21	18	16	15	14	
E16	9	97	66	36	23	21	18	16	15	14	
E17	4	97	33	15	14						
E18	4	97	36	18	15	14					
E19	8	97	42	36	23	21	18	16	15		
E20	5	97	36	23	21	15					
E21	8	97	42	36	23	21	18	16	15		
E22	10	97	59	42	36	29	23	21	18	16	15
E23	3	97	33	15							
E24	4	97	33	21	15						
E25	3	97	33	15							

detector model G1314A set at 254 nm wavelength, and auto sampler model G1329A. Agilent Zorbax Eclipse XDB C18 4.6 mm × 150 mm, 5 m column was used for biogenic amine separation. Data were integrated and recorded using Chemstation Software program. The biogenic amines of *P. juliflora* pollen extracts were quantified by comparing their peak areas with those of corresponding biogenic amine standard solutions using the Chemstation data System Program. Stander error was calculated for each biogenic amine.

2.4. Cluster analysis

Data from the allergen protein analyses were scored for cluster analysis depending on the presence or absence of each molecular weight of proteins. If a product was present in a cultivar, then it was designated "1"; if absent, then the product was designated "0". Pairwise comparisons of genotypes were used to generate similarity coefficients based on the SIMQUAL module. The similarity coefficients were then used to construct a dendogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) using NTSYS-PC software version 2.0 (Exeter Software, New York) [17].

3. Results

3.1. Protein Allergens of P. juliflora

The study of protein allergens in all genotypes in both regions revealed the presence of all allergen proteins. The molecular weight of these proteins varied from 14 to 97 kDa. The type of protein varied between individual genotypes in both regions. Results showed the presence of 18 proteins with molecular weights 14, 15, 16, 17, 18, 19, 20, 21, 23, 29, 33, 36, 42, 45, 59, 65, 66, and 97 kDa. Regarding the results of each region, Al-Qassim region genotypes contained 12 different proteins with molecular weights 14, 15, 16, 17, 18, 19, 20, 21, 29, 42, 45, and 97 kDa (Table 3; Fig. 1), whereas the genotypes of the Eastern region revealed 14 proteins with molecular weights 14, 15, 16, 18, 21, 23, 29, 33, 36, 42, 59, 65, 66, and 97 kDa (Table 4; Fig. 1). It is obvious that the number of proteins in the genotypes of the Eastern region exceeds those in the Al-Qassim region. Both regions shared 8 proteins with molecular weights 14, 15, 16, 18, 21, 29, 42, and 97 kDa. Four proteins were in the Al-Qassim region with molecular weights 17, 19, 20, and 45 kDa but were not detected in the Eastern region. In addition, 6 proteins were found in the Eastern region with molecular weights 23, 33, 36, 59, 65, and 66 kDa but were not recorded in the Al-Qassim region. Results indicate that there is a variation in protein ratios among the genotypes. A 15-kDa protein was the most abundant protein of all proteins, with a ratio of 92%, followed by a 97-kDa protein with 80%, and both proteins were found in all Eastern region genotypes (Fig. 2). Moreover, an 18-kDa protein was found with a ratio of 72% and a 29-kDa protein with a ratio of 42%. The lowest ratio for any protein was 4%, observed for proteins of 65 and 66 kDa. Other proteins were found at varied ratios (Fig. 2). Furthermore, results indicated that the number of proteins ranged from 3 to 10 per genotype. Genotypes E7 and E22 from the Eastern region had 10

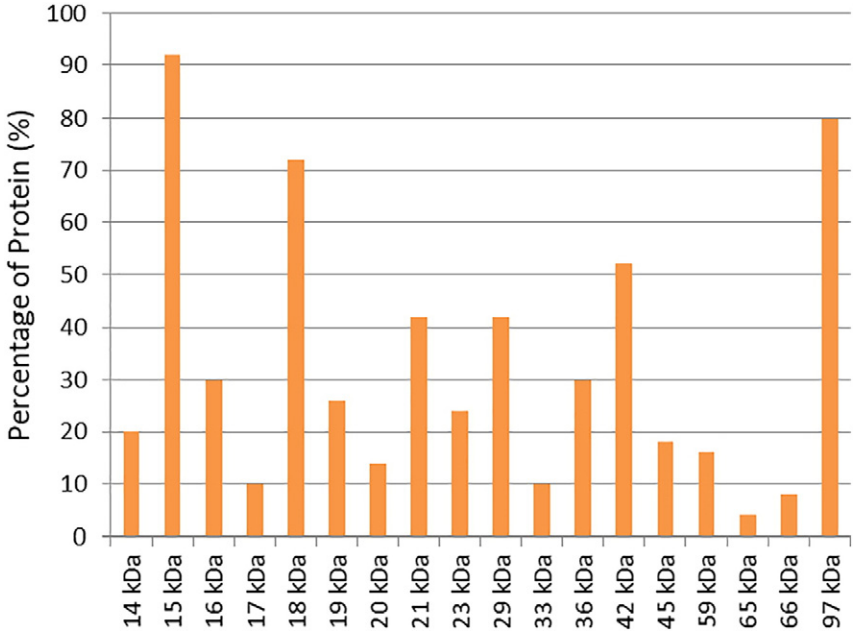

Fig. 2. Percentage of allergen proteins in *Prosopis juliflora* genotypes from both regions.

different proteins as the highest number, followed by the genotypes E15, E16, and E9 from the same region and genotype B17 from the Al-Qassim region with nine proteins. Genotypes E8, E9, E23, and E25 from the Eastern region had only three proteins as the least number (Table 3 and Table 4).

The cluster analysis (Dendrogram) for the allergen proteins of the genotypes divided all the genotypes of both regions into three main groups, with minimum similarity degree of 0.59 and maximum degree of 1.0 (Fig. 3). The first group included the genotypes B1, B2, B4, B12, B13, B17, and B19 (all of them from the Al-Qassim region) with similarity ranging from 0.62 to 1.0. The second group was divided into four subgroups: first of them included the genotypes B3, B7, B9, B13, B14, B15, and B16 (all from the Al-Qassim region) with similarity between 0.72 and 0.89 degree. The second subgroup included the genotypes B5, B8, B18, B21, B22, B23, B24, and B25 (all from the Al-Qassim region) with similarity between 0.80 and 1.0. The third subgroup included the genotypes E8, E9, E17, E18, E20, E23, E24, and E25 (all from the Eastern region) with similarity from 0.88 to 1.0. The fourth subgroup included only two genotypes, B10 and B11, from the Al-Qassim region with a similarity of 1.0 between the two genotypes and similarity 0.74 with the group. The third main group included the genotypes E1, E2, E3, E4, E5, E6, E7, E10, E11, E12, E14, E15, E16, E21, and E22 (all from the Eastern region) with similarity degrees ranging from 0.75 to 1.0. Similarity degrees recorded in the cluster analysis resulted from the amount of protein in each genotype and the resemblance of proteins among the genotypes within each region. Some proteins were found in one region and not in the other: the number of proteins in the Al-Qassim region was 12, while that in the Eastern region was 14.

3.2. Biogenic amine assessment in P. juliflora

The assessments of concentrations of bioamines in *P. juliflora* are summarized in Table 5 and Table 6. In the Al-Qassim region, genotype B3 had the highest concentrations of tryptamine, β-phenylethylamine, putrescine, histamine, and tyramine. Genotype 9 in the same region had the highest concentrations of both spermidine and spermine, while genotype 10 recorded highest concentration of cadaverine among all genotypes in this region. Regarding the lowest concentrations in the Al-Qassim region, genotypes B7, B23, and B25 were the lowest in the bioamines tryptamine, β-phenylethylamine, putrescine, and tyramine. The genotypes B1, B3, B4, B5, B6, B9, B11, B13, B16, B21, B23, B24, and B25 had the lowest concentrations of cadaverine among all genotypes in the Al-Qassim region. The genotype B23 was the lowest in histamine and spermidine content (Table 5). Moreover, both B7 and B11 genotypes recorded the lowest concentrations in spermine. Regarding

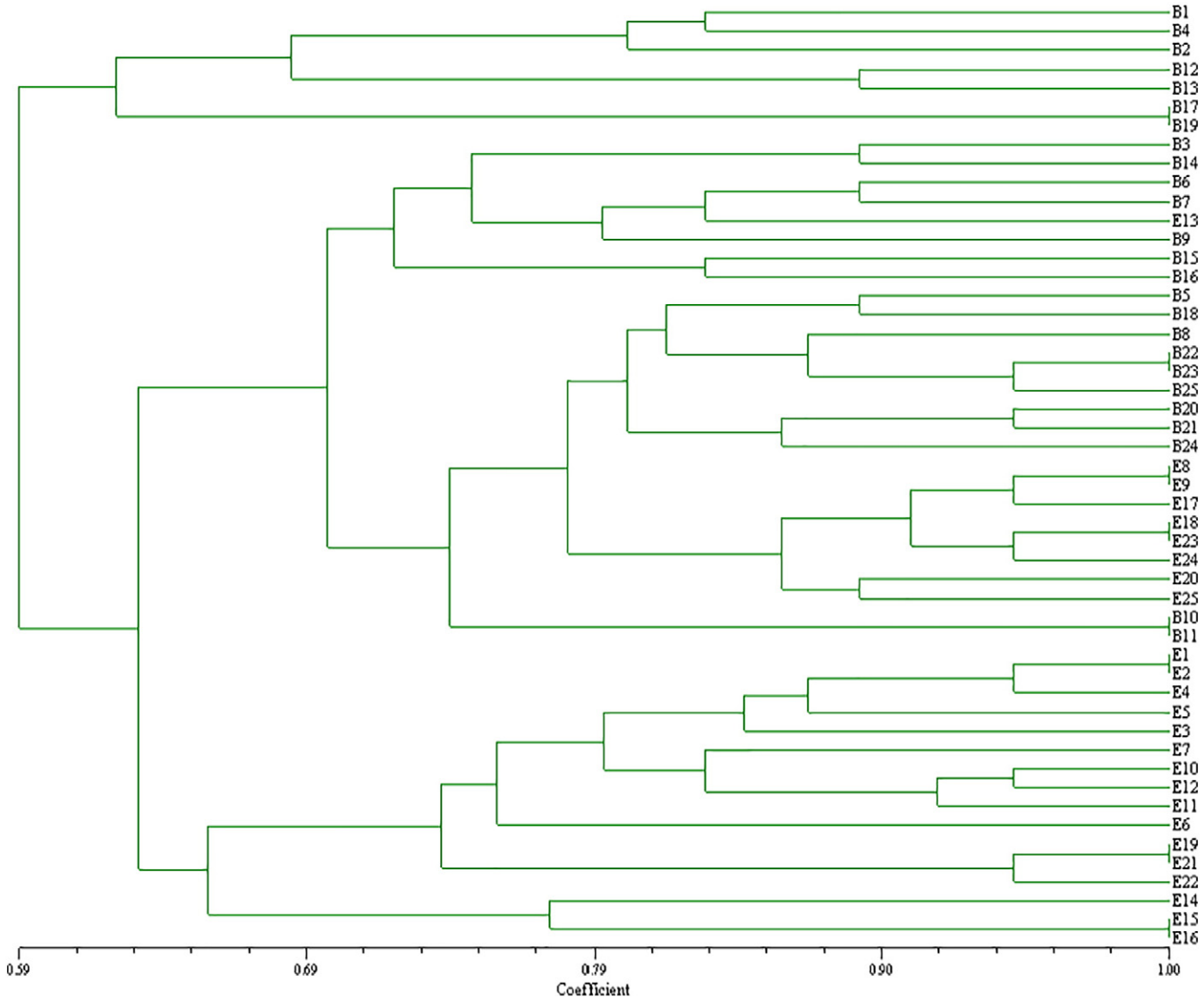

Fig. 3. Dendrogram of allergen proteins from *Prosopis juliflora* genotypes from both regions.

Table 5
Biogenic amine concentrations in *Prosopis juliflora* genotypes from the Al-Qassim region.

Genotypes	Tryptamine μg/g	β-phenylethylamine μg/g	Putrescine μg/g	Cadaverine μg/g	Histamine μg/g	Tyramine μg/g	Spermidine μg/g	Spermine μg/g
B1	54.4	45.6	45.6	7.4	18.6	132.2	17.5	4.4
B2	45.2	12.1	25.7	5.2	4.7	55.6	7.5	3.9
B3	83.3	47.0	126.3	79.5	37.0	396.2	49.5	7.4
B4	27.5	21.3	19.6	5.5	6.6	55.2	6.6	2.7
B5	61.5	38.3	66.2	9.0	17.7	174.1	24.8	5.6
B6	60.5	26.0	46.5	7.2	9.4	90.4	16.5	7.2
B7	11.4	4.3	5.5	10.3	1.3	13.7	1.6	0.2
B8	22.0	41.2	17.1	33.1	9.6	205.2	9.0	1.8
B9	35.0	36.8	98.6	4.6	30.2	154.1	92.5	30.0
B10	53.3	41.9	39.9	183.8	9.2	198.2	25.1	15.9
B11	24.1	15.0	18.0	4.8	8.3	63.0	4.4	1.0
B12	20.6	35.2	22.4	16.2	2.9	232.0	17.3	11.9
B13	37.5	34.2	39.8	7.4	9.2	191.0	24.7	6.9
B14	61.7	25.1	75.1	31.0	19.3	198.2	30.1	11.1
B15	46.7	40.5	63.7	39.0	7.7	224.6	45.5	12.4
B16	37.8	33.2	58.1	4.5	10.1	235.7	34.8	16.9
B17	28.1	20.8	54.3	77.7	11.0	213.1	36.9	15.7
B18	25.2	22.1	20.5	88.3	12.5	190.0	9.8	5.2
B19	27.0	21.8	32.5	93.6	15.5	247.0	22.5	13.5
B20	34.8	16.7	42.4	16.9	4.1	108.2	32.4	16.4
B21	40.8	11.7	24.8	6.7	7.9	112.6	6.8	6.5
B22	75.1	27.1	59.9	21.7	17.2	99.2	22.1	5.6
B23	9.1	4.6	6.0	0.83	0.9	25.9	3.0	4.1
B24	39.6	41.4	52.3	6.5	10.1	218.0	31.5	17.0
B25	12.6	6.2	13.9	4.6	4.3	58.0	8.1	5
SE*	±3.9	±3.1	±5	±8.6	±1.8	±17.4	±3.9	±1.3

* SE= Standard Error; difference between two means ≥SE indicates significant difference.

the Eastern region, it is worth mentioning that samples of genotypes E15, E23, E24, and E25 were not present in enough quantity to be analyzed. The genotype E4 had the highest concentration of the bioamines β-phenylethylamine, histamine, and spermine (Table 6). Moreover, the genotype E4 along with genotype E14 showed the highest tryptamine and putrescine content; however, genotypes E4 and E6 had the highest concentrations of spermidine. Genotype E6 alone was the highest in tyramine content among all Eastern region genotypes. It was also noticed that genotype E1 had the highest concentration of cadaverine. Concerning the lowest concentrations in the Eastern region, great variability was noticed between genotypes for each bioamine. The genotypes E7, E11, E16, E17, E18, E19, E20, and E21 showed the lowest concentrations of both tryptamine and β-phenylethylamine. For putrescine, E9, E11, E16, E17, E18, E19, E20, and E21 showed the least concentrations. In Eastern region, genotypes E2, E4, E5, E6, E7, E8, E9, E14, E17, E20, and E22 showed the least concentrations of cadaverine. Furthermore, genotypes E9, E11, and E19 were the lowest in histamine concentrations, whereas this bioamine was not found in genotypes E18, E20, and E21 in the Eastern region. The genotypes E9, E11, E16, E18, E19, E20, and E21 had the lowest concentrations of the bioamine tyramine. The concentration of spermidine was in the least in genotypes E9, E11, E17, E18, and E19 and was not recorded in genotypes E20 and E21. Among all Eastern region genotypes, E9, E12, and E22 showed the lowest concentrations of spermine, while it was

Table 6
Biogenic amine concentrations in *Prosopis juliflora* genotypes from the Eastern region

Genotypes	Tryptamine μg/g	β-phenylethylamine μg/g	Putrescine μg/g	Cadaverine μg/g	Histamine μg/g	Tyramine μg/g	Spermidine μg/g	Spermine μg/g
E1	45.3	16.8	49.6	263.7	11.3	94.0	23.8	5.2
E2	39.2	11.16	23.4	6.0	7.9	89.1	6.4	5.8
E3	33.6	22.5	22.1	94.5	13.1	95.3	8.4	4.0
E4	141.0	45.1	159.9	7.8	23.7	156.6	33.3	10.9
E5	53.4	17.7	96.8	3.2	9.4	123.7	31.1	13.3
E6	61.4	21.2	100.2	12.3	11.5	216.0	35.7	12.6
E7	9.4	4.4	17.3	12.1	4.2	65.1	6.2	2.5
E8	22.2	11.9	30.7	3.4	8.1	114.9	16.6	7.5
E9	12.2	9.6	7.0	6.2	1.3	8.7	0.8	0.9
E10	51.3	15.3	39.8	42.3	8.7	56.1	11.3	3.6
E11	9.3	4.9	4.9	37.8	1.6	12.9	0.7	ND
E12	39.1	9.2	33.3	22.2	6.3	147.9	6.3	0.9
E13	44.0	7.5	40.2	21.6	12.8	51.3	16.4	5.9
E14	143.3	24.9	141.3	4.7	12.1	155.1	22.8	2.9
E16	9.1	8.4	9.3	173.1	3.6	16.8	ND	ND
E17	4.4	4.5	5	15.5	2.0	36.1	0.9	ND
E18	6.8	4.2	3.1	48.4	ND	2.8	0.4	ND
E19	11.3	6.8	8.4	43.7	0.8	12.5	1.2	ND
E20	3.4	5.4	2.0	1.9	ND	1.6	ND	ND
E21	4.5	5.5	3.5	142.4	ND	4.3	ND	ND
E22	36.1	16.1	28.3	10.9	7.6	133.4	10.4	1.7
SE*	±8.6	±2.1	±10.1	±14.9	±1.3	±15.4	±2.7	±0.9

* SE= Standard Error; difference between two means ≥SE indicates significant difference.

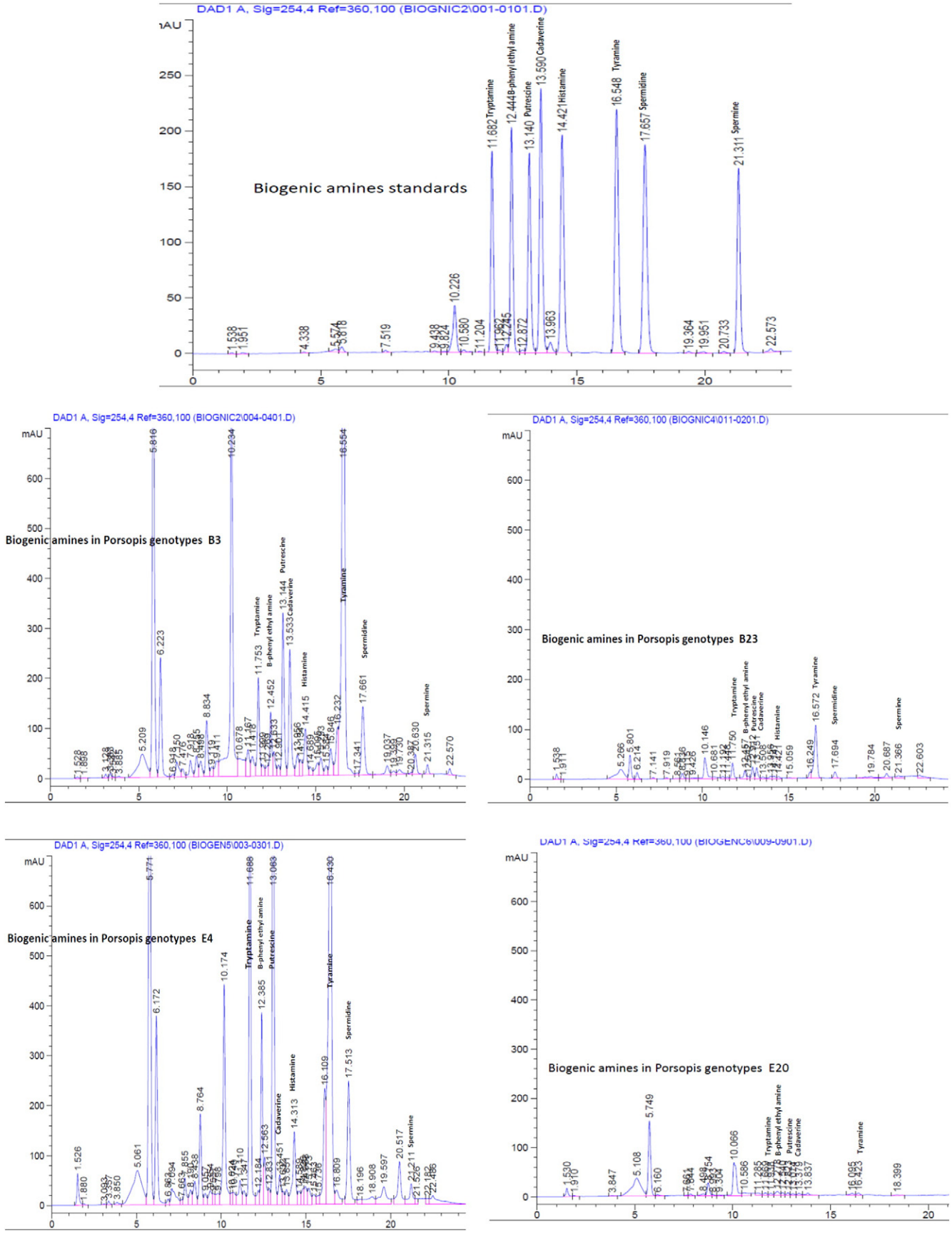

Fig. 4. Biogenic amine chromatograms of some of the *Prosopis juliflora* genotypes (B3, B23, E4, and E20) from both regions and biogenic amine standard.

not found in E11, E16, E17, E18, E19, E20, and E21. The biogenic amine chromatograms of some *P. juliflora* genotypes from both regions are shown in Fig. 4.

4. Discussions

Results showed that all proteins found in the pollen grains of *P. juliflora* trees were the same as the proteins reported in previous studies and had nearly the same molecular weights. In this study, molecular weights ranged from 14 to 97 kDa, and the total number of proteins was 18, whereas Dhyani et al. [18] reported 16 allergen proteins with molecular weights ranging from 14 to 97 kDa. Killian and McMichael [19] identified 13 proteins with molecular weights ranging from 11 to 99 kDa. Studies by Pham and Baldo [20], Weber [21], and Sastre et al. [22] reported results for allergen proteins with molecular weights of 15, 18, 19, 20, 40, 46, and 60 kDa. More et al. [11] identified allergen proteins with molecular weights 59 and 66 kDa from the pollen grain extracts of *P. juliflora* flowers. Recently, Assarehzadegan et al. [9] reported that there were 8 proteins with molecular weights ranging from 10 to 85 kDa extracted from the pollen grains of *P. juliflora* flowers that can cause allergy. It can be noted that there were differences in molecular weights between the present investigation and other mentioned studies. Moreover, some proteins were found in some studies and not detected in others. The reason for this variation may be the slight differences in molecular weights, which could be explained by the variations in flower extracts, methods of computing molecular weights of protein components, and methods of protein extraction from pollen grains [18]. Assarehzadegan et al. [9], Dhyani et al. [18], Killian and McMichael [19], Pham and Baldo [20], Weber [21], and Sastre et al. [22] conducted studies on respiratory allergy patients to determine allergen proteins through allergy skin tests and included the identification of allergen proteins in patient serum. These studies revealed that all proteins from *P. juliflora* trees with molecular weights from 8 to 97 kDa are allergens. Among the patients studied, 90% gave a positive response. The present study, unlike previous studies, focused on estimating the proteins in pollen grains, and we can claim that all identified proteins from mesquite trees from both regions (Eastern and Al-Qassim) cause allergies in patients who are sensitive to pollen grains. The above studies reported that these proteins can be used as diagnostic and therapeutic agents for patients sensitive to *P. juliflora* pollen. Previous studies [18,23] showed that proteins with molecular weights 20, 52, 59, and 66 kDa are more reactive and affect more among the sera of positive sensitivity test individuals. Our studies showed the presence of proteins with molecular weights 20, 59, and 66 kDa. The 66-kDa protein was found in the genotypes E8, E14, E15, and E16 in the Eastern region with 8% ratio, and the 59-kDa protein was found in the genotypes 2, 3, 4, 7, 10, 11, and 12 in the Eastern region trees with 16% ratio. In the Al-Qassim region, the 20-kDa protein was found in the genotypes B6, B9, B10, B11, B12, B17, and B25 with 14% ratio, whereas the 52-kDa protein was not detected in the genotypes of either region.

The current research is the first to study the allergen proteins in *P. juliflora* pollen grains in the genotypes from the Al-Qassim and Eastern regions. The present investigation clearly explained the contents of the bioamine proteins, which were not reviewed before. The results herein explained that the reason for the effect of allergen proteins in pollen grains of *P. juliflora* could be due to bioamine content, in particular, histamine and tyramine, which are more effective in showing allergy symptoms. Exposure to *P. juliflora* pollen grains encourages the body to produce antibodies to histamine and subsequently allergy symptoms such as runny nose, eye tearing, or itching appear through histamine receptors [24,25]. In this study, some genotypes (B23, E20, and E21) did not show the presence of histamine or showed little concentration of tyramine, which implies that exposure to the pollen grains of these genotypes will not result in symptomatic allergy. We can conclude from these results that not all *P. juliflora* trees cause allergy as the main reason for allergy is the presence of histamine.

Data of the concentrations of bioamines indicate that in the Al-Qassim region, some genotypes contain very little concentration of histamine compared with other genotypes. Genotype B7 contained 1.3 μg/g, genotype B23 contained 0.9 μg/g, and genotype B3 contained 37 μg/g histamine. The concentration of tyramine varied between genotypes in both regions. It was noticed that genotypes free of histamine or containing little concentrations of histamine also showed little concentrations in tyramine. This implies that there is a direct relationship between histamine and tyramine. Killian and McMichael [19] reported that mesquite (*P. juliflora*) plant is considered a serious allergen. Exposure to mesquite varieties or hybrids could result in different IgE banding patterns, especially considering the worldwide distribution of *P. juliflora*.

5. Conclusions

It could be concluded from this study that environmental conditions, especially humidity, did not have any effect on allergen protein or their bioamine contents, e.g., histamine. Allergen proteins were found in the genotypes of both the Eastern region, which has a humid environment, and the Al-Qassim region, which has dry weather. Subsequently, there were no effects of environmental conditions on the presence or absence of the bioamines, including histamine. Thus, the presence or absence of allergen proteins is attributed mainly to the genotypic structure of *P. juliflora* trees. Bioamines, except histamine and tyramine, were recorded at varying concentrations in different genotypes and can be related to toxicity rather than allergy.

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