Using Two-Dimensional Gel Electrophoresis Approach for Characterizing of the Ole e 1, an Olive Pollen Major Allergen

A. Soleimani[1], J.D. Alché[2], A.J. Castro[2], M.I. Rodríguez-García[2] and A.R. Ladan Moghadam[3]
[1]Horticultural Department, Faculty of Agriculture, University of Zanjan, Zanjan 313, Iran
[2]Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008, Granada, Spain
[3]Islamic Azad University of Garmsar, Garmsar, Iran

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Abstract

Ole e 1 is a member of the "pollen proteins of the Ole e 1 family" which produces IgE-reactivity in >70% of patients allergic to olive pollen. In order to precisely determine the nature of the differences in the protein expression pattern of Ole e 1 for cultivars of different characteristics, 'Rowghani' and 'Zard', crude protein was extracted, and isoelectric focusing (IEF) was performed in a IPGphor device (Pharmacia), and analytical gel electrophoresis was also performed according to Laemmli. Gels were transferred onto PVDF membrane and then probed with a monoclonal antibody to Ole e 1. An Alexa Fluor 488 anti-mouse IgG antibody made in goat (molecular probes) was used at as the secondary antibody. Approximately 750 spots could be detected by silver staining in the 2-D map of each sample. Up to 9 of these spots were immunoreactive to the Ole e 1 antibody after Western blotting experiments in 'Rowghani', whereas up to five immunoreactive spots only were detected in 'Zard'. The high degree of polymorphism displayed by Ole e 1 in a cultivar-specific manner may represent part of a unique recognition system. Moreover, its probable relation to different biological behaviors of corresponding cultivars is discussed.

INTRODUCTION

Olive (*Olea europaea* L.) is a woody and an important oil-producing tree species. The olive industry was first developed on a large scale in Iran almost a decade ago using two major cultivars, 'Rowghani' and 'Zard'. Among seasonal allergies, olive tree pollen allergy is a major health problem for humans throughout the Mediterranean area (Rodriguez et al., 2002). Thus, it seems that respiratory allergy to olive's pollen in Iran will become a serious problem in the near future. The major allergen from olive tree pollen is Ole e 1, representing about 1% of the total dry mass of the mature pollen. However, the protein content of pollen extracts depends on the cultivar as well as on environmental factors such as geographical and seasonal conditions (Castro et al., 2003). Hence, the aim of this study is to study Ole e 1 protein polymorphism in two main Iranian cultivars.

MATERIALS AND METHODS

Olive pollen was obtained from two main Iranian cultivars 'Rowghani' and 'Zard', cultivated at the olive research centre of Tarom-Zanjan and stored at -80°C. Crude protein extracts were obtained by stirring 1 g of each pollen sample in 1 ml of extraction buffer (Tris-HCl, pH 8.8, 40 mM, TritonX-100, 2% v/v, ascorbic acid 1 mg/ml, protease inhibitor cocktail 10 L/ml, DTT 5 mM, polyvinyl polypyrolidone 5% w/v), for 4 h at 4°C. The extracted protein was dissolved in chloroform/methanol (2:1) and precipitated in TCA/acetone. After 3X washing with cold acetone, the final pellets were dried and dissolved in 2-D rehydration solution (Tris-HCl 40 Mm, pH 8.8, urea 7 M, thiourea 2 M, CHAPS 4% w/v, IPG buffer 3-10, 0.2% v/v, bromophenol blue trace, tributyl phosphine, 'TBP' 5 mM). The protein samples were alkalined by adding iodoacetamide to a final concentration of 200 mM. For 2-DE, samples were treated in 270 L of 2-DE rehydration solution by reswelling 18 cm Immobiline Dry Strip (pH 3-10, Bio-Rad, UAS) for 12 h.

IEF was performed in IPGphor (Pharmacia Biotech, USA) at 20°C, applying 0 V for 6 h, 30 V for 7 h, and an increasing voltage up to 8,000 V for a total time of 12 h. Focused strips were equilibrated using dithiotheritol and iodoacetamide solution. For SDS/PAGE the Laemmli buffer system was used to cast 4% stacking and 13% resolving gel. Separated polypeptides were transferred into PVDF membrane at 25 V/30 min. All the previous mentioned steps were followed and the reaction was detected using Blot Scanning Alex Fluro, Bio-Rad.

RESULTS

Figure 1 shows the bidimensional pattern of the crude extract immunostained with monoclonal antibody and alex fluor 488 goat antimouse IgG as secondary antibody. Separation pattern of Ole e 1 can be improved by using SDS-PAGE plus isoelectro-focussing (IEF), because it allows us to separate by charge many proteins that share similar molecular masses. Ole e 1 appears as many spots of different pI values (5-7) which is in agreement with the polymorphic character of the protein.

DISCUSSION

This study demonstrates the presence of conspicuous quantitative and qualitative difference between two cultivars. Ole e 1 is a glycoprotein of 146 amino acids, which exhibits in SDS-PAGE a pattern of multiple bands, as it has two main forms: glycosylated (20 kDa) and non-glycosylated (18.5 kDa). Minor variants at 22 kDa (hyperglycosylated component) are frequently present. The presence of glycan reagents in allergenic sources could explain the high degree of cross-reactivity observed among allergens from pollen, vegetable food and invertebrate animals (Aalberse and Van Ree, 1997). Ole e 1 protein in 'Rowghani' was separated by 2-DE as 2, 7 and 3 spots with molecular weights of 18.5, 20 and 22 KDa respectively. Five immunoblot spots were detected in 'Zard' with molecular weight of 20 KDa. PI values exhibited by 2-DE gels ranged in 4.8-6.5. This high degree of protein spots polymorphism is a characteristic of plant pollen allergens such as short ragweed (Bond et al., 1991) and birch (Swoboda et al., 1995) among others. Furthermore, in the case of olive, the presence of isoallergens is also attributed to glycosylation and with more probability phoshporylation variants. Phosphorylation of Ole e 1 might be a signaling system which regulates the initiation, maintenance and even the guidance of pollen-tube growth, thus enabling successful fertilization as proposed in previous studies (Alche et al., 1993, 2004). The high amount of protein clearly underlines that Ole e 1 probably plays an essential biological role within pollen. Although the biological function of Ole e 1 is unknown, some important clues are available. Recent studies have demonstrated that Ole e 1 is involved in germination and/or pollen tube growth; however, the low germination capacity of pollen grains from 'Rowghani' which has a high amount of Ole e 1 in comparison with 'Zard' should be studied in more detail. Current genetic improvement programs of olive culture industry around the world are directed to solve agronomic and commercial problems such as the production of autofertile plants (Rugini and Pesce, 2006) since olive is partially self-incompatible (Quero et al., 2002). It could be interesting to speculate about the potential role of Ole e 1 in the self-compatibility reactions since previous studies have documented 'Rowghani' as a partially self-compatible and 'Zard' as a partially self-incompatible cultivar (Zinanlu et al., 2002).

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Figures

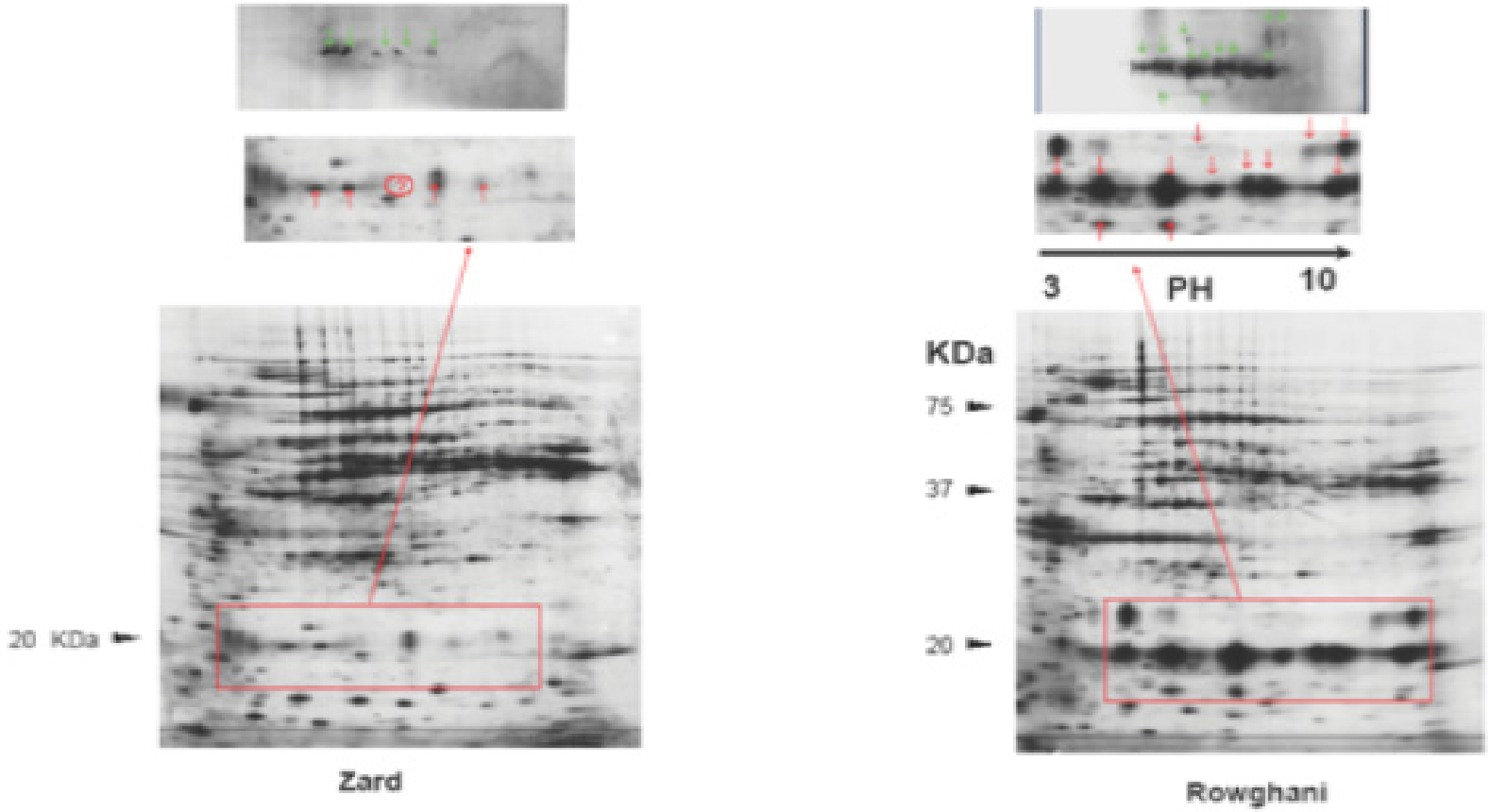

Fig. 1. Two-dimensional map of the Ole e 1 pattern of olive pollen extracts from two cultivars, 'Zard' and 'Rowghani'. Separation by electrofocusing plus SDS-PAGE, and further immunostaining with a monoclonal first antibody and Alex Fluor 488 goat antimouse IgG (H+L) as a secondary antibody. Isoelectric point (pI) and molecular mass (kDa) values of standard proteins are indicated.