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# Ultra Structures Assessment and Comparison of Allergenic Features of Mature and Immature Pollens of *Quercus persica* L.

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**Abstract**

There are extensive Persian Oak forests in the west and south west of Iran. Since the pollens are one of the most plants allergenic factor, and 80 up to 90 percent of plant's allergen is pollen based, therefore in the present study the allergenic features of Persian Oak's mature and immature pollens were studied, using cello logy and anatomical methods. The pollen samples were fixed by FAA. SEM analysis showed spherical shaped pollen along with tricolpate and warty shape exine. Pollen's extract achieved using salt phosphate buffer. Electrophoresis profile of proteins showed total 16 bands in the range of 16 to 116 Kd. Mature pollen also had one distinct 52 Kd band. Allergenic test using guinea pig (350-400 Kg and 4-6 old) carried out. The blood tests, based on the numbers of eosinophils, neutrophils and immunoglobulins content of sample and treatments showed significant differences to the control. The mature pollen extract also was more allergenic than that of immature.

**Keywords:** vegetative organs, pollen ultra structures, allergenic features of pollen, ontogenetic features, *Quercus persica* L.

## 1. Introduction

Allergenic features of pollen is in the first category of hypersensitive responses. Studies have shown that IgE content is increased by pollen caused allergy (Hosseini et al., 1991). Proteins of pollen are one of allergenic factor which are embedded in cytoplasm and the pollen's coat. Minerals absorbed from environment also can activate the allergenic proteins of pollen. Interaction of pollen and air contaminators brings about releasing of allergenic aerosol of pollens. These particles penetrate into breathing system more than pollen itself (Chehregani et al., 2004). Previous studies have released that some *Leguminosea* related species have different patterns of allergenic features (Robinson et al., 2005). Persian Oak belongs to *Fagaceae*, and *Quercus* genus, by more than 500 species, is distributed throughout the word in the shrub and arboraceous forms. The fruit of Persian oak have been being used by Americans, European, Asian and African for more than thousands of years (Ozcan, 2006). This tree has extended leaves, 20 meters in height and smooth trichoms. Oaks have spirally arranged leaves, with lobate margins in many species; some have serrated leaves or entire leaves with smooth margins. The fruit is a nut called an acorn, borne in a cup-like structure known as acupule; each acorn contains one seed. Many deciduous species are marcescent, not dropping dead leaves until spring. In spring, a single oak tree produces both male flowers (in the form of catkins) and small female flowers. The live oaks are distinguished for being evergreen, but are not actually a distinct group and instead are dispersed across the genus (Ebrahimi et al., 2008). Regarding of wide distribution of Persian oak in the western forests of Iran, in the present study therefore, The allergenic feature of mature and immature oak' s pollen was assayed base on electrophoresis profiles.

## 2. Materials and Methods

Vegetative structures were collected near city of Khoram abad (Iran) for following anatomical assay and extraction. Buffer extract was used to prepare the proteins profile. To do this, PBS buffer (phosphate saline) by following protocol was used; 1 g of mature and immature pollen extract were separately mixed with 6 ml of PBS. The mixtures then were riled for 24 h in 4 °C on the shaker. Cold centrifuge in -4 °C then was carried out by 13000 g. Upper phase was kept in -20 for next experiments.

*2.1 Animal Samples*

Guinea pig in the range of 350- 500 gr were used as animal sample. The samples were isolated in certain environment (22±2 °C, 55±5 humidity, 12 light, 12 darkness photoperiodism) and fixed diet feed, for adaption to new experimental condition. The samples were randomly categorised in to three groups; group 1 were injected by mature pollen extraction, group 2 by immature pollen extract and third group were injected by salty Phosphate buffer as control sample. Injections were done once a week for 5 continues weeks by 100 µl of extraction, as a peritoneum injection (subcutaneous injection for last time injection). A week after the last injection, giving blood were done from heart of animal samples. The numbers of eosinophils using CBC, IgE using ELISA test (IU/mL) and blood sugar level (Mg/dl) were then measured for all groups. SDS-PAGE electrophoresis was used to assay the protein band profiles. 20 µl of extraction for all samples, as well as polyacrylamide gel 12% were imposed to do this. R-25 coomassie blue stain was used for dyeing the gels.

*2.2 Ontogenic Studies*

FAA fixator was used to fixation of flower buds of Oak. Following staining was done several weeks after fixation, by hematoxylin and eosin. Light microscope was then used for scanning of samples. Antheridium were dried and the result powder in range of 70-230 µm, was scanning by SEM.

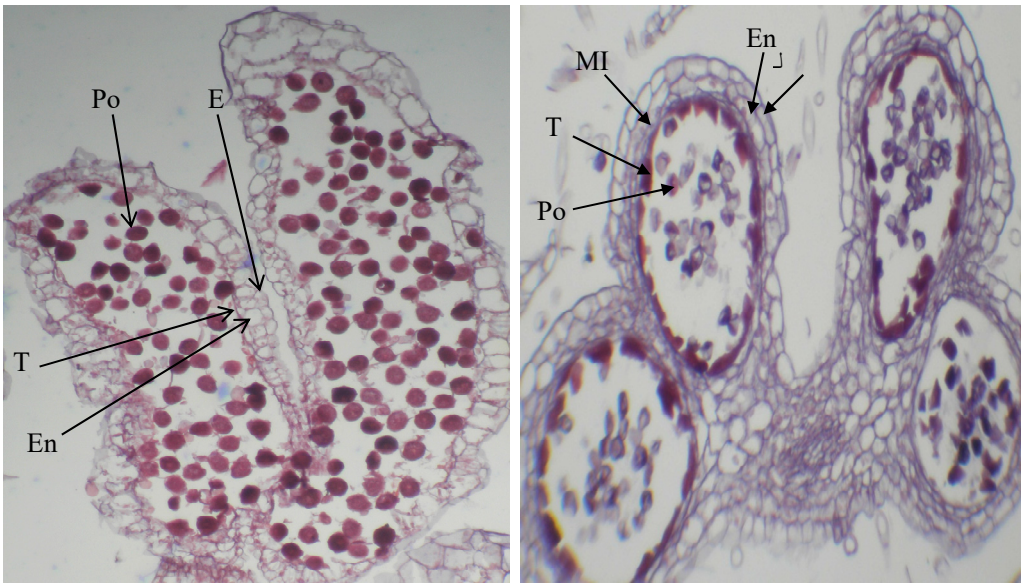
*2.3 Statistical Analysis*

SPSS ver16. was used for statistical analysis. Means were compared using the duncan test at P<0/05, level of significance to distinguish the differences between treatments and control samples. There were three replicates for all experiments.

**3. Results**

*3.1 Structure of Mature and Immature Antheridium*

The observed results showed that antheridium consist of a layer of cutin cells, which protect the antheridium. In the immature one, mechanical layer was seen under epidrem, and under the transition cells (into the immature antheridium sac), topi layer was seen. Immature pollens embedded into the antheridiumsac sac. Epidrmal cells and mechanical layer are shown in figure 1. Transition cells were wasted in the mature antheridium, and cells of topi layer along with mature pollens were seen inside the mature antheridium sac.




(1) (2)

Figure 1. Mature antheridium with mature pollen (1). Immature antheridium with young microspores (2). E: Epiderm, En: Mechanical layer, MI: Transition cells layer, T: Topi layer cells, Po: Pollens

Microscopic study showed that mature pollen of Persian oak, have more oval and longer than that of immature one. The pollen were tricolpate, and the exine was warty shape (Figure 2). Mature pollens have deeper colpate, in comparison with immature one (Figure 3).

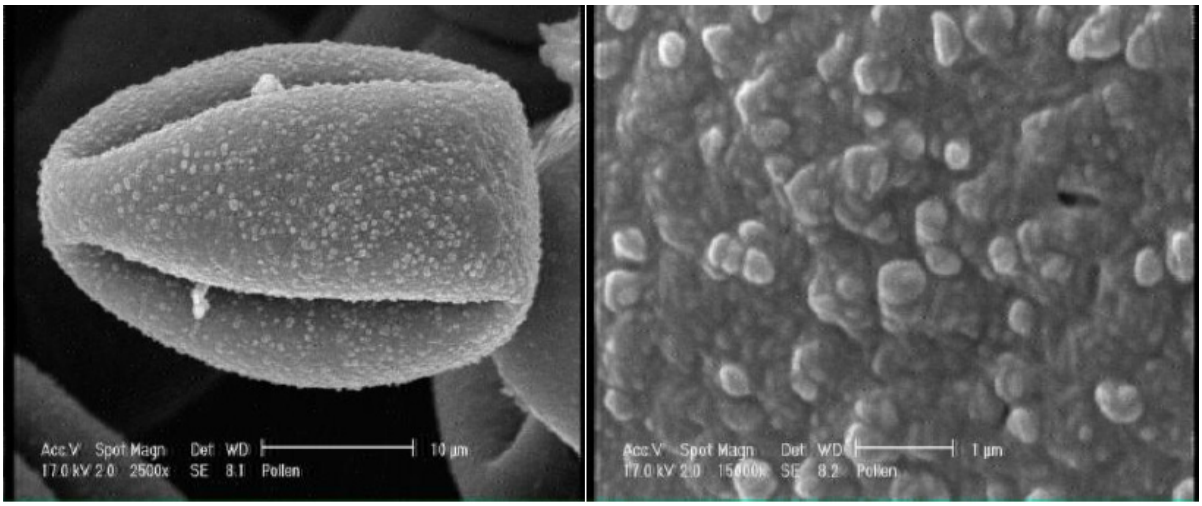



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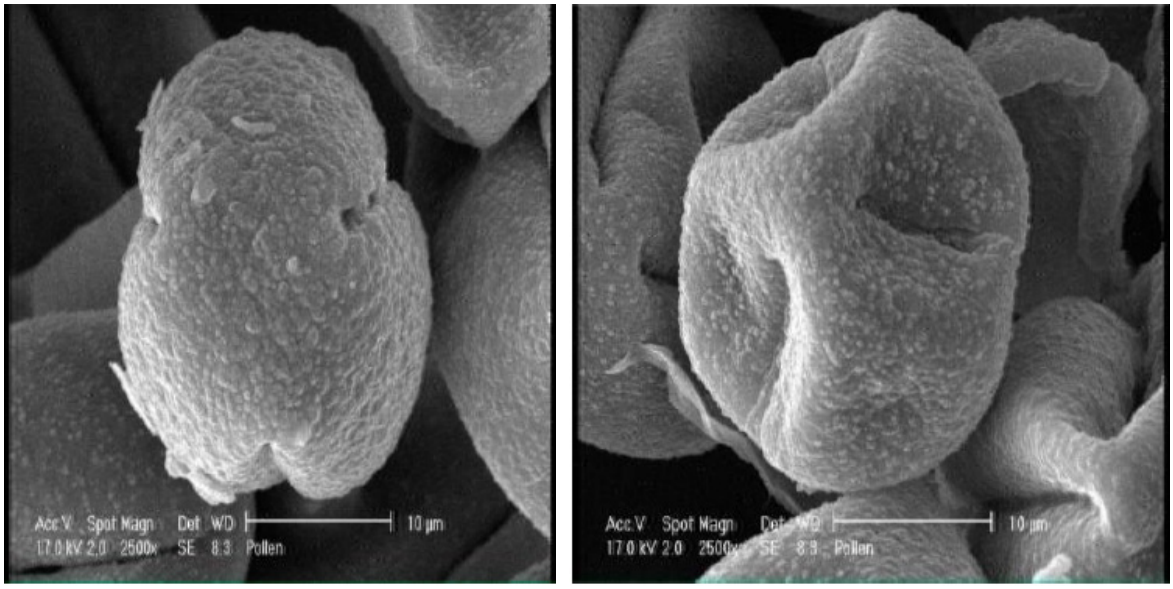
Figure 2. Microscopic image of Persian oak pollen. Mature pollen (1). Warty shape Exine (2)




(1) (2)

Figure 3. Colpate of (1) mature and (2) immature pollens

*3.2 Electrophoresis Profile of Oak Pollen*

16 proteins band in the range of 16,18,23,25,30,35,34,40,43,52,66,86,91,96 and 166 kb as well as one distinct heavier 116 kd band, were seen in the electrophoresis profiles of pollen's proteins extract. Profile of mature pollen had one more bolder 52 kd band, compared to immature one (Figure 4).

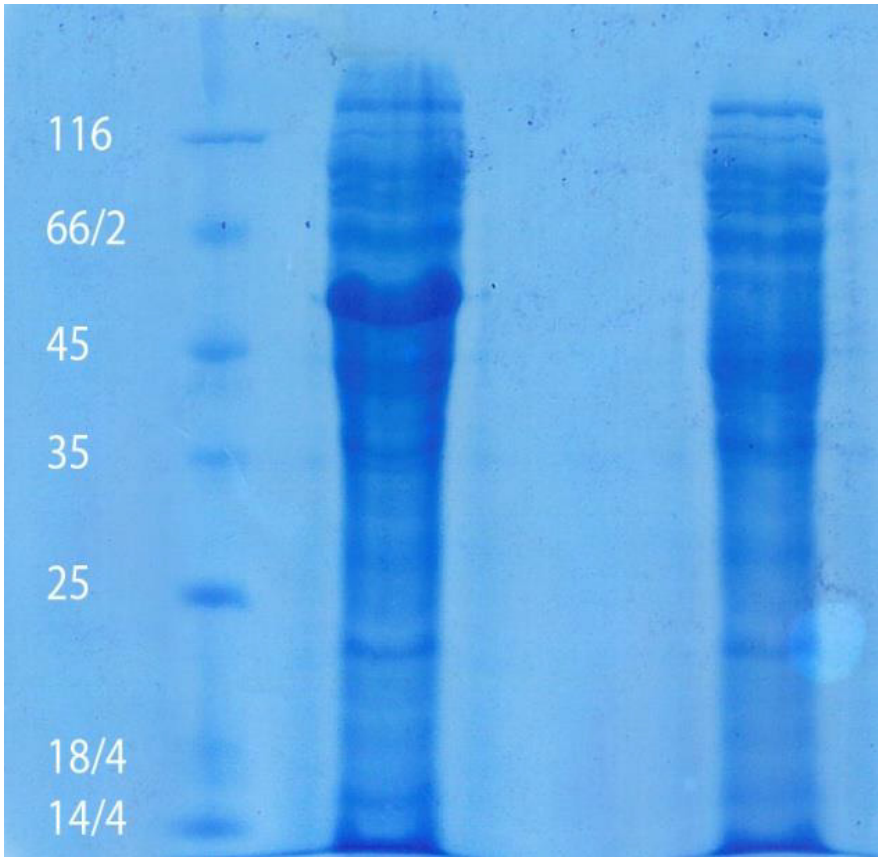
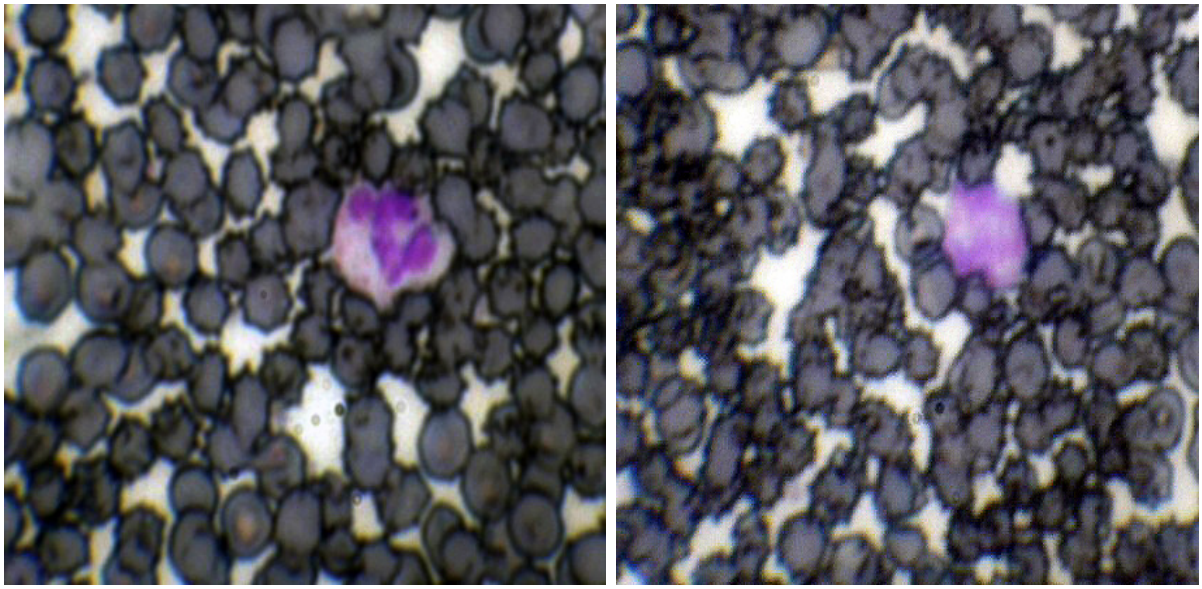



Figure 4. Electrophoresis profile of mature (left) and immature (right) pollens

*3.3 Allergenic Assay*

In the Figure 5, it is shown that eosinophils numbers in the blood of animal samples injected by mature pollen extract, were more than that of control and immature pollen extract injected one.



(1) (2)

Figure 5. Eosinophils (1) and Lymphocyte (2) in the blood test of mature pollen extract injected samples

Both mature and immature pollen extract of Oak, brought about itching of eyes and Sneezing of animal samples, 30 min after dropping the extracts. The allergenic effect was more by the mature pollens (Figure 6). However, the results showed no significant difference in the skin allergenic test of control and immature pollen injection. As the figure 7 shows, mature pollen extract caused more extensive red corona (3 mm diameters) than control and immature treatment samples (about 0.3 diameters).

A B C

Figure 6. The eye allergenic test of (A) control, (B) immature pollen extract, and (C) mature pollen extract

A B C

Figure 7. Skin allergenic test of (A) control, (B) immature pollen extract, and (C) mature pollen extract

*3.4 Blood Tests Results*

The blood sugar level of animal samples was normally 120 mg/dl. By the injection of phosphate saline buffer this level increased by 155 mg/dl. It was also raised up to 196 mg/dl and 159 mg/dl by mature and immature pollen extract injection, respectively. Eosinophils leve of control samples was 1.3%. Mature and immature pollen extract treatment increased it up to 2.7 % and 1.9 %, respectively. Basophils level of control sample was also about 1.1%, but increased by the mature (2.1%) and immature pollen extract (1.3%). Immunoglobulin level of control sample was about 4 U/ml. For mature and immature pollen extract injected samples, 10 and 8 U/ml were reported respectively (Figures 1, 2, and 3).

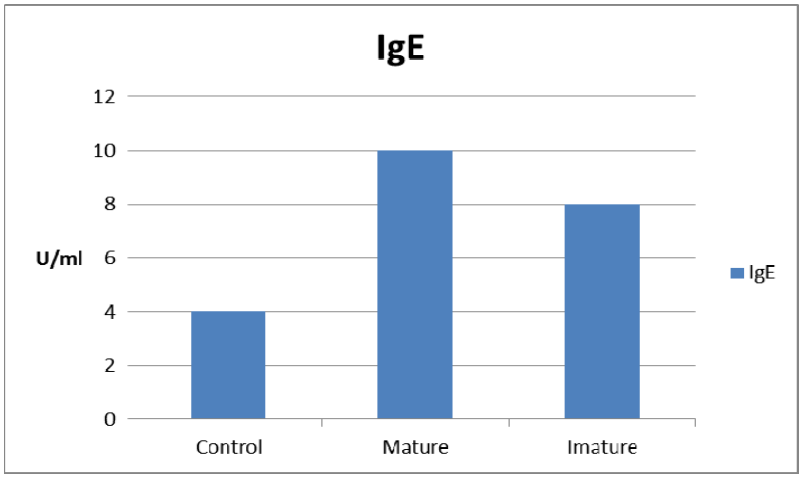



Figure 1. The serologic test; IgE levels

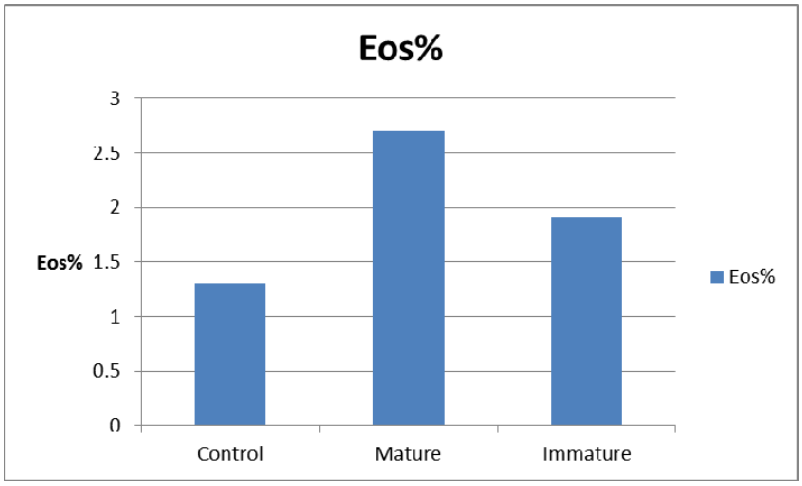



Figure 2. The serologic test; Eosinophils levels

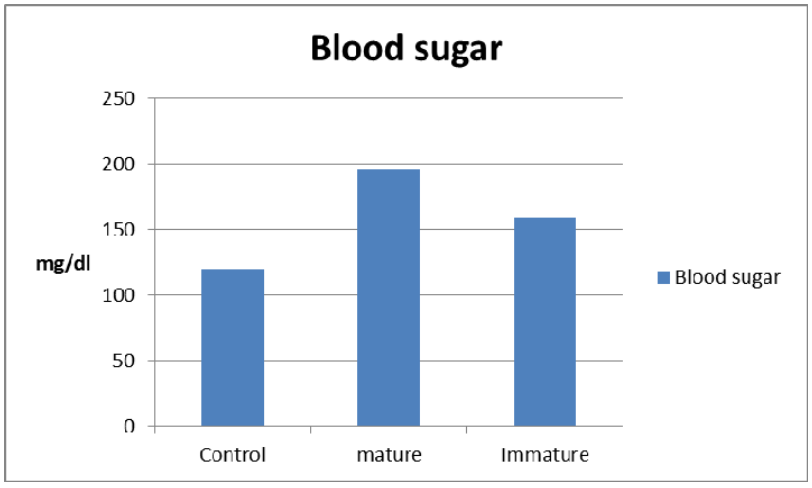



Figure 3. Comparison of blood sugar in treatments and control samples

## 4. Discussion

The results of present study about the general features of Persian oak which reports tricolpate pollens along with warty shape exine, was previously confirmed by Shah (2005). The nutritious cell layer that was Secretory from the beginning, are visible up until the last development phase of microspors in the margin of pollen sac. This layer is eventually changed into Amoeba- like structures. Transition layer was also wasted during the developmental process to feed the pollens (Majd et al., 1997).

Due to the lack of complete development of pollen's wall and exine and intine colpate where allergen factors are placed. The allergenic capacity of immature pollen is not as severe as mature one. Regarding of the results of skin test, it can be concluded that Persian oak pollens cause hypersensitive responses type I. Itching after 30 min of treatments also confirms the hypersensitive. Chehregari et al. (2003) and Rezanejad et al. (2003) reported the same results.

Proteins electrophoresis profile showed significant differences between mature and immature pollen, so that in mature pollens profile, one more distinct and bold band was seen. It shows synthesizing and accumulation of proteins up until the last phase of development, and also the effect of pollen size on allergenic features. Rezanejad et al. (2007) and Sing et al. (1993) confirmed the mentioned results. More IgE level in animal samples injected by mature pollen extract compared to controls, was already reported by Sing et al. (1993). Asarnoj et al. (2010), assayed the allergenic effects of Birch pollens on the human. They reported that based on the ages of samples and treatment duration, the allergenic results were different. Geroldinger et al. (2011), reported allergenic features of Birch pollens. Pérez et al. (2010) showed the effect of environmental factors on the presence of allergenic pollens of *Cupressaceae, Fraxinus ، Olea Platanus* and *Quercus*. Arbabian et al. (2011) reported more allergenic effects of Wheat pollens in the polluted aria than unpolluted one and light level of blood sugar, IgE and eosinophils. Sharif shooshtari et al. (2013) also studied the allergenic features of mature and immature pollens of *Leucanthemum*. They reported more eosinophils, and IgE content in the animal blood samples of mature pollen injected samples. Skin sensitivity was also seen in treated samples.

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