

AN INVESTIGATION OF POLLEN GRAIN THERMAL DIVERSITY ON SPECIES LEVEL

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ABSTRACT. In the current study, the thermal stability of four selected *Pinus* L. species was analyzed in order to test the hypothesis that wethers pollen grain from same genus exhibit any variation on the species level. For this purpose *Pinus sylvestris* L., *Pinus brutia* Ten., and *Pinus nigra* Arn. were subjected to dynamic thermogravimetric analysis. The obtained thermograms the selected four *Pinus* species revealed no significant variation among their thermograms. For all the tested species five main degradation stages have been observed. The dTG_{max} values for *P. griffithii* Mc. Clelland, *P. sylvestris*, *P. nigra* and *P. brutia* were recorded at 442.6 °C, 439.1 °C, 442.2 °C, and 437.6 °C respectively. Considering the results from the current study it can be clearly concluded that the thermal stability of pollen grains from the same genus do not exhibit any significant variation.

1. INTRODUCTION

In plants, the pollen grains are the carriers of genetic materials needed for pollination [1]. Different species contains pollen grains with varying surface morphology and dimension [2-3]. Structurally pollen grains are comprised of two shells, an inner layer, known as intine, is a cellulosic structure, and an external layer known as exine. The the structural makeup of external layer is composed of a polymer called sporopollenin [4]. These layers enable the pollen to protect the genetic material in an efficient way [5]. In addition, researchers are now focusing on the utilization of pollen grains for different applications including drug delivery, adsorption studies [6-8]. As is known that physicochemical properties play a decisive role in determining the type of application for which a specific material will be used. These physicochemical properties show variation depending upon the type and source of a material. Among these characteristics, thermal stability is the most important property defining key features of a material. Like all other polymeric materials, it thought that the physicochemical properties of all pollen grains could not be the same and they could vary from plant to plant and genus to genus. Pollen diversity and identification on the basis of physicochemical properties in different species can be checked by using thermogravimetric analysis (TGA) technique.

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Thermogravimetric (TG) thermal analysis examine the change in mass due to desorption, absorption, vaporization, oxidation, reduction and decomposition of a sample as a function of temperature [9-10]. TGA is an analytical tool used for analysing the thermal behaviour of thermoplastics, thermosets, composites, films, fibers etc., [7, 11-13]. There are three main types of TGA; 1) Isothermal or Static TGA, in which change in overall weight of sample is recorded keeping the temperature. 2) Quasi-static TGA where sample with constant weight is heated and changes at temperature at different intervals is recorded. 3) Dynamic TGA, is a type of thermal analysis the sample is heated at constant temperature range increasing at gradual rate, usually in relation with time. This technique is also known as to as thermodilatometry (TD) if the dimensions of analyte are record under trivial load [9-10, 14]. In the current study, in order to analyse the pollen grains diversity on the species level, we used dynamic thermogravimetric analysis (dTGA).

The current study was aimed to differentiate the pollen grains on species level using thermal stability as the main criteria. For this purpose, four different *Pinus* L. species were selected and subjected to dTGA analysis.

2. MATERIAL AND METHODS

2.1 Sample collection

Here in this study, we tried to explain the thermal properties of four different *Pinus* species. Four species of *Pinus*, i.e., *P. sylvestris*, *P. brutia*, *P. griffithii* and *P. nigra* were used in the current research. Polen samples of *P. griffithii* were collected from the garden of Ankara University Science Faculty and the pollen samples of the remaining three species were collected from Kastamonu province, Turkey.

2.2 Dynamic thermogravimetric analysis (dTGA)

Dynamic thermal gravimetric analysis (dTGA) was used to investigate the thermal stabilities of four *Pinus* species. Briefly, about 5 mg, for each sample, was put in the porcelain cup and heated from 25 to 650 °C using EXSTAR S11 7300 at a heating rate of 10 °C min⁻¹. The thermograms were obtained at the end of the analysis and used for results interpretations.

3. RESULTS AND DISCUSSIONS

In order to determine that whether thermal stabilities of pollen from same species of the same genus variate or not four *Pinus* species were subjected to TGA analysis. Thermograms of four tested *Pinus* species revealed a very similar pattern of weight loss and degradation curves (Fig. 1). For all the tested four species, weight loss occurred in five degradation stages. In all the thermograms the first mass loss can be ascribed to the evaporation of adsorbed water from the polymeric structure of pollen grains [6, 15]. The second, third and fourth mass loss can be ascribed to the degradation of intine (cellulosic structure, genetic material etc.) [15]. In a previous study conducted on pollen weight loss in relation to temperature revealed that the pollen of *P. thunbergii* Lamb. is composed of 9.12% moisture, 4.46–4.91% crude ash, 17.01–18.72% crude protein, 2.50–2.75% crude fat, 2.46–2.71% crude starch, 4.72–5.19% invert sugar and 1.91–2.10% reduced sugar [16]. In another report the increasing temperature results in the step by step degradation of different components at different temperature range. Carbohydrates were the first component to be modified (starting from 45 °C and culminating at 183 °C) followed by proteins (up to 183 °C), in agreement with the Maillard reaction and Strecker degradation mechanisms. The lipid fraction proved to be the most stable, and lipid markers were still recognized up to 300 °C [17]. The fifth mass loss around 400–650 °C is thought to be due to the degradation of exine (sporopollenin).

In the current study, TGA results of the four tested *Pinus* species did not reveal any significant differences in terms of their thermal stabilities. The dTG_{max} for *P. griffithii*, *P. sylvestris*, *P. nigra* and *P. brutia* were recorded at 442.6 °C, 439.1 °C, 442.2 °C and 437.6 °C respectively (Table 1). Considering these results, it can be clearly stated that the thermal properties of pollens in the same genus exhibit high level of similarity.

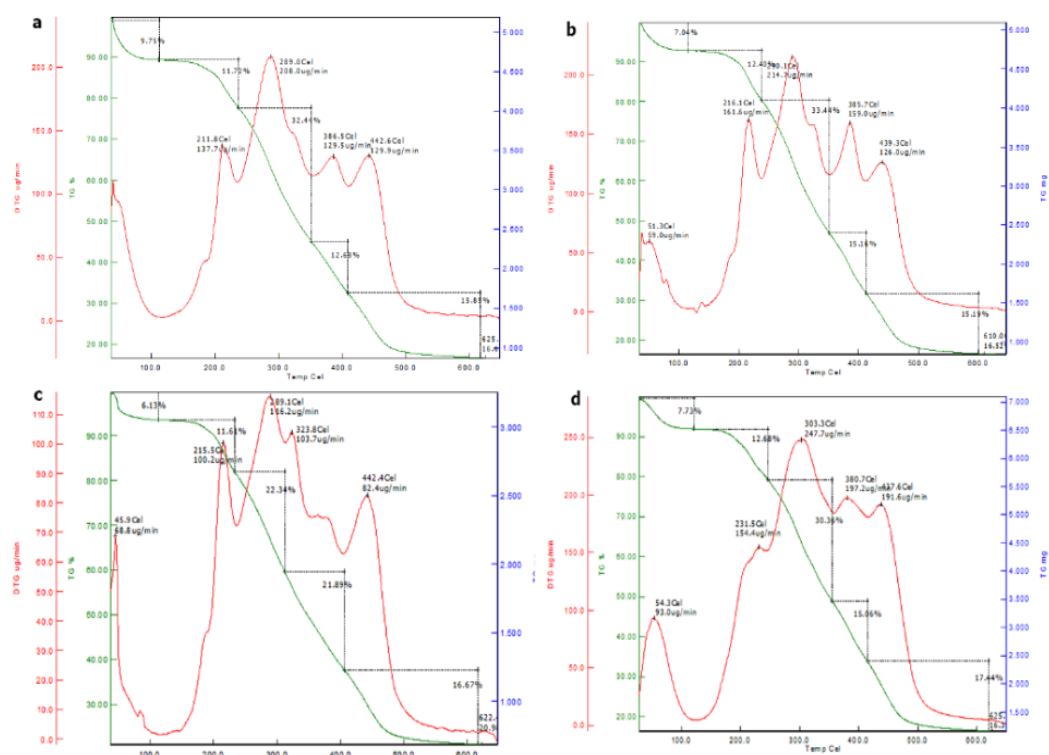


FIGURE 1. dTGA thermograms of four *Pinus* species; a) *P. griffithii*, b) *P. sylvestris*, c) *P. nigra* and d) *P. brutia*

TABLE 1. Thermogravimetric analysis of *P. griffithii*, *P. sylvestris*, *P. nigra*, *P. brutia*

Species	1st weight loss (%)		2nd weight loss (%)		3rd weight loss (%)		4th weight loss (%)		5th weight loss (%)	
	25-100	DT _{Tmax} (°C)	100-250	DTG _{Tmax} (°C)	250-300	DTG _{Tmax} (°C)	300-400	DTG _{Tmax} (°C)	400-650	DTG _{Tmax} (°C)
<i>P. griffithii</i>	9.75	49	11.72	211.8	32.62	289.0	12.68	386.5	15.85	442.6
<i>P. sylvestris</i>	7.04	51.3	12.40	216.1	33.44	200	15.19	385.7	15.16	439.1
<i>P. nigra</i>	6.13	45.9	11.61	215.5	22.34	289.1	21.89	323.8	16.67	442.2
<i>P. brutia</i>	7.73	54.3	12.68	231.5	30.36	303.3	15.06	380.7	17.44	437.6

4. CONCLUSION

Thermal stability is considered to an important property of any polymeric material as it played a decisive role in a certain application for which that specific material is intending to be used. In the same regard, pollen grains from different plant species are gaining interest from divergent fields of science in terms of a broad set of application such as pharmaceuticals, packaging and cosmetic industry. In the same regard in the current study, the thermal stability of four *Pinus* species was investigated. Results revealed that thermal stabilities of pollen grains isolated from different species of the same genus do not exhibit significant variation. The findings of the current study will be helpful for the future applications of pollen as a material in different fields.

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