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Research Article

Plantago lanceolata L. Seed Coat Variation and Its Implications on the Usage of Seed Micromorphology Among Widespread Taxa

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ABSTRACT

Seed coat micromorphology has been great help in systematic studies over the past century. It is useful in most plant groups, including the widespread taxa. Although phenotypic plasticity in plants is a well-known phenomenon, there are not many micromorphological studies investigating whether plants with wide distribution areas show variation in seed coat characteristics. *Plantago lanceolata* L. (Plantaginaceae), as one of the most widespread *Plantago* taxa, is used in this study to investigate the presence of seed microsculpture variation among different populations. The seeds of 50 populations from across Turkey were analyzed using Stereomicroscope and SEM. Some seed surface characters of *P. lanceolata* were found to be consistent (cell shapes and anticlinal wall shapes), whereas others were variable (anticlinal walls, periclinal walls and surface sculpture). Irregularly wrinkled surface is reported in *P. lanceolata* seeds for the first time in this study. No correlation between geographical differences and seed characters were found. These results indicate that caution should be taken when dealing with widespread taxa with high levels of variation.

Keywords: *Micromorphology, Plantago, SEM, Seed coat*

Plantago lanceolata L. Taksonunda Tohum Kabuk Varyasyonu ve Bunun Yaygın Bitki Taksonlarında Tohum Yüzey Karakterlerinin Kullanımına Etkileri

Öz

Tohum kabuğu mikromorfolojisi, geçtiğimiz yüzyılda sistematik çalışmalara çok yardımcı olmuştur. Yaygın taksonlar dahil olmak üzere, çoğu bitki grubunda faydalı karakterler vermektedir. Bitkilerde fenotipik plastisite iyi bilinen bir kavram olmasına rağmen, geniş yayılış alanlarına sahip bitkilerin, tohum kabuğu karakterleri özelinde değişiklik gösterip göstermediğini araştıran çok fazla mikromorfolojik çalışma bulunmamaktadır. En yaygın sinirliot türlerinden biri olan *Plantago lanceolata* L. (Plantaginaceae), bu çalışmada farklı popülasyonlar arasında tohum mikromorfolojisinde varyasyonun varlığını araştırmak için seçilmiştir. Türkiye genelinden 50 popülasyonun tohumları ışık mikroskobu ve tarama elektron mikroskobu kullanılarak analiz edildi. *P. lanceolata*'nın bazı tohum yüzey karakterlerinin tutarlı olduğu (hücre ve antiklinal duvar şekilleri), diğerlerinin ise değişken olduğu (antiklinal duvarlar, periklinal duvarlar ve yüzey özellikleri) tespit edilmiştir. *P. lanceolata* tohumlarında düzensiz buruşuklara sahip yüzey özelliği, ilk kez bu çalışmada rapor edilmiştir. Coğrafi

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farklılıklar ile tohum karakterleri arasında bir ilişkiye ise rastlanmamıştır. Bu sonuçlar, yüksek düzeyde varyasyona sahip yaygın taksonlarla tohum mikromorfolojisi çalışılırken dikkatli olunması gerektiğini göstermektedir.

Anahtar Kelimeler: *Mikromorfoloji, Plantago, SEM, Tohum kabuğu*

I. INTRODUCTION

Plantago L. (Plantaginaceae) taxa, like many others, have been subject to a number of seed micromorphology studies. Among these taxa, *Plantago lanceolata* L. is one of the most geographically widespread and popular subjects for study. It is economically important for medicinal uses [1]. Both natural and naturalized populations contribute to its wide distribution around the world. *Plantago lanceolata* may be considered a successful weed as it can adapt to a variety of environmental conditions. Its wide distribution and adaptability have resulted in wide phenotypic variation. Thus, it has been subject to numerous studies on plasticity [2-5].

Phenotypic plasticity is an organism's response to environmental conditions through changes in its phenotype. It has long been known that the growth habit of *P. lanceolata* is highly variable, and most of its vegetative and reproductive traits vary in parallel [6].

The seeds of some *Plantago* taxa are important sources of medicine [7-9], as well as cosmetics and nutrition [10]. It is of vital importance to correctly identify plants when it comes to ethnobotanical uses. However, information on *Plantago* seed characteristics in literature vary as much as the *Plantago* itself [11]. This is probably due to the wide distribution and high adaptability of some widespread plantains.

Seed surface micromorphology is considered a good character in the majority of plant groups and has found wide usage in systematic studies. Micromorphological studies on the seeds of various *Plantago* taxa have been carried out in recent years [12-15]. All of these studies found seed microsculpture to be useful in systematics but none of them mentioned variation of this trait within or between populations.

The aim of this study is to understand the limits of plasticity of seed surface sculpture characteristics using herbarium specimens collected throughout Turkey and to provide insight into the usage of seed micromorphology in systematic studies within widespread taxa.

II. MATERIAL AND METHODS

A. PLANT MATERIAL

The plant material used in this study was taken from the collection of Istanbul University Science Faculty Herbarium (ISTF). Plants were collected from across Turkey in 1941-2002. 50 specimens with mature seeds in good condition were selected for use in this study and two seeds were taken from each specimen. Detailed information on specimens is given in Table 1.

Table 1. *Collection information of the specimens examined in this study.*

ISTF No	Location	Habitat and altitude	Date	Collector
1102	Kayseri		2.8.1941	A. Heilbronn, M. Heilbronn
15794	İstanbul: Kilyos		24.6.1956	B. Tutel
22329	İstanbul: Tuzla	roadside	30.6.1967	R. Bulut

22343	Tekirdağ: Marmara Ereğlisi		1.7.1969	G. Sanlı
22528	Balıkesir: Edremit, Akçay	seaside, sea level	6.8.1967	B. Tutel
22686	Kocaeli: Bayramoğlu-Çayırova	field edge	23.8.1967	B. Tutel, A. Aydın, R. Bulut
22829	İstanbul: Bostancı-Altıntepe	roadside	30.10.1967	A. Aydın
23330	Tekirdağ: Barbaros, Kumbağ	roadside	1.8.1968	R. Bulut
23390	Konya: Beyşehir-Avşar gardens	damp places	18.8.1968	R. Yiğit
23402	İstanbul: Yalova	seaside, sea level	29.8.1968	B. Tutel
23867	Adana: Feke, around Çardak	roadside, field edge, 736 m	25.6.1969	A. M. Eld.
23964	Trabzon: Akçaabat-Başikdüzü	hills, 20 m	1.7.1969	H. Demiriz
24068	Antalya: Güzeloba	damp places, 18 m	5.7.1969	G. Kaynak
24086	İzmir: Kemalpasa, Bornova	roadside	6.7.1969	H. Demiriz
24291	Rize: İyidere, Fethiye	field edge, 50 m	21.7.1969	A. Çırpıcı
24302	Rize: İyidere, Fethiye, Çanakçeşme valley	250 m	21.7.1969	A. Çırpıcı
24370	İstanbul: Levent, Sarıyer, Etiler	field edge	29.7.1969	B. Tutel
24431	Erzurum: Palandöken, mouth of Kırkdeğirmen river	stony roadside, 2230 m	3.8.1969	H. Demiriz, O. Özbay, S. Özyurt
24592	İstanbul: Silivri, Semizkum	20 m	20.8.1969	H. Demiriz
24608	İstanbul: Sarıyer, Bahçeköy, Forestry Faculty	meadow	4.9.1969	H. Demiriz, B. Tutel, S. Ozyurt
24610	İstanbul: Sarıyer, Bahçeköy, Neşet suyu	meadow	4.9.1969	H. Demiriz, B. Tutel, S. Ozyurt
24617	İstanbul: Sarıyer, Kemerburgaz-Ayazaga, Cendere valley	roadside, field edge	4.6.1969	B. Tutel, S. Özyurt, H. Demiriz
24618	İstanbul: Sarıyer, Kemerburgaz-Ayazaga, Cendere valley	roadsidei field side	4.9.1969	H. Demiriz, B. Tutel, S. Özyurt
25042	İstanbul: Kadikoy, Selamicesme-Ciftehavuzlar	damp places	27.7.1970	A. Aydın
25588	Düzce: Gümüsova, Cukuralan	damp places	21.6.1971	M. Tüzün
26417	Kocaeli: İzmit-Golcuk	roadside, field edge	25.6.1972	B. Tutel
26553	İstanbul: Şile, around Fener	hills	28.7.1972	B. Tutel, A. Aydın, İ. Delice
26612	İstanbul: Kartal, Aydos, S. facing slopes	meadow, 450 m	16.8.1972	İ. Delice
26659	İstanbul: Florya	seaside, 10 m	29.8.1972	B. Tutel
27342	Bitlis: Tatvan, Kirkor mountain S slopes	hills, 1900 m	18.7.1973	H. Pesmen
27399	Samsun: Terme, Unye	roadside	10.8.1973	N. Tutel
28239	Rize: İyidere, Fethiye Mah.		28.9.1974	A. Çırpıcı, İ. Delice
28317	Erzurum: Tortum, Kaledibi, Tortum river valley		1.10.1974	H. Demiriz, A. Çırpıcı, İ. Delice
29739	Kayseri: Bünyan-Pınarbaşı, Pazarören road fork	dry meadow	14.7.1976	A. Çırpıcı
29807	Bingöl: Yolçatı, Bilaloğlu	stony roadside, 1570 m	17.7.1976	H. Demiriz, B. Tutel, A. Çırpıcı
29913	Bitlis: Tatvan, Küçüksu-Gevas, Van Lake S shore	stony roadside, 1630 m	19.7.1976	B. Tutel, H. Demiriz

30008	Diyarbakır: Lice, Hazro, Uzunargit, Değirmen	damp places, 890 m	3.8.1976	S. Alakuş, H. Demiriz, H. Olgaç
32126	Nigde: Aksaray, Hamidiye		22.6.1978	T. Altug
32660	Kütahya: Gediz, Murat Mountain			A. Çırpıcı
32724	Edirne: Enez, University Camp	damp places	19.8.1978	T. Çelebioglu, T. Altuğ
35936	Denizli: Pamukkale	meadow	13.8.1991	B. Tutel
35942	Edirne: Keşan, Erikli köyü, Yali mv.	roadside, field edge, sea level	23.8.1991	B. Tutel
35985	Aksaray: Güzelyurt, Ihlara valley	meadow, 1271 m	29.10.1991	S. Kuş
36101	Antalya: Beydağları, Tünektepe	roadside, field edge, 670 m	3.6.1993	B. Tutel
36182	Amasya: Taşova, Borabay lake, S slope	dry hills, 1080 m	7.8.1993	E. Üzen
36440	Eskişehir: Kümbet, Yazılıkaya		15.7.1994	E. Üzen
36454	Afyon: Çay, Doğanköy, Eber lake	damp places, 994 m	16.7.1994	E. Üzen, F. Kurt
36473	Antalya: Manavgat	damp places, 20 m	25.7.1994	E. Üzen
37026	İstanbul: Kıraç, Bahçeşehir, W slopes	50 m	4.7.1997	E. Üzen, S. Kuş
40641	İstanbul: Süleymaniye, Botanical Garden		18.6.2002	E. Üzen

B. MACROMORPHOLOGY

Seed photos were taken with Olympus SZX7 stereomicroscope connected to a Canon DSLR camera. Measurements were made using Kameram v3.1 software.

C. SURFACE MICROMORPHOLOGY

Seeds were cleaned with a soft brush dipped in 70% ethanol and air dried before fixing them dorsal side up on aluminum stubs with double-sided carbon tape. They were then coated with gold-palladium for 45 seconds using a EmiTech Sputter Coater. They were examined and photographed with Jeol Benchtop SEM at 100x, 300x, 1000x and 3000x magnifications.

III. RESULTS

The seeds of the studied populations are between 1.55-2.49 (average 2.07 ± 0.25) mm in length and 0.69-0.34 (average 2.07 ± 0.25) mm in width. The length to width ratio is 0.3-0.7 (avg. 0.5). Color varies from brown to almost black (Figure 1).

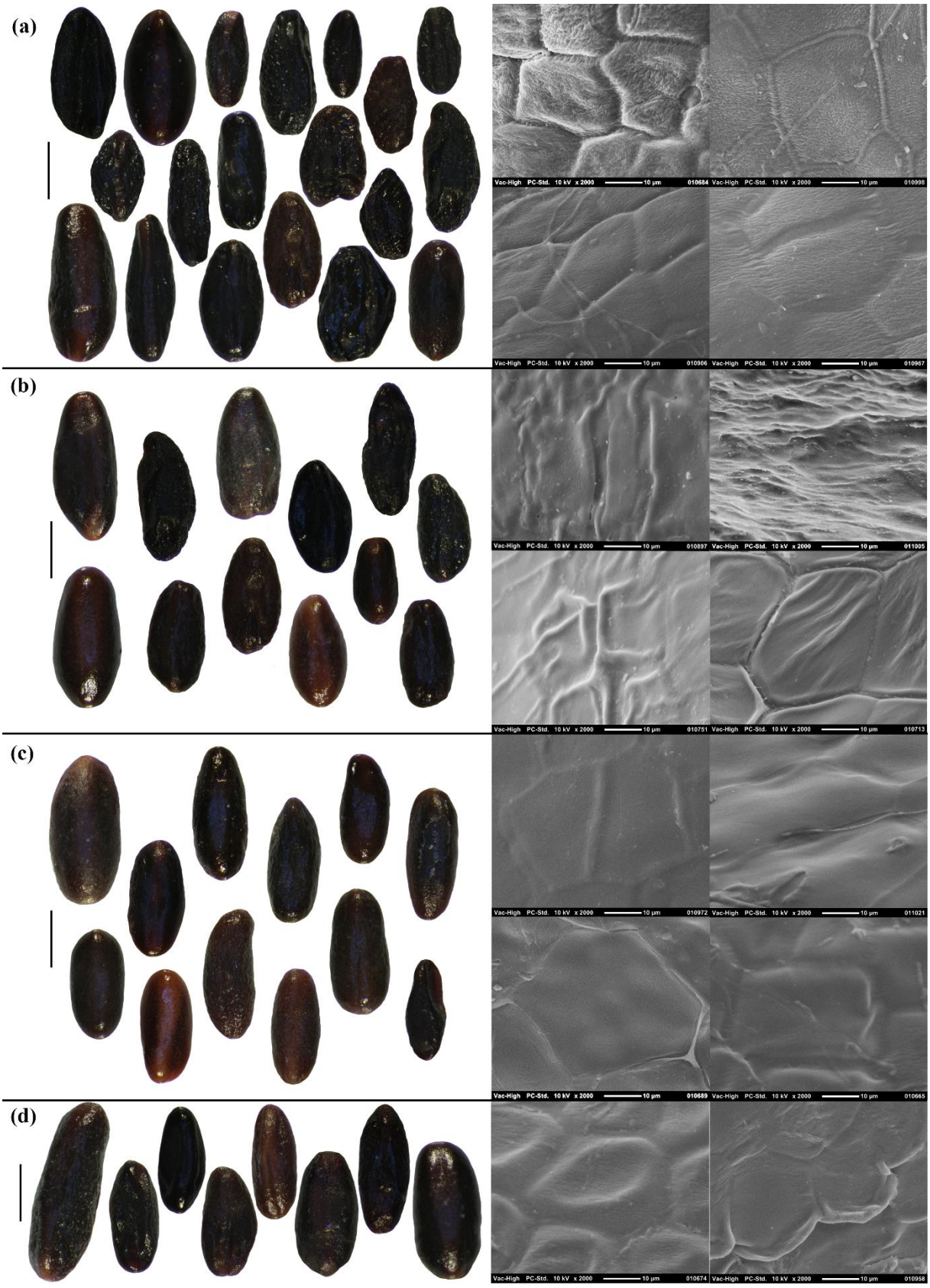

Figure 1. *Seed macro- and micromorphological variation. Left column shows stereomicroscope photos of studied seeds (the scale bar is 1 mm) and right column shows examples of seed surface characteristics.* ***(a)*** *Seeds with reticulate seed surface* ***(b)*** *Seeds with irregularly wrinkled seed surfaces* ***(c)*** *Seeds with smooth seed surface* ***(d)*** *Seeds with slightly rugose seed surface.*

Seed surface microstructure is variable; however, cell and anticlinal wall shapes are invariably isodiametric and straight, respectively. Anticlinal wall levels are raised or grooved, with grooved being the more dominant characteristic found in 78% of the seeds. Periclinal walls are flat (68%), convex (20%) or concave (12%). Seed surface ornamentation is reticulate, irregularly wrinkled, smooth or slightly rugose. The most frequently observed surface ornamentation types for *P. lanceolata* seeds are reticulate (Figure 1 a) and irregularly wrinkled (Figure 1 b) surfaces. Smooth (Figure 1 c) and slightly rugose (Figure 1 d) surfaces are the other two surface ornamentation observed. Despite the fact that the irregularly wrinkled surface has not previously been reported, it was commonly observed in this study. Although surface characteristics varied between populations, no correlation was detected in relation to geographic distribution or habitats (Figure 2).

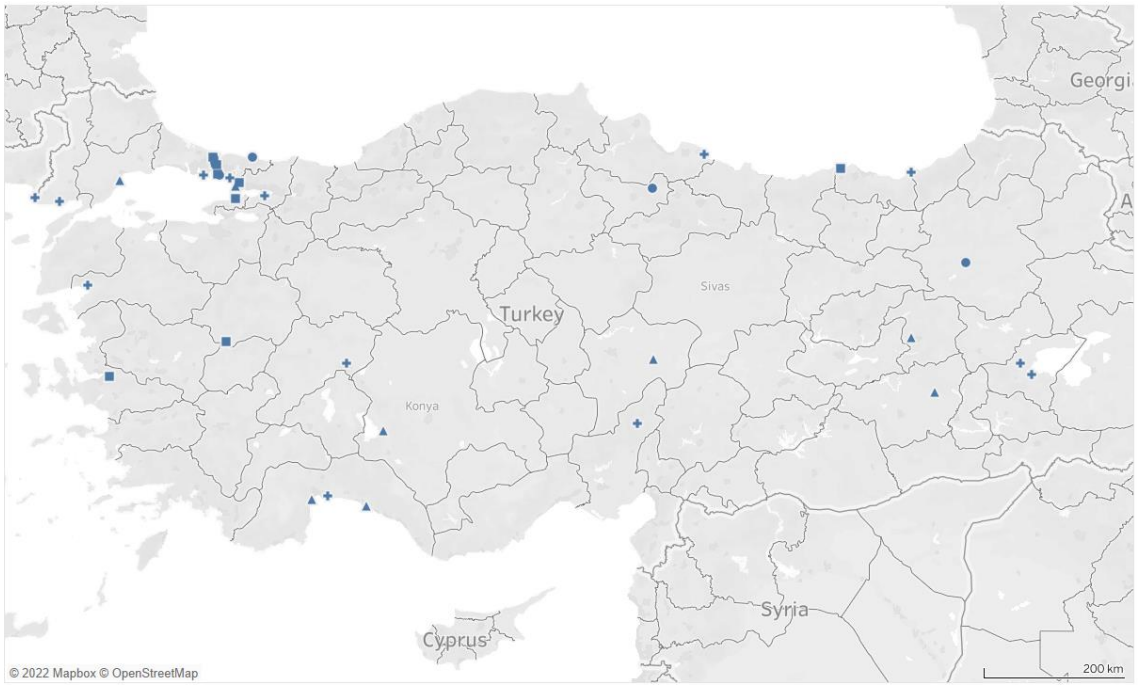

Figure 2. *Distribution map of the populations studied. The shapes indicate surface sculpture (square: irregular wrinkles; cross: reticulate; round: slightly rugose; triangle: smooth). The map was created with Tableau public v.2022.1.1.*

IV. DISCUSSION

Previous studies on *Plantago* seed micromorphology have generally found *P. lanceolata* seed coat structure to be reticulate. Although the terminology differs between literature, the surface characteristics are similar but not exactly the same as those of other studies. Surface characteristics similar to those of the current study were described by Shehata & Loutfy [13], Klimko *et al.* [14] and Jun-Zhe *et al.* [15] as "scalariform to reticulate," "reticulate" and "reticulate," respectively. This is the most common surface type of *P. lanceolata* (41% of all 50 studied populations) found in the current study. The other common surface type in previous studies is smooth [12, 16], which was found in about 25% of populations studied here. Although *P. lanceolata* seeds with irregular wrinkles had a high rate of occurrence (20% of the populations), such ornamentation has not been reported in previous studies. The surface termed "slightly rugose" in this study has a frequency of 14%, and is similar in appearance to a *P. lanceolata* surface in another study [17], where it was described as "negative reticulate".

This study shows that seed coat characteristics in *P. lanceolata* vary in anticlinal and periclinal wall levels as well as surface sculpture. Although this is an expected outcome, the surface sculpture showed no correlation between habitats, altitude, collection dates or location of the plants. However, a previous study [3] has shown that in *P. lanceolata*, lighter or darker flowers are formed depending on the

temperature, and by doing so they can control the embryonic development of their offspring. Similarly, although effects of temperature or other ecological factors on seed surface properties have not yet been studied, it may have an effect on seed surface micromorphology considering high plasticity of *P. lanceolata*. However, a detailed study with more precise ecological parameters and thorough sampling would be needed to draw a solid conclusion.

This may be considered a preliminary study due to its limited geographical range and lack of infra-population comparison. However, it is clear that there is considerable variation between seed surfaces of different populations of *P. lanceolata*, therefore caution should be taken when using seed coat characters in systematic work in the case of widespread taxa with high morphologic variation.

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