COMPARATIVE ANATOMICAL-PHYSIOLOGICAL STUDY OF CALCICOLE AND CALCIFUGE SPECIES

F. Zarrinkamar

Zarrinkamar, F. 2000 08 01: Comparative anatomical-physiological study of calciole and calcifuge species. *-Iran. Journ. Bot. 8(2): 279-286.* Tehran.

This research analyzed the different anatomical-physiological characteristics of two leguminous plants: the calcifuge plant *Lupinus luteus* was compared with the calcicole plant *Vicia faba.* The difference in structures, the dense glandular hair coverage, the presence of crystals and tanniferous cells in *V. faba*, together with physiological factors, are among the reasons why calcicole plants are more resistant to excesses of calcium.

Fatemeh Zarrinkamar, Research Institute of Forests and Rangelands, P. O. Box 13185-116, Tehran, Iran.

Key words. Lupinus luteus, Vicia faba, anatomy, calcifuge, calcicole, physiology.

مطالعهٔ آناتومی-فیزیولوژی دو گونه Calcicole (آهک پسند) و Calcifuge (آهک گریز)

فاطمه زرّین‌کمر

در طی این تحقیق تفاوتهای آناتومی-فیزیولوژی دو گونه از خانوادهٔ پروانه آسا به نامهای *Lupinus luteus* L. (آهک گریز) با گونهٔ *Vicia faba* L. (آهک پسند) مورد مقایسه قرار گرفت. وجود ویژگیهایی از قبیل تراکم بالای کرکهای غده‌ای در واحد سطح اپیدرم، وجود کریستال و ترکیبات تانینی در بافتهای مختلف مربوط به گونهٔ *V. faba* به همراه عوامل فیزیولوژی دلایل لازم را برای سازگاری این گونه با شرایط آهکی فراهم می‌نماید.

INTRODUCTION

The differing sensitivities of plants to excesses of calcium has long attracted the attention of ecologists and agronomists. The difference between the less common "calcifuge" species and their "calcicole" equivalents (more common in certain regions), being among the major factors governing distribution of species, has been recognized since 1880. However, it is only recently that coherent hypotheses have begun to be formulated concerning the mechanisms which allow calcicole plants better to resist excesses of calcium. Calcium absorption has been studied on two leguminous plants the *Lupinus luteus* and the *Vicia faba*, which are distinguished essentially by their capacity to absorb certain mineral ions, particularly calcium. The *L. luteus* is a calcifuge plant which does not grow naturally on calcareous soil. However, *V. faba* is a calcicole plant which grows naturally on a soil which is rich in calcium carbonate (Salsac 1970). The difference in behavior between calcifuge and calcicole species is that the former become intoxicated by excesses of calcium, while the latter manage to regulate intake in cases of excess. This was the conclusion drawn from the very detailed experimental studies by Robert (1915) and Iljin (1938, 1951, 1952), which examined calcium levels in some 200 species. Salsac (1969, 1970, 1973) systematically examined the kinetics of net calcium absorption by the excised roots of the "calcicole" *V. faba* and *L. luteus* the "calcifuge" demonstrating that calcium absorption by the roots was apparently not directly linked to metabolism. Jacobson, Moore & Hannapel (1960) suggest that calcium absorption is largely a matter of surface fixation on walls and membranes. Leggett & Gilbert (1967) are of the same opinion. Mattson (1948) and Wacquant & Passama (1971) suggest that surface absorption phenomena play a primary role during the first stage of absorption. The calcium is fixed at a limited number of sites, located for the most part at the cell-medium interfaces. Depending on the time factor (Salsac 1973), highly concentrated external calcium accumulation is more pronounced in the roots of calcifuge plants than in those of calcicole. With high concentration of Ca++, the "calcicole" limits the element's endo-cellular penetration, unlike the "calcifuge" the element's invasion of endo-cellular compartments is certainly one of the causes of the calcifuge species'

intolerance of calcareous soils. In the course of our experiments to compare the anatomy of the two types of plants. we observed differences and, accordingly, drew certain conclusions.

MATERIALS AND METHODS

The seeds of *Vicia faba* and *Lupinus luteus* were obtained from seed bank at the Bastions University, Geneva and planted in phytotron for 4 or 5 weeks.

Transverse sections of leaf, stem and root were prepared by hand cutting. Sections were stained in Safranin, Fast-green and Carmino-Vest. In addtion material were embedded in Paraffin Wax, then sections Stained with safranin, Gentian violet. Observations were carried out with Stereo-Microscope, Light Microscope and Scaning Electron Microscope.

In order to study venation and stomata density, the diafanization technique (Dizeo de Strittmater 1973) was employed.

OBSERVATIONS

Foliar anatomy

General characteristics: surface view of the diafanized leaf, epidermis of quadrangular cells, the cuticle thick, stomata and hairs usually on both surfaces. the stomata superficial (fig. 1, A, B, F). In *L. luteus*, density is high on the abaxial surface, stomata are generally anisocytic and anomocytic, the latter being more frequent in *L. luteus* (fig. 2, A). Hair of single-series non-glandular type, with short basal cells and an elongated terminal cell (fig. 2, B). In *V. faba*, glandular-type hair, spherically-topped glands with abundant short basal cells between the cuticles (fig. 1, C, E, F).

Vascularization

The midvein principal is enlarged, without major ramification of the vascular network. The areola are large polygons with ramified venules, most areola having a single venule which ends in a scleroid. The venules are simple, linear or curved, with only rare ramification (fig. 1, D).

Transverse section of the leaf

General characteristics: unistratified epidermis with quadrangular cells, both leaf surfaces have thick cutiele and stomata superficial. There are one or two layers of hypodermis on the adaxial surface. Mesophile types are usually dorsiventral

(fig. 1, G; fig. 2, C). The central layers of the mesophyle are occupied by law-chlorophyll cells and often, but not always, with brown-colored tanniferous cells. In *V. faba* the mesophyle is uniform, but in *L. luteus*, it is made up of several small groups of palisade cells. The vascular bundles are collateral, formed of xylem and phloem elements and an abaxial sclerenchymatic cap; this is made up of lignified sclereids interspersed with gelatinous fibers layers S1 and S2 lignified and S3 non-lignified (fig. 1, H; fig. 2, D).

Crystals in Vicia faba

Standing alone and accompanied by vascular bundles in the leaf. Crystals in the form of styloid bars in the palisade cells.

Stem

The primary stem in *V. faba* is of a U-shape but, when developed, is quadrangular. In *L. luteus*, it is circular and the medulla at the center is large. It shows a unistratified epidermis with quadrangular cells, with a thicker external tangential wall and thick cuticle. The hair is generally of the same type seen on the leaves. Hypodermis consist of one or two layers interrupted by the substomatical chambers. At the corners of *V. faba* stem, the thick cuticle and external part of the primary cortex are often collenchymatous. The cortical parenchyma is composed of small non-lignified cells, with few layers. The inter-fascicular parenchyma is made up of iso-diametric cells, single-punctuation. The vascular bundles are collateral and arranged in a circle; each bundle has a cap of sclereids and fibers (fig. 1, J, K; fig. 2, E, F). In the adult stem, a secondary structure is barely discernible. The peripheral zone of the cylindrical vascular complex includes the phloem. The periphlomatic fibrous cap is the first feature to be distinguished. The phloem consist of sieve tube with companion cells and phlomatic parenchyma cells and fibers. The rays are of the thickness of a cell. Within the rays, the cells are isodiametric, with a thin wall. The xylem is diffuse; the bundles are small and short. The "brown" colored tanniferous cells and sometimes proteins and mucilage, are often found in the primary or secondary phloem and, less frequently, in the primary cortex.

Root

General characteristics of transverse-section: the root structure of the two

species is uniform. Young roots are of a tetrachic structure; there is a secondary structure which, looking inward, shows a generally fine suber, with small thin-walled cells.

The phloem consists of sieve tubes with companion cells, phlomatic axial parenchyma, fibers and rays. The cells of the parenchyma are large, with a primary wall. The xylem includes a vessel elements. A perforation plate is single, alternated pits and rays in series of 4 or 6, mixed with the axial parenchyma, fibers and sclereids (fig. 1, L; fig. 2, H). The center of each structure is occupied by the large-cell medullar parenchyma, a mixture of fibers and sclereids.

CONCLUSIONS

Our experiments confirm that calcium absorption by the plant is not directly linked to cellular metabolism. Cytoplasmic membranes play an important role in calcium absorption by vegetables. Fixation is particularly marked on plasmalemma. It appears that calcifuge plants become intoxicated by an excess of calcium because they cannot limit fixation as calcicoles can. In plasmalemma, an excessive intake of calcium could lead to changes in the permeability of organelles. This suggests a process where by, in calcicoles, calcium fixation is regulated by the phospholipid membranes. The anatomical characteristics analyzed in the course of this research have shown that there are not many structural differences between the two species. The major difference is in the hairs: the *V. faba* has a high density of glandular hairs. In general, glandular hair is involved in the secretion of various substances such, for example, as saline solutions. The presence of crystals is also noted in *V. faba*. These constitute a mechanism for eliminating excesses of calcium. Also, the brown-colored tanniferous cells sometimes contain mucilage in the primary cortex. These are all reasons why the plants adapt to an environment with excessive calcium. The observations make it possible to conclude that the plants compared resemble a complex laboratory. There are both internal and external factors which allow the plant to opt for the best, positive and valid solution.

REFERENCES

Iljin, W. S. 1938: Calcium content in different plants and its influence on production of organic acids. -Bull. de

Í Assoc. russe pour les recherches scientifique Á Prague, 7, 43-76.

- 1951: Metabolism of plants affected with lime-induced chlorosis. -Plants and Soil 3: 339-351.

- 1952 - Metabolism of plants affected with lime-induced chlorosis. -Plants and Soil 4: 11-28.

Jacobson, L., Moore, D. P. & Hannapel, R. J., 1960: Role of calcium in absorption of monovalent cations. -Plant Physiol. 35: 352-358.

Leggett, J. E. & Gilbert, W.A. 1967: Localization of the calcium-mediated apparition (?) selectivity in the cross-sectional volume of soybean roots. -Plant Physiol. 42: 1658-1664.

Mattson, S., 1948: Laws of ionic exchange. III. donnan (?) equilibria in plant nutrition. -Ann. Roy. Ager. Coll., Sweden 15: 308-316.

Robert, T. 1915: Recherches sur le role physiologique du calcium chez les végétaux. Sc. Nat., Paris, 167p.

Salsac, L., 1969: Caractéres particuliers de l absorption du calcium. -Bull. Soc. Fr. Physiol. Vég 15: 213-236.

- 1970: Absorption du calcium par les racines de féverole (calcicole) et de L. luteus jaune (calcifuge). Thése Doct., Etat, Sc, Nat, Paris, 146p.

- 1973: Absorption du calcium par les racines de féverole (calcicole) et de L. luteus jaune (calcifuge). -Physiol. Vég. 11: 95-119.

Fig. 1. A - L, Anatomy of *Vicia faba*; A - C, F, observation in SEM; A, adaxial surface; B - F, abaxial surface showing distribution and position of stomata and hairs; G - H, transverse section of the leaf; I, petiole in TS; J, K, stem in TS; L, root in TS (A = 12μm; B,C,F = 14μm; D,E,H = 50 μm; G, I, J, L = 100 μm).

Fig. 2. A - H, Anatomy of *Lupinus luteus*; A - B, surface views of adaxial foliar epidermis showing distribution and position of stomatas and hairs; C, D, transvers section of the leaf; E,F, stem in TS; G, petiole in TS; H, root in TS (A, D, F= 50 μm; B, C, G, H = 100 μm).

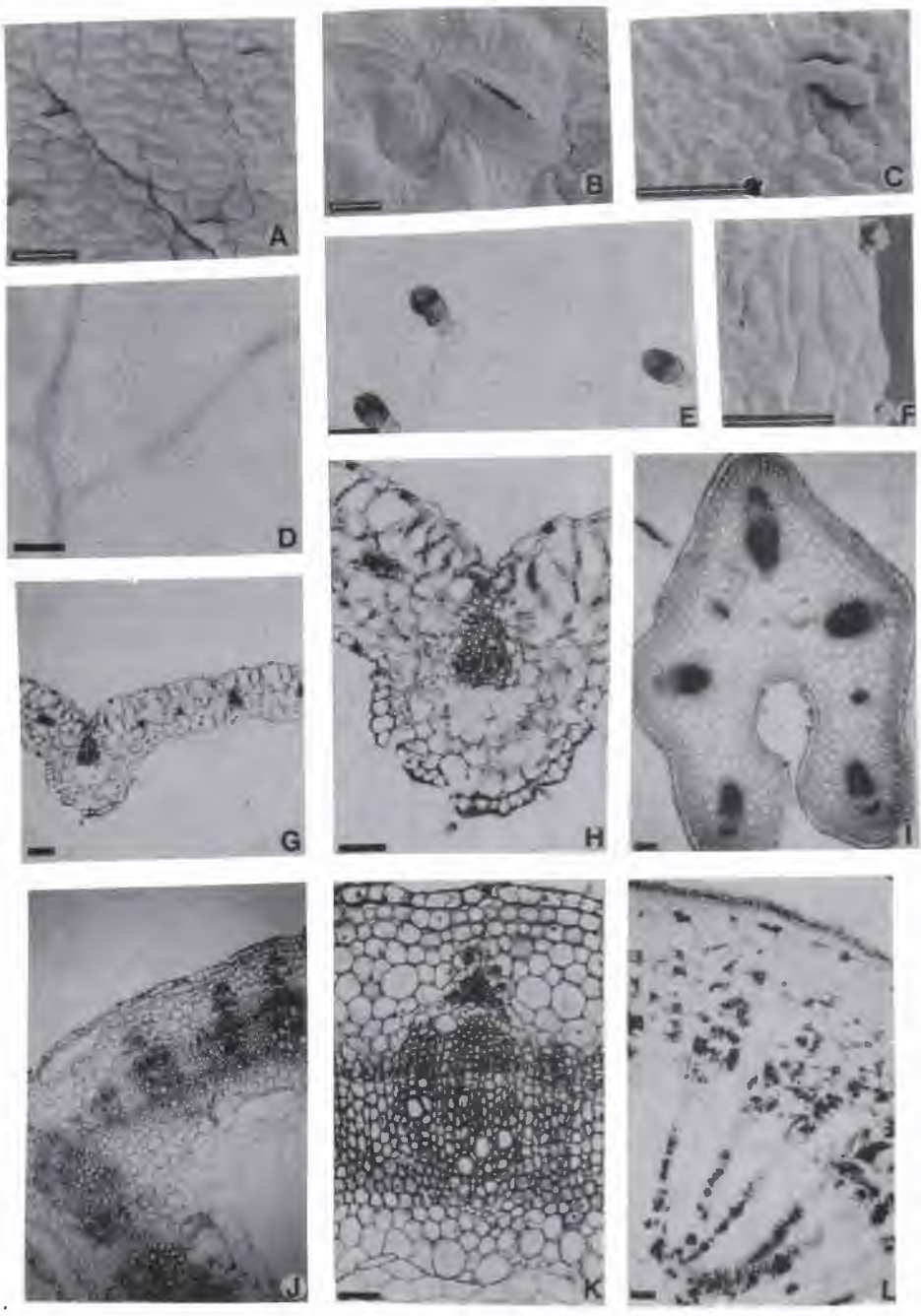

Fig. 1.

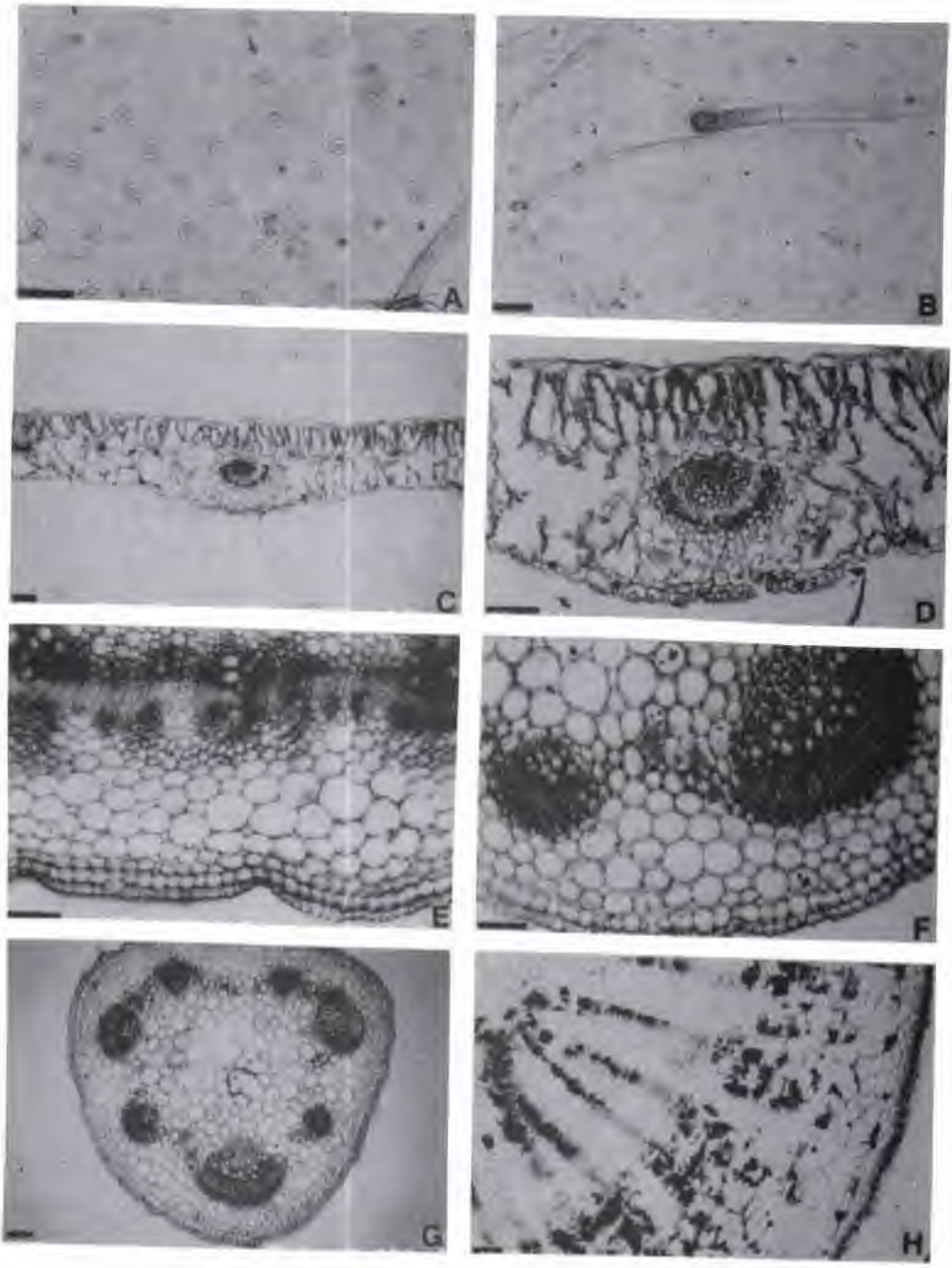

Fig. 2.