

ANATOMICAL CHANGES OF LENTIL (*LENS CULINARIS* MEDIK.) UNDER PHOSPHORUS DEFICIENCY STRESS

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Abstract

Phosphorus deficiency in lentil (*Lens culinaris* Medik.) resulted in the development of abundant long root hairs which were rudimentary in plants fed with phosphorus. Root and stem diameter as well as thickness of leaf reduced under phosphorus deficiency. The cortical zone of the stem and root was found to decrease under phosphorus deficiency. Vascular area became smaller with less number of xylem vessels and smaller size of the cavity due to phosphorus deficiency. The pith area was increased in the stem under phosphorus deficient condition.

Introduction

Plants have evolved to adapt to their environment. Therefore, their morphology, anatomy and physiology are likely to provide clues towards their adaptation to various growing conditions (Chandler and Bartels 2008). One of the most important inputs to agricultural systems is phosphorus (P) and the issues associated with P availability and acquisition by plants represent problems of global proportions (Stutter *et al.* 2012). Phosphorus is an essential nutrient required for plant growth and reproduction (White and Brown 2010), but due to its strong reaction with soil and subsequent lack of mobility it is estimated that 30 - 40 % of the world's arable land is limited by P availability (Runge-Metzger 1995). Plants are profoundly affected by phosphorus deficiency because phosphorus is an indispensable constituent of nucleic acids and membrane phospholipids. Moreover, phosphorus plays a pivotal role in energy transfer, as a regulator of enzyme activity, and in signal transduction (Kavanová *et al.* 2006). Under P deficient conditions, however, plants have evolved several strategies to enhance P acquisition and reduce the need for P to act on multiple levels, including morphological and biochemical changes, transcriptional activation and physiological responses (Czarnecki *et al.* 2013).

Lentil is one of the oldest and most popular food legumes in Bangladesh. It provides a valuable and balanced protein source that, coupled with its ability to thrive on relatively marginal lands and under adverse environmental conditions, has ensured its survival as a crop. It is the second most important pulse crop in area and production, but stands first in the consumer's preference in this country (Uddin *et al.* 2008). It is a rich source of dietary protein and micronutrients for majority people in the country, and is consumed as a soup called 'dal' with rice (<http://www.ais.gov.bd/>). The productivity of the crop suffers from several biotic and abiotic factors. Very little is known about the influence of abiotic stress like phosphorus deficiency on internal morphology of lentil. So the present investigation was initiated to study the changes in anatomical structures in lentil due to phosphorus deficiency.

Materials and Methods

Lentil (*Lens culinaris* Medik. var. BARI Masur-4) was taken as experimental material and seeds were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Seeds were surface sterilized using sodium hypochlorite solution and plants were raised

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in pots under sand culture (Hewitt 1966) method. Modified half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) containing phosphorus (+P) and no phosphorus (-P) were used to moisten the sand at regular intervals (twice in a week). The sand of +P pots were moistened with +P nutrient solution while that of deficient pots with -P nutrient solution. Roots (3 cm below sand surface), stem segments (fourth internode) and leaves (7th number) were collected from 28-day-old plants and preserved in FAA solution for anatomical study. Free hand sectioning of root, stem and leaf was done with the help of a razor blade. The sections were stained in safranin and fast green and studied with the help of a compound microscope. Micrographs of the sections were taken using a digital camera (Nikon UFY-11A, Japan) attached with microscope.

Results and Discussion

Phosphorus deficiency resulted in smaller diameter of root compared to control (Fig. 1 A, B). The most important feature is the development of abundant root hairs in phosphorus deficient condition (Fig. 1B) which is not found in control (Fig. 1A). Fohse *et al.* (1991) observed smaller diameter in the root of spinach under phosphorus deficiency. Li *et al.* (2004) reported an increase in number, length and density of root hairs in rice due to phosphorus deficiency. The epidermis of root was composed of thin-walled single layered regularly arranged cells (Fig. 1A) which are smaller and irregularly arranged in phosphorus deficient condition (Fig. 1B). The cortical parenchyma was composed of 9-10 layers of cells in +P plant while it was reduced to 7-8 layers in -P condition. Secondary growth was noticed in both +P and -P condition. The vascular cylinder became smaller in -P condition compared to +P. Xylems vessels were less in number under -P condition than +P. Liu *et al.* (2004) also found decreased the number of xylem vessels with smaller area in *Vigna* seedlings under phosphorus deficiency.

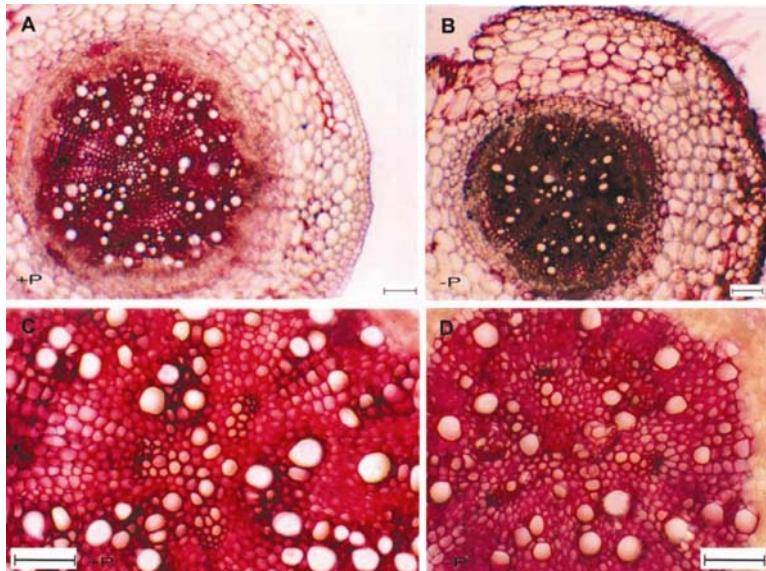


Fig. 1. Transverse sections of root of 28-day-old lentil grown in sand culture containing phosphorus (+P) (A,C) and no phosphorus (-P) (B,D). Bar = 100 μ m.

Stem diameter was found to decrease under -P condition compared to +P (Fig. 2 A, B). This smaller diameter was related with the reduction in cell size and also with reduction in size of the

vascular elements. A similar result of decreased stem diameter under phosphorus deficiency was also recorded in maize by Lyon and Garcia (1944). Epidermal cells were smaller in -P plant (Fig. 2B) than that of +P plant (Fig. 2A). The cuticle deposited over the epidermis was thickened in plant grown under phosphorus deficient condition. Cortex consisted of 7 - 8 layers of parenchymatous cells. The cortical cells were larger with intercellular spaces (Fig. 2A) in the plant fed with phosphorus than without phosphorus (Fig. 2B). Two types of vascular bundles were noticed. One type occurred in the stellar region and the other was found in the cortical region where four patches of vascular bundles were found. Conspicuous groups of sclerenchyma were superimposed upon the phloem. Vascular area with vascular elements was declined in phosphorus

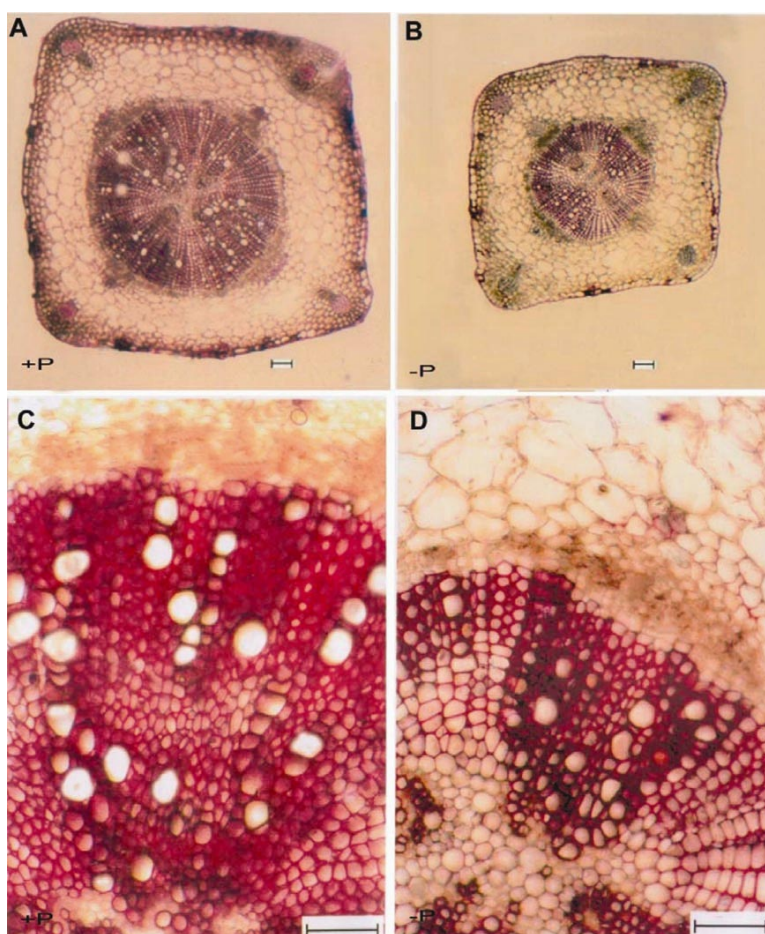


Fig. 2. Transverse sections of internode of 28-day-old lentil grown in sand culture containing phosphorous (+P) (A,C) and no phosphorous (-P) (B,D). Bar = 100 μ m.

deficient stem. Numbers of metaxylem and protoxylem vessels under -P were fewer as compared to that of +P (Fig. 2 C,D). Smaller size of the vessel cavities was noticed under P deficient condition. Lovelock *et al.* (2006) found that addition of P to P-deficient dwarf mangroves increased the diameter of xylem vessels and area of conductive xylem tissue of the canopy. Xylem

ray cells were larger in P deficient stem than that of +P plant. In phosphorus deficient plant, pith region was increased compared to that of +P.

Leaf thickness was decreased due to phosphorus deficiency (Fig. 3 A, B). Monika *et al.* (2005) reported that phosphorus deficiency reduced the leaf thickness in grass. Upper and lower epidermal cell became smaller in size in phosphorus deficient leaf (Fig. 3B). Chiera *et al.* (2002) showed that the cell size and thickness of epidermal cell were decreased under phosphorus deficient conditions in soybean leaf. In +P plant, cuticle was thinner than that of -P plant. Palisade and spongy parenchyma were more chlorophyllous and comparatively larger size in the +P than that of P deficient plant (Fig. 3 A, B). Phosphorus deficiency limited the leaf size in wheat by producing fewer cells of mesophyll tissue per leaf (Daniel *et al.* 1998).

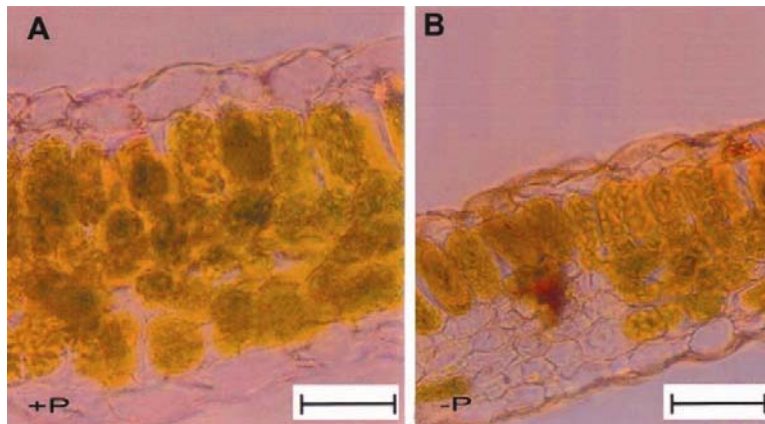


Fig. 3. Transverse sections of the 7th leaf of 28-day-old lentil grown in sand culture containing phosphorus (+P) (A) and no phosphorus (-P) (B). Bar = 100µm.

Crop plants are better equipped with an appropriate type of anatomy, largely constitutive in nature, to cope with the surrounding environment (Rieger and Litvin 1999). However, environment also seems to play a major role in modifying the anatomical features. Lentil plants showed different degrees of anatomical changes in the stem, root and leaf under phosphorus deficiency. Modifications of root growth and architecture are the most obvious and best-documented responses of plants to P starvation (Raghothama 1999, Williamson *et al.* 2001). Under P deficient conditions, root diameter decreases and the number of root hairs as well as their length increases which help enhance P uptake by increasing soil surface area contact (Peret *et al.* 2011, Brown *et al.* 2012). Under conditions of P deficiency plants allocate more photosynthate to root production thereby promoting root growth and allowing the root system to explore greater volumes of soil for P (Nielsen *et al.* 2001). The advantages of this have been demonstrated, for example, in bean (*Phaseolus vulgaris*) where genotypes with highly branched, actively growing root systems have been shown to be more P efficient than genotypes lacking such root traits (Lynch and Brown 2001). This has also been confirmed in brassicas (Hammond *et al.* 2009) and demonstrated for production of lateral roots (Zhu and Lynch 2004). Leaf growth depression under phosphorus deficiency is also well documented (Chiera *et al.* 2002, Assuero *et al.* 2004, Kavanová *et al.* 2006).

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