

Anatomical Alterations Associated with White Fluorescent Light Exposure of *Phaseolus vulgaris* L. Plants

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Abstract: The biological effect of white fluorescent light exposure (emitted from an ordinary 30 Watt, 220V fluorescent bare lamp) on the anatomical architecture of *Phaseolus vulgaris* L. plants obtained from pre-treated seeds was obtained. Both soaked and dry seed lots were separately exposed to light source for 1, 2, 3 & 4 discontinuous periods of one hour with a break time of a complete hr. between each two successive periods. Another chronic dose of 4 continuous hrs. of exposure was undertaken. Other control sets of untreated seeds were used for comparison. Cultivation experiment lasted for 50 days (in plastic pots in the home garden), during which continuous monitoring of the morphological changes were recorded. By the end of the experiment, anatomical studies of roots, stems & leaves of treated plants compared with the untreated ones were undergone. Roots resulted from soaked seeds, subjected to light for 4 discontinuous hrs. were discriminated by being transformed in shape from normal tap roots to storage roots with distinctive anomalous anatomical features. Advanced secondary thickening with a continuous cylinder of 2ry elements, high lignifications of xylem parenchyma, presence of air cavities in phloem region, presence of phloem fibers, enlarged pith parenchyma and a hollow central cavity were the most conspicuous distinctive features. Plants resulted from 1hr. exposed dry seeds produced a 4-grooved 2ry xylem ring with high lignification and lacked pith. Epidermal cork with variable thicknesses was also a feature of the soaked-seeded exposed plants. Stems of the same T4 soaked treatment were completely lacking 2ry thickening. Metaxylem vessels were also characterized by their narrow diameter and lowered number. Leaves resulted from 3hrs. of exposure (soaked & dry) and the 4hr. exposed soaked seeds were distinguished by their lower values of diversion angle of their leaf blade halves. T3 & T4 soaked-seeded plants had the lowest trichome density, lowest Tpal/Tm value and lowest number of lateral veins (smaller vascular bundles). These features point out to the decreased photosynthetic activity of plants.

Key words: white fluorescent light, *Phaseolus vulgaris*, anatomy.

INTRODUCTION

The tremendous importance of *Phaseolus vulgaris* L. as an immemorial legume plant exactly lies in its nutritional value. The immense content of protein, especially in seeds, makes the plant amenable of scientific research. In the majority of developing countries, the problem of food security is of prime importance in restraining starvation threats. These plants and others are daily subjected to a number of environmental stresses. Human beings are in perpetual strike for progress in using the source of light in accordance of their prospective aspirations and urbanization. Beginning with the primitive fire to the kerosene light bulbs-as incandescent sources-passing with gas-discharge, electric discharges and lasting to the fluorescent lamps are successive trials for this progress. All these sources have their adverse environmental disorders on living organisms. The International Programme on Chemical Safety-IPCS-(1994) explained that the amount of ultraviolet radiation emitted from different radiators increases with the increase in the temperature of material used and its formation. They concluded that the emissions of radiations from the tungsten filament lamps is negligible with the human health. They measured the amount of UV radiations emitted from the different types of fluorescent lamps used and found that the bare lamp, normally used in offices and homes, is the highest radiant one. Berkely (2002) pointed to the release of very small amounts of mercury to the environment from fluorescent lamps compared to the incandescent light bulbs. Lorelei (2003) recommended using the incandescent light bulbs than fluorescent ones. He explained that the incandescent light originates from heating a thin tungsten filament by electricity which is a form of firelight, while fluorescent light comes from continuous electrical discharges which make the mercury atoms produce an excess energy in the form of ultra

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violet light. This UV light turned into visible light by a combination of phosphorus and form fluorescent layer inside the tube. Barnes *et al.* (1993) referred to the effect of light source and its intensity on the photosynthetic photons flux.

Bugbee (1994) studied the effect of radiation quality emits from the six most common electric lamps on photosynthesis and growth of wheat and soybeans plants. He found that all the examined types of lamps have little effects on photosynthesis rate, but they alter the growth by changing the branching and internodes elongation.

Ibrahim & Mostafa (2007) studied the effect of UV radiation on the biomass and the Relative Growth Rate (RGR) of *Azolla caroliniana*. They obtained that there was a significant decrease in these growth criteria and they returned these results to the significant decrease in chlorophyll a & b. They explained the obtained results to the decrease in 5-aminolaevulinic acid which is the precursor of chlorophyll.

Accordingly, this study is carried out to investigate the effect of white fluorescent light originated from the ordinary fluorescent bare tube, commonly used in glass houses, homes and offices on the anatomical features of *Phaseolus vulgaris* L. plants grown from both soaked and dry seeds.

The present investigation has been undertaken to examine how the structure and organization of the root, stem and leaf of the studied species is affected by the adverse environmental conditions due to harsh exposure to light.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L, were obtained from the Agricultural Research Center, Giza, Egypt. Choice of the species was undertaken due to its wide use in research experiments, fast growth and easily observable changes in the morphological characters of its plants.

Seeds were divided into two lots, each of 300 seeds, the first of which was soaked for one hour in ordinary tap water before the experiment, while the second remained dry.

Seeds of either lots were exposed to 30 Watt fluorescent lamp, 220Volt with a frequency of 60 Hertz, from a distance of one foot (about 33 cm) for different periods: 1(T1), 2(T2), 3(T3) & 4(T4) hrs.; with a break period of one hour between each two hours of exposure .60 seeds were used for each period. Another 60 seeds from each lot (T4c) were exposed to lamp for four continuous hours (chronic). Two control sets of seeds (unexposed) for either lots were used for comparison.

All seeds (10/lot) were cultivated in 1:1 (Pitmos: sand) soil and daily irrigated using 25 ml tap water . Six replicates were applied for each treatment.

Experiment was carried out under natural conditions in home garden. Continuous record of morphological observations was monitored for a period of 50 days, after which desiccation was slightly creeping to the plants of all treatments excluding the control one. Samples of roots, stems and leaves were conserved for anatomical studies. Measurements were undertaken using the linear optical micrometer.

Data concerning the anatomy of either treated and untreated plants were analyzed by analysis of variance, ANOVA-SPSS, Version 17-(SAS Institute, 1985) to verify the significance of difference between the means at both 0.01 & 0.05 levels of probability

Anatomical Procedure:

1-Plant specimens were conserved in 70% ethyl alcohol.

2-Manual sectioning of specimens was carried out using a sharp blade.

3-Double staining of plant sections was undertaken as follows:

-Sections were embedded in safranin solution (1 g safranin was added to 100 cc .distilled water and another 1 g to 100 cc. 95%ethyl alcohol .The two constituents were then mixed then filtered) for five minutes so that the lignified tissues are staining red.

-Sections were washed in 70% ethyl alcohol.

-Sections were then transmitted to a series of different dilutions of ethyl alcohol (80, 90 then 95% consequently) for the aim of dehydration.

-Sections were then rinsed for few seconds in 1% alcoholic light green solution (1 g light green +100 cc 95% ethyl alcohol, shaking then filtration) in order to stain all the non-lignified tissues with the green colour.

4-Sections were washed in a consequent series of : 95% alcohol , absolute alcohol, equal amounts of absolute alcohol & xylol then in absolute xylol.

5- Finally, sections were permanently fixed on slides in Canada palsam for forthcoming examinations.

6-Examination was carried out using an electric microscope provided with a digital camera.

7-Photos were picked up using (40X) magnification power.

RESULTS AND DISCUSSION

Root Anatomy:

The anatomical features of roots of the soaked *Phaseolus vulgaris* L. seeds were affected to a great extent by light exposure than the dry seed. There was a general high significant decrease in the root diameter for all exposed soaked and dry-seeded plants evenly. Roots resulted from soaked seeds that were subjected to light for 4 discontinuous periods of 1hr. were highly affected. It is to be mentioned that these roots turned from normal tap roots to storage hollow cylindrical ones with terminal tapering end. It realized a diameter of 5.96 mm if compared with 8.32 mm for the unexposed plants (Tab. 1). Consequently, all plant roots showed a highly significant decrease in their vascular cylinder diameters when compared to the control. The diameters of 2ry xylem vessels were exceedingly wider for these anomalous roots. On the other hand, T3 treatment of soaked seeds (exposed for 3 discontinuous periods of 1hr.) showed a sign. difference in their 2ry xylem vessel diameter. Average number of 2ry xylem vessels/root showed exceedingly lower values especially, for soaked-seeded plants. Control roots were distinguished by several anatomical characters such as: possession of thick subepidermal cork layer, high 2ry thickening, broad parenchyma rays in front of the 1ry xylem arches, high lignification of 2ry xylem parenchyma, huge number of 2ry xylem vessels (Aver. of 28) and narrow cortex with compressed parenchyma. A general feature of low production of xylem vessels, wide cortex and presence of pith was obvious for soaked-seeded plants, especially in treatments T1, T2 & T3. T4 treatment was characterized by a squared, narrow vascular cylinder in the terminal tapering part of the root whereas, the storage root has a hollow central cavity, a complete layer of lignified parenchyma cells in which the 2ry xylem vessels are embedded. Also, the conducting tissues (primary xylem & 1ry phloem) are in the form of complete rings and the presence of patches of phloem fibers was monitored. All treated roots possessed an epidermal cork layer. T1 roots produced from dry exposed seeds were discriminated by its possession of 4-grooved vascular cylinder. It is to be noted that the number of 2ry xylem vessels produced from most of dry seeds, especially T1, T3 & T4c, was not affected by light exposure. Loss of pith was a general feature nearly for all dry seed treatments. Other anatomical diagnostic features of roots were detected as follows: presence of external cork layer of variable thicknesses (67.5 – 90 μm for T1 & T2 soaked plants, 135 – 155 μm for T3 soaked plants & 135 – 202 μm for T4c soaked plants). Also, some aspects for 2ry thickening were observed. These are the presence of 2ry vascular elements in addition to the 1ry 4-armed ones with a special advance for the storage root in T4 soaked plants. The presence of phloem fibers & possession of broad wedge-like parenchyma rays in front of the 1ry xylem arches, especially for the treated T4c soaked & T1 dry-seeded plants were also recorded.

Stem Anatomy:

Generally, *Phaseolus* stems resulted from all treated seeds, including the untreated ones are hexagonal. Epidermal strips between corners are nearly straight. Conspicuous prominence of restricted corners was only obvious for T1 (a single period of 1hr. exposure to light) of dry-seeded plants when it seemed grooved. Stem diameters for all treatments ranged between 1.67 & 3.34 mm comparing with 2.34 mm for control (Tab. 2). Stem diameter was only highly significant for T3 soaked treatment. Sign. difference was recorded for T3 dry & T4 (soaked & dry) treatments. Epidermis is generally uniseriate. Its thickness ranged from 37.5 to 82.5 μm , compared with 56.3 μm for the untreated plants. No sign. diff. was observed. Secondary thickening was variable for the different treated plants. It was limited for treatments T1, T3 & T4 (soaked) and for T1-T4 (dry) while was high for treatments T2 (soaked) & T4c (dry). No obvious 2ry thickening was recorded for T4 (soaked) stems. Consequently, the length of vascular bundle varied for the various treatments. Highly significant increased vascular bundle (1.69 & 2.13 mm) were only achieved for treated T4 & T4C (dry) plants compared to 1.05 mm for untreated plants. Data (Tab.2) also showed a high sign. decrease in the diameter of metaxylum vessels, especially for treatments T1 (157 μm), T3 (97.5 μm) & T4 (165.0 μm) soaked and for T1 (75 μm), & T2 (105 μm) dry plants as compared to (240 μm) for the control. Lowered average number of xylem vessels / bundle (3.5) was obtained for T4 soaked plants. Appearance of interfascicular 2ry vascular bundles was recorded for all treated plants, excluding treated T3 & T4 (soaked) plants when the 2ry thickening was restricted to the vascular bundles or absent. Microscopical detection of tannin sacs in pith was observed.

Table 1: Anatomical features of *Phaseolus vulgaris* L roots growing from seeds exposed to white fluorescent light for different time periods. Nb: N = Normal root S = Storage root.

Treatment	Root characters			
	Av. no. of 2ry xyl. vessels	Metxyl .vessel diam.(mm)	Vascular cylinder diam.(mm)	Root diam. (mm)
Soaked seeds				
Control	0.080±8.32	6.987±0.116	0.140±0.006	28
T1	0.099±1.95* *	1.09±0.032* *	0.135±0.013n.s	10
T2	0.135±2.100* *	1.106±0.019* *	0.210±0.033n.s	11
T3	0.198±2.288* *	1.200±0.038* *	0.124±0.064n.s	2
T4	N 1.069±0.056* *	1.069±0.056* *	0.281±0.000n.s	7
	S 4.613±0.056* *	4.613±0.056* *	0.240±0.067n.s	20
T4C	.0.033±2.531* *	1.425±0.099* *	0.112±0.130n.s	14
Dry seeds				
T1	0.227±1.969* *	1.455±0.007* *	0.135±0.013n.s	28
T2	0.676±3.112* *	1.556±0.082* *	0.165±0.020n.s	12
T3	0.033±3.656* *	1.763±0.099* *	0.150±0.015n.s	28
T4	0.019±2.794* *	1.388±0.099* *	0.150±0.008n.s	14
T4C	2.681±0.049* *	1.425±0.038* *	0.150±0.015n.s	20

Table 2: Anatomical features of *Phaseolus vulgaris* L. stems growing from seeds exposed to white fluorescent light for different periods.

Treatment	No. of xylem vessels/bund.	Diam. of metaxylem vessels (µm)	Length of vascular bundle (mm)	Thickness of epidermis (mm)	Stem diameter (mm)	No. of vascular bundles	
						1ry	2ry
Control	2.344±0.104	5.3±6.49	1.050±0.750	240.0±19.84	3.7±0.33	13	8
T1	2.268±0.114n.s	63.8±3.73n.s	1.069±0.032n.s	157.5±12.99* *	7.3±0.67*	15	9
T2	2.119±0.068n.s	37.5±3.37n.s	0.863±0.037n.s	172.5±3.73n.s	6.0±0.58*	14	12
T3	2.625±0.135* *	67.5±2.99n.s	0.863±0.037*	97.5±15.00* *	5.0±1.15n.s	16	-
T4	1.9±0.498*	52.5±7.5n.s	0.600±0.037*	165.0±15.00* *	3.5±0.33n.s	12	-
T4c	2.325±0.135n.s	41.3±3.73n.s	1.013±0.064n.s	120.0±7.50*	6.0±0.58*	24	20
T1	2.438±0.209n.s	52.5±3.77n.s	0.788±0.064n.s	75.0±7.50* *	4.0±0.33n.s	24	12
T2	2.287±0.131n.s	48.7±3.77n.s	1.125±0.113n.s	105.0±7.50* *	5.0±0.58n.s	24	16
T3	2.381±0.099n.s	48.8±7.50n.s	1.069±0.086n.s	135.0±12.99n.s	5.7±0.67n.s	28	4
T4	2.963±0.099*	63.8±3.73n.s	1.688±0.064* *	165.1±7.50n.s	6.3±0.33*	24	8
T4C	3.338±0.099*	82.5±7.50n.s	2.126±0.159* *	247.5±12.99n.s	4.4±0.33n.s	20	3

Leaf Anatomy:

The most prominent visual diagnostic feature of *Phaseolus* leaves was the variation in the measurement of the adaxial diversion angle between the two halves of the leaf blade (Tab. 3). It ranged from a minimal value of 40° to a maximum value of 137° when compared with 148° for untreated plants. It is noticeable that the most coincidence was observed for treatments T3 (60°), & T4 (53°) soaked plants and for T4 (40°) dry plants. This coincidence diminishes the leaf exposure area to light and-in turn-affects photosynthetic activity of plants. Density of trichomes was estimated per millimeter length of leaf epidermis in its transverse section. It is astonishing that the least number of trichomes mm⁻¹ of surface area was recorded for T3 (7.5) & T4 (7.7) soaked plants. It is to be mentioned that *Phaseolus* leaves are characterized by possessing papillose epidermis that contains solitary crystals and a variety of hair types. These hair types are the simple uniseriate-with short basal cells and a long terminal one-, hooked hairs-with one or two basal cells and a larger terminal one that bents as a hook - and a few-celled glandular club-shaped hair with or without distinctive stalk. Tanniferous contents were also seen in the mesophyll region. The ratio of palisade layer thickness/mesophyll layer thickness in the leaf transverse section (=Tpal/Tm) was determined to detect the photosynthetic activity of treated and untreated plants. It is of great importance to point out to the extremely lowered value of this ratio for the T3 (19.2%) soaked plants and the mild ratio (30.1%) for treatment T4 soaked plants compared to the value of 36.7% for untreated plants. All dry treatments besides T1(soaked) one showed higher values of Tpal/Tm ratio, that ranged between 41.7 & 97.7 % (Tab.3). The number of xylem vessels forming the main vascular bundle was detected. It is nice to indicate that treatments T3 & T4 (soaked) realized an equal lower values of 7 vessels compared with 25 vessels for the untreated plants. A least value of 4 vessels was recorded for T2 (dry) treatment while the highest value of 11 vessels was for T2 (soaked). The number of lateral veins denoting the positions of smaller lateral vascular bundles ranged between 2 & 6 for all treated plants while being 4 for the untreated plants.

Table 3: Internal architecture of *Phaseolus vulgaris* L. leaves arised from seeds exposed to white fluorescent light for different periods.

Treatment		Leaf characters				
		Diversion angle (°)	Density of trichomes (mm ⁻¹)	Tpal/Tm (%)	No. of vessels of main vasc. bund.	No. of lateral vasc. bund.
Control		148	23.90±1.70	36.7±1.66	25	4
Soaked seeds	T1	130	10.33±1.48* *	97.7±1.45* *	9	4
	T2	120	14.80±1.59*	53.1±1.82*	11	5
	T3	60	7.50±0.72n.s	19.2±0.85* *	7	3
	T4	53	7.67±0.57* *	30.1±5.07n.s	7	3
	T4c	115	16.74±2.82* *	32.8±6.15n.s	9	6
Dry Seeds	T1	130	5.87±1.47* *	51.7±1.67*	8	3
	T2	110	12.60±3.70n.s	57.3±9.91n.s	4	2
	T3	120	13.83±2.61n.s	39.0±2.08n.s	10	2
	T4	40	35.90±6.78n.s	45.0±2.89n.s	5	2
	T4C	137	16.20±2.26n.s	41.7±1.67n.s	5	3

Discussion:

Living organisms – including plants – were daily exposed to ordinary solar radiation within the entire solar band (400-3000 nm). Direct exposure to the visible and invisible solar emissions may be hazardous to these living organisms, especially at noontide.

In order to detect the effects that would rely on the exotic seed exposure to different doses of artificial white fluorescent light (emitted from a 30-Watt fluorescent bare lamp), one should take into consideration that most crop seeds prefer to germinate under dark conditions (inside the soil) and are even stored and preserved under dark conditions also. This exposure – indeed – represents an environmental stress since a 10-Watt fluorescent light produces a magnetic field greater than 1 milligauss as mentioned by Tuberoso (2007). Not only the seed germination and seedling growth are affected by exposure to magnetic, electrical, or thermal effects, Muller & Shykoff (1999); Taia & Salha (2008) but also the growth and flowering of plant species are promoted or delayed depending on the type of treatment (Yamada *et al.*, 2008). Later authors mentioned that the exposure of *Eustoma grandiflorum* to the daylight – type fluorescent lamp delayed flower budding by 4 days, compared with 66 days necessary for flower budding induction of untreated plants and 46 days for plants exposed to a far-red fluorescent lamp. They also proved that the light source regime had slight or no effect on the time of flowering after flower bud formation. They added that the daylight-type fluorescent lamp increased the number of stem nodes and reduced the internode elongation of plant compared to the other light sources. Meanwhile, there was a concordance of these results and those declared by Heo-Jeong Wook *et al.* (2007). They indicated that blooming period of flowers under white fluorescent light was shorter as compared with the red exposed plant.

Root induction of Cherry plants was correlated via their exposure to white fluorescent light in addition to the presence of hormones (Lacona *et al.* 2003).

On the other hand, Piszczek & Gowacka (2005) proved that the exposure of cucumber either to daylight or blue fluorescent lamp – with equal light intensities – induced the possession of big, dark-green leaves with high chlorophyll and dry matter content as compared to other yellow and green lamp exposure. Hajaam *et al.* (2007), emphasized the enhancement of plant propagation on exposing the *in vitro* culture media of *Chlorophytum borivilianum* to the white fluorescent light (2000-3000 Lux). Following the same trait. Minas (2007a; b; c & d) induced the propagation of different species through their exposure to white fluorescent light. In the present investigation, either soaked or dry *Phaseolus* seeds were separately subjected to different periods of white fluorescent light. Effect of light stress on the anatomical diagnostic features of roots, stems & leaves was studied. Roots of soaked treatments were highly affected by light exposure than the dry treatments. Roots of T4 soaked plants were transformed from normal tap roots to storage ones. These alterations might be a means for escaping from death and searching for survival depending on the stored food, air, or water. These roots also showed a high significant decrease in metaxylum vessel diameter.

Similar transformations of roots were recorded by Pilati & Souza (2006) when being ramified and tetrarch or diarch. They also proved the presence of 2ry thickening of both hypocotyl and epicotyl of the seedlings, in addition to the thick periderm.

Data also indicated the weakness of 2ry thickening of T3 & T4 soaked stems. In these treatments no 2ry vascular bundles were formed. The same treatments were distinguished by several leaf diagnostic features such as: 1-decrease in measurement of the diversion angle and more coincidence of the leaf blade halves and this might be a means for minimizing the exposed leaf area. 2-The lessening of Tpal/Tm ratio reflects the decreased photosynthetic activity of leaves. 3- Shortage in the number of xylem vessels of main vascular bundle and in

the number of lateral vascular bundles as a result of the preceding two characters. Generally exposing *Phaseolus* soaked seeds to white fluorescent light for 3 & 4 discontinuous periods of one hour produced weak plants and this – indeed – will affect their maturation and productivity which is the goal of its cultivation.

It is to be noted that the dry exposed seeds were less affected by light exposure if compared with soaked ones and this emphasizes the high resistance of dry seeds to the drastic environmental stresses, including light. Kovacs & Keresztes (2002) returned the biological effect of gamma-rays to its interaction with atoms or molecules in the cell, particularly water, to produce free radicals which can damage different important compounds of plant cell. They added that gamma-rays accelerate the softening of fruits due to the breakdown of the middle lamella in cell wall. They also influence the plastid development and function, such as starch-sugar interconversion. The authors also attributed the biological effect of UV to its great energy that destroys the chemical bounds, causing a photochemical reaction. They also continued that UV radiation influences plastid structure (mostly thylakoid membranes) and photosynthesis.

Ibrahim & Mostafa (2007) ensured the effect of UV – radiation in decreasing the biomass and relative growth rate of *Azolla caroliniana*. They also attributed the significant decrease in chlorophyll a and b contents to the decrease in 5-aminolaevulinic acid content (precursor of chlorophyll).

Albert *et al.* (2008) mentioned that exposure to UV – radiation might also affect other parts of photosynthetic machinery, such as the Calvin cycle.

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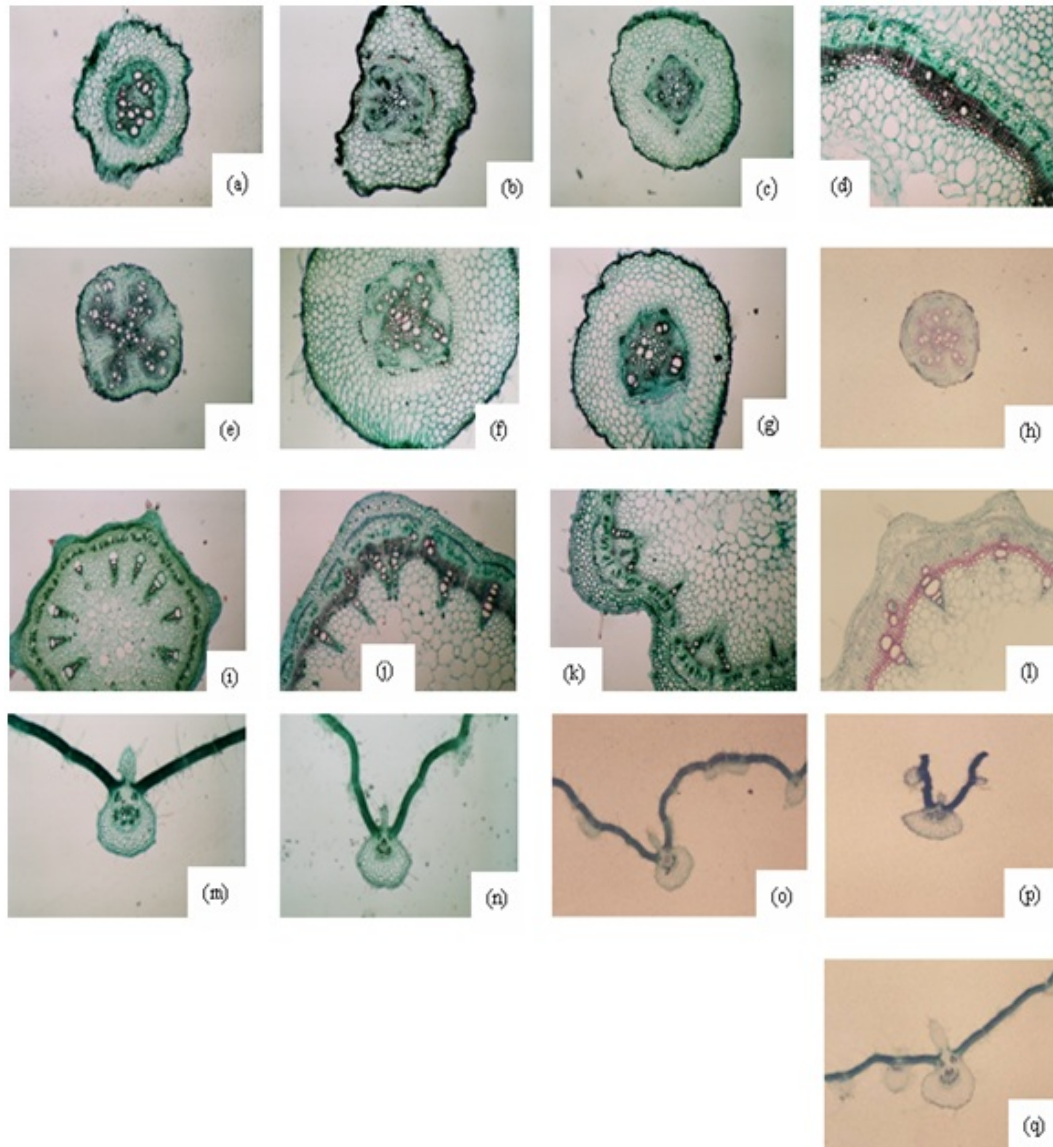
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Anatomical structure of *Phaseolus vulgaris* L. plants at different light treatments:

N.b.: magnification power for all photos is even (40X).

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|-----------------------|-----------------------|
| (i) T4 soaked stem. | (a) T1 soaked root. |
| (j) T4 C soaked stem. | (b) T3 soaked root. |
| (k) T1 dry stem. | (c) T4 N soaked root. |
| (l) control stem. | (d) T4 S soaked root. |
| (m) T2 soaked leaf. | (e) T1 dry root. |
| (n) T3 soaked leaf. | (f) T3 dry root. |
| (o) T4 soaked leaf. | (g) T4 dry root. |
| (p) T1 dry leaf. | (h) Control root. |
| (q) control leaf. | |