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# Effect of seed weight on stem anatomical characters in white lupine (*Lupinus albus* L.) cultivars

**M.A. Hasan[1], S.K. AL-Taweel*[2], J.H. Hamza[3] and W.M. Jewad[3]**

Department of Field Crops,
College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadiriya, Baghdad, Iraq.
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## ABSTRACT

An anatomical study was carried out at the College of Agricultural Engineering Sciences, University of Baghdad, in 2017, on lupine crop (*Lupinus albus*) as a comparison guide of three seed weights of three lupine cultivars *viz*. 'Giza-1', 'Giza-2' and 'Hamburg'. The nested design was used with four replications. The results showed that cultivars had a significant effect on stem anatomical traits. 'Hamburg' cultivar recorded the highest stem diameter, cortex thickness and xylem vascular diameter, while cultivar 'Giza-1' recorded the lowest values for the same traits as well as the highest collenchyma layer thickness, vascular bundle thickness, and xylem thickness. Cultivar 'Giza-2' recorded the lowest vascular bundle thickness and xylem thickness. The interaction between cultivar and seed weight was significant. 'Hamburg' cultivar was superior to both 'Giza-1' and 'Giza-2' anatomically. 'Hamburg' cultivar with lower seed weight was found best for field sowing to get higher yield with lower seed cost.

**Key words:** Anatomical markers, Collenchyma, Cortex, Epidermis, Seed size, Vascular bundles.

## INTRODUCTION

White lupine (*Lupinus albus* L.) belongs to the Fabaceae family which has more than 200 species. Lupine is cultivated as a source of feed, food and input for pharmaceutical industries (Ainouche and Bayer, 1999). White lupine as an annual or biannual plant is grown in Australia, Russia and Mediterranean countries. The seed of lupine are rich in protein (30-35%), carbohydrates (35%), oil (18-28%), and fibers (10-11%) soluble in the water, antioxidants and low in starch free gluten, alkaloids for medical uses. The high contents of monounsaturated and polyunsaturated fatty acids in Lupin seed's flour are appeared it's important for both humans and animal's nutrition (Al-Hamdani and Al-Anbari, 2017).

An anatomical study using physiological factors by Ali *et al*., (2009), showed that spraying broad bean leaves with different concentrations of zinc significantly increased stem diameter and vascular cylinders. Rady *et al*., (2016) showed that proline enhanced the anatomical traits of Giza 1, Giza2 varieties grown under salt stress. Ali *et al*., (2009) and Eisa and Ali (2014), reported an increase in the thickness of vascular bundles with increased concentrations of nutrients used for bean seed. Gomaa *et al*., (2015) reported that foliar application of salicylic acid to lupine led to increased stem diameter through increased stem wall thickness and hollow pith diameter. It was attributed to increased epidermis thickness, cortex, fiber strands, tissues of phloem and xylem in addition to parenchymatous area thickness of the pith, and vessel diameter. Hasan *et al*., (2018) recommended soaking the broad bean seed with salicylic acid to improve the seedling anatomical traits and their tolerance to salt stress. The influence of seed size on physiological and biochemical seed quality was evaluated in many studies. Large seed size of barley showed significant differences in germination percentages, seedling dry weight and vigor index (Massimi, 2018), furthermore the seed size had a positive association with seed quality parameters such as protein content, dehydrogenase and a-amylase activity in chick pea (Anuradha *et al*., 2009).

This experiment was carried out to study anatomical features of the stem in lupine plant in the early stage of growth and to understand the physiological traits due to different seed weights of three white lupine varieties.

## MATERIALS AND METHODS

An anatomical study was carried out at the College of Agricultural Engineering Sciences, University of Baghdad, in 2017. Three cultivars of white lupine were obtained from the Agricultural Research Center in Egypt. 'Hamburg' is an Australian sweet taste variety characterized by small vegetative growth and small seed size. 'Giza-1' is Egyptian cultivar having bitter taste, strong growth and large seed size. 'Giza-2' is another Egyptian cultivar having bitter taste, strong growth and early blooming as compare to 'Giza-1'.

*Corresponding author's e-mail: sudad.altaweel@coagri.uobaghdad.edu.iq
[1]Department of Biology, College of Pure Sciences, Ibn Al-Haiytham, University of Baghdad, Baghdad, Iraq.
[2]Medical and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
[3]Department of Field Crops, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

The seed were sown in the field during mid-November in 2017. The treatments consisted of three cultivars *viz.* 'Giza-1', 'Giza-2' and 'Hamburg'; and three seed weights *viz.*, lower weight, medium weight and higher weight (Table 1). Plant samples were collected when the height of the plants reached 20-25 cm in the vegetative stage and the stem samples were cut from the middle of the stem and fixed in formalin acetic acid alcohol (FAA) solution for 24-48 hours as per the procedure of Johanson (1940). The samples were washed twice after fixing by ethyl alcohol concentration 70% to prepare samples for manual cutting. Fresh plant samples were sectioned using hand sectioning method as per Hutchinson (1954) with some modification according to Al-Hadeethi (2016) and stem diameter, epidermis thickness, collenchyma layer thickness, cortex thickness, vascular bundle thickness, xylem thickness and diameter of xylem vessels were recorded. The statistical analysis of the data was done according to the variance analysis of the nested design with four replicates. The means of treatments were compared with a test of least significant difference (LSD) at 5% probability (Steel and Torrie, 1981).

## RESULTS AND DISCUSSION

### Anatomical Study

**General description of stem cross section of white lupine plant:** The stem of lupine plant is circular or quadrangular in the cross section surrounded by a layer of the epidermis comprised of one row of narrow cells followed by cortex, which in turn consists of two main types of the cells, *viz.*, collenchyma cells and parenchyma cells, The collenchyma's cells are located below the epidermis and their number of rows differed with weight of seed from 2 to 3 rows of cells or 3 to 5 rows of cells and this layer was significance in giving the stem strength, stiffness and flexibility. The parenchyma cells contain many chloroplasts important in the process of photosynthesis. The pericycle followed the cortex and connected ribbons of sclerenchyma cells surrounding the vascular bundles or found in groups above the phloem called bundles cup fiber. The vascular bundle consisting of xylem and phloem the type of it was open type. The pith located in the central part of the stem. The previous features mentioned in the general description of the cross section of the lupine stem were observed in Hamburg cultivar with some minor differences (Fig.1). The improved stem

**Table 1:** Mean weight of 50 seed (g) of white lupine cultivars.

| Cultivars | Seed symbol | Weight of 50 seed (g) | | | | Mean weight of 50 seed (g) | Standard Deviation |
|---|---|---|---|---|---|---|---|
| | | Sample1 | Sample 2 | Sample 3 | Sample 4 | | |
| Giza-1 | Lower weight | 11.12 | 11.25 | 11.20 | 10.90 | 11.12 | ±0.15 |
| | Medium weight | 14.95 | 14.33 | 14.73 | 14.46 | 14.62 | ±0.28 |
| | Higher weight | 22.76 | 22.25 | 22.24 | 22.63 | 22.47 | ±0.27 |
| Giza-2 | Lower weight | 9.76 | 9.82 | 9.14 | 9.30 | 9.51 | ±0.34 |
| | Medium weight | 15.23 | 15.34 | 15.42 | 15.68 | 15.42 | ±0.19 |
| | Higher weight | 20.25 | 20.02 | 19.85 | 19.98 | 20.03 | ±0.17 |
| Hamburg | Lower weight | 9.70 | 10.08 | 9.36 | 9.98 | 9.78 | ±0.32 |
| | Medium weight | 13.60 | 13.21 | 13.70 | 13.09 | 13.40 | ±0.30 |
| | Higher weight | 15.57 | 15.40 | 15.55 | 15.32 | 15.46 | ±0.12 |

**Table 2:** Effect of cultivars and seed weight on stem anatomical characters of white lupine.

| Cultivars | Stem diameter (μm) | Epidermis thickness (μm) | Collenchyma layer thickness (μm) | Cortex thickness (μm) | Vascular bundle thickness (μm) | Xylem thickness (μm) | Xylem vessels thickness (μm) |
|---|---|---|---|---|---|---|---|
| Giza-1 | 1120.2 | 3 | 7.1 | 34 | 125 | 89.9 | 5.9 |
| Giza-2 | 1121 | 3.2 | 7.2 | 34.3 | 81.9 | 51.3 | 7.1 |
| Hamburg | 1155.4 | 3.1 | 6.3 | 40.7 | 94.1 | 65.6 | 7.2 |
| **LSD 5%** | **1.2** | **0.1** | **0.4** | **1.8** | **4.4** | **2.0** | **0.5** |
| **Cultivars ×Seed weight** | | | | | | | |
| Giza-1 × Lower | 1114.3 | 3.2 | 8.1 | 29.3 | 95.8 | 60.8 | 8.4 |
| Giza-2 × Lower | 1121.7 | 3.3 | 8.6 | 39 | 40.8 | 20.8 | 3.2 |
| Hamburg × Lower | 1226.3 | 3.7 | 7.1 | 44.6 | 147.7 | 127.1 | 5 |
| Giza-1 × Medium | 1126 | 2.8 | 6 | 35.9 | 101.3 | 74.6 | 3.9 |
| Giza-2 × Medium | 1120.3 | 3.3 | 6.1 | 37.7 | 108.1 | 79.5 | 8.2 |
| Hamburg × Medium | 1117.7 | 2.6 | 5.8 | 28.7 | 48 | 28.4 | 11.7 |
| Giza-1 × Higher | 1120.3 | 3 | 7.4 | 36.7 | 178 | 134.2 | 5.3 |
| Giza-2 × Higher | 1121 | 3.1 | 6.6 | 26.2 | 96.9 | 53.7 | 9.8 |
| Hamburg × Higher | 1122.3 | 3 | 6 | 48.9 | 86.7 | 41.3 | 4.9 |
| **LSD 5%** | **2.2** | **0.3** | **0.7** | **3.1** | **7.7** | **3.4** | **0.8** |

anatomy provided an opportunity for good translocation of the absorbed nutrients via healthy cells to be used in different metabolic processes positively reflecting in vigorous growth and improving yield under normal and adverse conditions such as salinity (Semida *et al*., 2014).

**Stem cross section of 'Hamburg' cultivar:** The cross section of the stem distinguished as elliptic form in lower seed weight and in secondary growth stage mean diameter of internal tissue after complete the primary growth increased. Further the epidermis of stem consisted of unicellular and uniseriate hairs in addition to cells of epidermis were ovoid in shape followed by cortex layers consisting of 3-5 layers of supported collenchyma's. The pericycle consisted of group of sclerenchyma cell covered the secondary phloem and the secondary xylem could not be distinguished in to metaxylem and protoxylem so the pith was narrow (Fig. 1).

The cross section of stem from the medium seed weight was elongated in shape at primary growth stage. The epidermis cells were oval in shape consisting of unicellular and uniseriate hairs followed by cortex consisted of 2-3 layers of supported collenchyma's. The pericycle consisted of connected ribbons of sclerenchyma cells surrounding vascular bundles. The vascular bundles consisted of xylem and phloem so the pith area broader (Fig. 2).

The cross section of stem from higher seed weight was ovule to elongate in shape in the early and secondary growth stage. The epidermis was lignified and narrow and ovule cells were free of hairs followed by cortex layers consist of 2-3 layers of supported collenchyma cells. The pericycle was ovoid in shape and stem consisted of group of sclerenchyma cells covering the phloem. The vascular bundles consisted of xylem and phloem so the pith area was broad (Fig. 3).

**Stem cross section of 'Giza-1' cultivar:** The cross section of the stem was quadrant form in lower seed weight and in secondary growth stage. The epidermis of stem consisted of ovoid cells free of hairs. The cortex layers consisted of 3-5 layers of supported collenchyma's. The pericycle consisted of group of sclerenchyma cell covering the secondary phloem. The secondary xylem could not be distinguished in to metaxylem and protoxylem so the pith was broad. The cross section of stem taken from medium seed weight in secondary growth stage had distinguished epidermis layer with ovule to elongated shape of cells free of hairs. The vascular bundles consisted of secondary xylem and secondary phloem and the pith area was narrow. The cross section of stem taken from higher seed weight showed epidermis layer having ovule to elongated shape free of hairs and the vascular bundles in the primary growth stage. The pith area was broad (Fig. 4).

**Stem cross section of 'Giza-2' cultivar:** The cross section of the lupine stem was quadrant form in lower seed weight and in secondary growth stage. The epidermis of stem

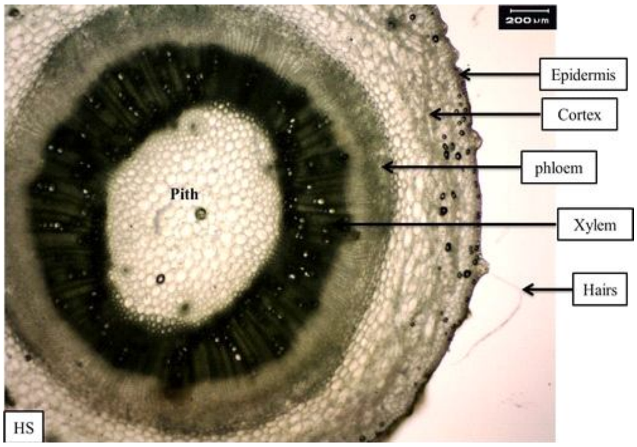


**Fig 1:** Stem cross section of lower seed weight (S) of Hamburg (H) cultivar.

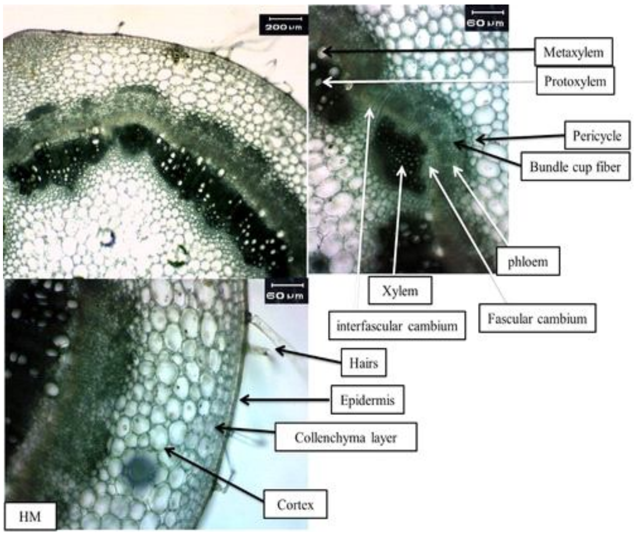


**Fig 2:** Stem cross section of medium seed weight (M) of Hamburg (H) cultivar.

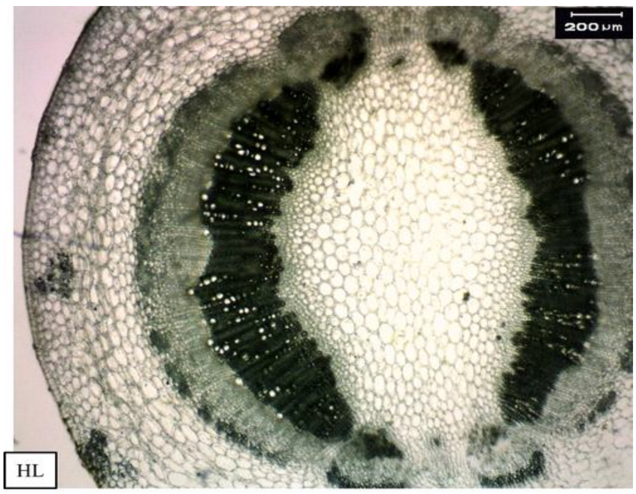


**Fig 3:** Stem cross section of higher seed weight (L) of Hamburg (H) cultivar.

consisted of ovoid cells free of hairs. The cortex layers consisted of 3-5 layers of supported collenchyma and the pericycle consisted of group of sclerenchyma cells. The secondary phloem and the secondary xylem could not be distinguished into metaxylem and protoxylem so the pith was narrow. The cross section of stem taken from medium seed weight in secondary growth stage had epidermis layer with ovule to elongated shape cells free of hairs followed by cortex consisting of 2-3 layers of supported collenchyma's. The vascular bundles consisted of secondary xylem and secondary phloem and the pith area very narrow. The cross section of stem was taken from higher seed weight in early secondary growth stage. The epidermis layer consisted of small ovule cells free of hairs. The sclerenchyma layers covered the phloem. The pith area was broad (Fig. 5).

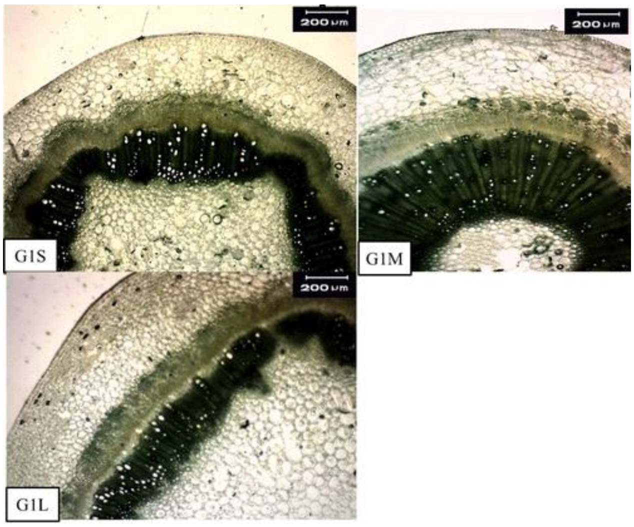


**Fig 4:** Stem cross section of Giza-1 (G1) cultivar in lower seed weight (S), medium seed weight (M) and higher seed weight (L) of seed.


**Fig 5:** Stem cross section of Giza-2 (G2) cultivar in lower seed weight (S), medium seed weight (M) and higher seed weight (L) of seed.

**Physiological study**

**Stem diameter:** 'Hamburg' cultivar recorded the highest diameter of stem while the lowest diameter was observed in 'Giza-1' cultivar, and it was at par with 'Giza-2' cultivar. It is possible that the diameter of the stem increases with the thickness of its internal tissues. 'Hamburg' cultivar with elongated elliptical stem and cortex along with pith occupied a large area. The vascular cylinder was also very thick. The interaction between cultivars and their seed weight had a significant effect. The 'Hamburg' cultivar with lower seed weight recorded the highest diameter of the stem. 'Giza-1' cultivar with lower seed weight recorded the lowest mean diameter. This is result may be attributed to higher thickness of the vascular bundle including xylem and phloem in the 'Hamburg' cultivar as compared to 'Giza-1' cultivar (Table 2).

**Thickness of epidermis:** The cultivar 'Giza-2' recorded the highest thickness in the epidermis and this was at par with that of 'Hamburg' while the lowest thickness was recorded in the 'Giza-1' cultivar. The interaction between the cultivars and their seed weight was significant. Cultivar 'Hamburg' with lower seed weight recorded highest thickness in the epidermis while the cultivars 'Giza-1' and 'Hamburg' of medium seed weight recorded the lowest epidermis thickness. This may be attributed to the fact that due to lower seed weight, their stem was in the secondary growth stage and the epidermis started to peel off and replaced with thick cork layer which helped in stem protection from the external influences. While, in higher seed weight, their stem was in the primary growth stage and the epidermis was simple and consisted of one layer of parenchyma cells (Table 2).

**Thickness of collenchyma layer:** The cultivar 'Giza-1' recorded the highest thickness of collenchyma layers and was at par with that of 'Giza-2', while the lowest thickness of collenchyma's was recorded in 'Hamburg' cultivar. The interaction between the cultivars and their seed weight was significant. The cultivar 'Giza-2' with lower seed weight recorded highest thickness and it was par with that of cultivar 'Giza-1' with lower seed weight. The cultivar 'Hamburg' with medium seed weight recorded lowest thickness of collenchyma's. This may be due to higher number of collenchyma layers in the stem of small seed as compared to fewer number of collenchyma layers in the stem in medium and large size of seed (Table 2).

**Thickness of cortex:** The thickness of cortex in the cultivar 'Hamburg' was higher as compared to cultivar 'Giza-1'. This has been attributed to the difference in cortex shape in cross section of stem among the cultivars. The shape was ovule to elongate in cultivar 'Hamburg' and it was quadrant in the cultivars 'Giza -1' and 'Giza-2'. This shape was characterized by narrow cortex and distributed in the corner of stem in the cultivars 'Giza -1' and 'Giza-2' while it was continuous loop with a fixed thickness cortex in cultivar 'Hamburg' (Metcalfe, 1960). The cultivar 'Hamburg' with higher seed weight recorded highest thickness of cortex on the other hand the cultivar 'Giza-2' with higher seed weight recorded the lowest thickness of cortex. This is attributed to differences in the cross section shape of stem (Table 2).

**Thickness of vascular bundle:** The cultivar 'Giza-1' recorded highest thickness of vascular bundle, while the cultivar 'Giza-2' recorded the lowest thickness of vascular bundle. There was significant interaction effect between cultivars and their seed weight on the vascular bundle thickness. The cultivar 'Giza-1' with higher seed weight recorded the highest thickness of the vascular bundle, while 'Giza-2' with lower seed weighed recorded lowest thickness of the vascular bundle. This is attributed to variability in increasing the diameter and surface area, production of secondary xylem and phloem due to variable seed weight in the early stages of secondary growth (Table 2).

**Thickness of xylem:** The 'Giza-1' recorded the highest thickness of xylem, while the lowest thickness was recorded in cultivar 'Giza-2'. As for the interaction, the cultivar 'Giza-1' with higher seed weight recorded the highest thickness of xylem, while cultivar 'Giza-2' with lower seed weight recorded the lowest thickness of xylem. The plants with higher seed weight did not stop the initial growth in the tissues and continued to produce new xylem tissues in the early stages of secondary growth, while in the plants with less seed weight, the stem showed the trend towards increase in the diameter and surface area in the early stages only (Table 2).

**Diameter of the xylem vessels :** The cultivar 'Hamburg' recorded the highest diameter for the xylem vessel and did not differ significantly with the cultivar 'Giza-2', while the diameter of the xylem vessel in cultivar 'Giza-1' was lowest. Among the interaction effects, cultivar 'Hamburg' with medium seed weight recorded higher diameter of the xylem vessel, while the cultivar 'Giza-2' with lower seed weight recorded lowest diameter of the xylem vessel. The increase and decrease in diameters is based on the secondary growth stage. The increase in the thickness of xylem is inversely related to the decrease in the diameter of the xylem vessel. Increasing the thickness of the xylem led to the production of new vessels with small diameters (Table 2).

## CONCLUSION

The study showed that cultivars and their seed weight had a significant effect on the anatomical characteristics of the lupine stem. 'Hamburg' cultivar recorded the highest diameter of the stem, thickness of cortex and diameter of xylem vessel. Cultivar 'Giza-1' recorded the lowest diameter of stem, cortex thickness and diameter of the xylem vessel followed by cultivar 'Giza-2'. Lower seed weight of cultivar 'Hamburg' recorded the highest diameter of stem and epidermis thickness. The increase in stem diameter, thickness of the epidermis, stem hairs provide protection to the plant from diseases and a wider range of environmental stresses such as drought. Thus from the results of this study, it may be concluded that 'Hamburg' cultivar is superior to the cultivars 'Giza-1' and 'Giza-2' anatomically and the combination of cultivar 'Hamburg' and lower seed weight was better as compared to other combinations for the cultivation of white lupines.

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