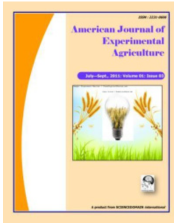


American Journal of Experimental Agriculture
1(4): 231-264, 2011

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anatomical Alteration in Response to Irrigation and Water Stress in Some Legume Crops

C. G. Abdel[1*] and Iqbal Murad Thahir Al-Rawi[2]

[1]*Department of Horticulture Dohuk University, Kurdistan Region, Iraq.*
[2]*Field crops Department, Salahalddin, University, Kurdistan Region, Iraq.*

Research Article

Received 27th May 2011
Accepted 7th June 2011
Online Ready 13th June 2011

ABSTRACT

Anatomical alteration of leaf tissues components were investigated in regards to adequate and inadequate watering in Mungbean, Vetch and three Lentil cultivars namely Baraka, Adlib and Nineveh. The possibility of mitigating the alteration of these adversities by the aid of GA_3 was also investigated. Lentil plants irrigated by 50% level appeared to be the most effective treatment. This treatment manifested the best results as it exceeded that of 75% level in terms of cuticle thickness (69.3%), epidermis thickness (12%), and spongy layer thickness (7%). Moreover, it also exceeded that of 25% level in epidermis thickness (22.6%), palisade thickness (2.8%), spongy layer thickness (21%), and thickness of lower epidermis (4.5%). Irrigation level of 75% exceeded that of 25% level in epidermis thickness (9.4%), palisade thickness (3.1%), spongy layer thickness (13.1%), and thickness of lower epidermis (13%). Common Vetch irrigated by 25% level was the paramount treatment. It exceeded that of 50% irrigation level in terms of cuticle thickness, epidermis thickness, palisade thickness, spongy layer thickness, thickness of lower epidermis, and thickness of lower epidermis cuticle by 54.9, 12, 13.3, 37.1, 37.9, and 71.9%, respectively. Mungbean irrigated every 2 days exceeded that of Mungbean irrigated every 8 days in cuticle thickness (42.9%), epidermis thickness (22.2%), thickness of lower epidermis (5.1%), and thickness of lower epidermis cuticle (25%). 200 mgl^{-1} GA_3 Common Vetch treated plants exceeded that of untreated in term of stomata aperture length (22.4%), and stomata population (29.9%). Mungbean irrigated results every six days and sprayed with 200 $mgl^{-1}GA_3$ gave the highest stomata length (5.4 μm). Finally Baraka lentil cultivar revealed significant increases in stomata aperture 5.6% and 6.7%, as compared to Nineveh and Adlib, respectively. Baraka was also superior over Adlib stomata length (4%).

**Corresponding author: Email: caserabdel@yahoo.com;*

Keywords: Leaf anatomy; xylem; irrigation; Lentil,Mungbean, CommonVetch Gibberellic acid GA_3

1. INTRODUCTION

Chazen and Neumann (1994) showed that reductions in the plastic extensibility of the cell walls of expanding maize leaf tissues and accompanying reductions in leaf elongation rates can occur within 2 min of exposing the roots to non ionic polyethylene glycol 6000 solutions (-0.5 MPa) osmotic pressure. Increases in wall loosening in the tip tissues could facilitate root elongation even when severe water deficits have strongly inhibited cell expansion further behind the tip. This may help explain the fact that root growth is often more resistant to water deficits than shoot growth (Pritchard et al., 1991). Subsequently, wall loosening cannot account for the fact that longer term water deficits lead to a shortening of the root elongation zone and production of relatively small mature root cells (Silk, 1992).

Xylem vessels are bounded by pit membranes, through which water must pass to move from one vessel to the next. During air drying tissue shrinkage could cause pores to become larger than they originally were (Dickson, 2000). The structure of xylem vessels is seen as an important factor in determining the occurrence of water stress induced cavitations (Zimmermann, 1983). Shane et al. (2000) found that tissue drying could produce large holes in the pit membranes of maize (*Zea mays*) roots. They determined that the pore size of pit membranes in maize roots were close to 5 nm.

Harvey and van den Driessche (1997) stated that drought resistant hybrid poplar clones possessed stronger pit membranes than drought sensitive clones, as indicated by the increased damage suffered by membranes during preparation for SEM. Water stress induced embolisms result from heterogeneous nucleation of cavitations within xylem conduits (Pickard, 1981; Tyree et al., 1994). The pressure in the embolized vessel will slowly increase to atmospheric as air diffuses into the embolism from surrounding cells. The gas bubble will be trapped at the pit membrane pores until the pressure difference between the vessels becomes great enough for a small bubble to be drawn through pores into a functional vessel where it may nucleate new cavitations.

Rainfalls grown onion plant substantially increased stomata numbers per unit area, epidermal cell numbers per unit area, as compared to these obtained from supplementary irrigated onions. Irrigating cowpea plants whenever 25% of soil available water capacity is depleted profoundly exceeded the other two irrigation levels 50 and 75% in terms of stomata lengths, lengths of stomata aperture (Abdel and Al-Salem, 2010). The objective of this experiment was to track the role of irrigation on cell differentiation.

2. MATERIALS AND METHODS

These experiments were carried out during the Mungbean growing season 2009- 2010 at the research Field of field crops Department, College of Agriculture, Salahalddin University, located at Latitude (36^{o}10') and Altitude (415m) and 43^{o}E longitude. The objective of this study was to track the anatomical alterations in leaf tissues caused by these three supplementary irrigation levels.

Split Split plot arranged in Randomized Complete Block Design (Split Split- RCBD) was chosen for Lentil experiment. The main plot (A) irrigating plants whenever 25 (a1), 50 (a2), and (a3) 75% depletion from the soil water available capacity (AWC) to a soil depth of 30 cm. Sub plot was three Gibberellic acid rates 0 (b1), 100 (b2) and 200mgl^{-1}and finally factor C in experiment 1 and factor B in experiment 2 were similar, they were represented by lentil cultivars namely Nineveh (b1), Adlib (b2), and Baraka (b3). Consequently, 27 treatments for experiment 1 and 12 treatments for experiment 2 were encompassed. Each treatment was replicated three times and every replicate contained 1m^2 planted with 5 rows 20cm apart and 5cm intra plant space. Split plot arranged in Randomized Complete Block Design (Split-RCBD) was selected for common vetch. The main plot (A) was irrigating plants whenever 25 (a_1), 50 (a_2), and (a_3) 75% depletion from the soil water available capacity (AWC) to a soil depth of 30 cm. Sub plot was three Gibberellic acid rates 0 (b1), 100 (b2) and 200mgl^{-1}. Split Plots arranged in Randomized Complete Block Design (Split-RCBD) was adopted for Mungbeans trail. The main plots were irrigation frequencies (A): Where irrigating plants every 2 days represent (a_1), irrigating plants every 4 days (a_2), irrigating plant every 6 days (a_3), and irrigating plants every 8 days (a_4).Whereas, factor (B) was represented by GA_3 rates including 0mgl^{-1} (b_1), 100mgl^{-1}(b_2), and 200mgl^{-1}(b_3). Subsequently 12 treatments were included in this investigation; each treatment was represented by 3 replicates. One replicate was included in a plot of 1m width and 1.2m length sown with 4 rows 30cm apart and 10cm intra plant space.

All culture practices proceeded in accordance with farming request in Erbil. Light microscope, metric slide and graded lens 7X and 40X magnification lenses were used to determine stomata dimensions. The eye lens 7X was dissected into 10 fractions each is subdivided into 10 parts and one part is found to equal 0.9 micron. Thus, full expanded leaves were sampled from the Lentil, Common Vetch and Mungbean plants. Leaf samples were wetted in Petri dishes. Then, epidermis of the fresh leaves was pealed on the leaves by forceps and knife, thereafter pealed epidermis was mounted at a drop of distilled water on the slide. Latter on the sample was delicately covered by slide cover. Slide was wiped with tissue to absorb the seepage water in order to avoid slide stacking with microscope stage, and then examination was fulfilled under object length of 40x and grade eye piece 7x (Abdel, 2006).

Permanent slide preparation was commenced by full expanded leaves sampling killed, fixed, wax embed as the following: Formalin, acetic acid, and absolute ethyl alcohol were used to prepare the killing mixture (FAA), this mixture is composed of 90 ml, 70% Ethyl alcohol, 5 ml glacial acetic acid and 5 ml formalin. Leaves samples were submerged in FAA solution for 24 hours. Then were placed inside a desiccators connected to a mild vacuum pump for 5 minutes to remove air bubbles (Aspiration) from the tissue (Modified from Berlyn and Mksche, 1976). Olympus light microscope (Cx 21FS1), Japan manufacturing Co. was used for slides examining by Ocular micrometer after calculating their corresponding grades and revert them to µm. Cross and longitudinal sections were photographed by KRUSS (DCM35) German made. Procedure was preceded at Anatomy Laboratory, Science College, Salahaddin University.

3. RESULTS AND DISCUSSION

3.1 Stomata Behaviour

3.1.1 Stomata response to irrigation

Lentil results (Table 1 and Figure 2) exhibited that growing lentil cultivars under varying supplementary irrigation levels resulted in varying stomata responses. Therefore, exposing plants to 75% irrigation level substantially reduced stomata width (19.2%), stomata aperture (4.5%), and stomata population (25.8%), as compared to 25% irrigation level. Similar trends were also found when 75% level was compared with that of 50% in stomata aperture (4.5%). Stomata population (55.9%) reduction was detected. Regression analysis (Table 2) revealed that all detected parameters were linearly correlated to supplementary irrigation levels.

Common Vetch plants (Table 3; Figure 1) supplementary irrigated whenever 50% soil available water capacity treatment. It significantly exceeded that 25% level in stomata length (29.4%) and that of 75% by (8.9%). However, 75% level revealed superiority over 25% level in term of width of stomata (99.4%), width of stomata aperture (34.4%), stomata population (48.4%). Moreover, it highly exceeded that of 50% level in width of stomata (34.6%), width of stomata aperture (18.8%), stomata population (17.9%). Stomata length and stomata population showed quadratic correlation to varying supplemental watering. Whereas other studied traits revealed linear response (Table 4).

Mungbean irrigated results (Table 5 and Figure 3) every six days and sprayed with 200 mgl^{-1} GA_3 gave the highest stomata length (5.4 micron). While the highest stomata aperture length (3.75 micron) was accompanied to non- treated mash plants irrigated every 2 days. However the highest population of stomata was found in plants treated by (100 $mgl^{-1}GA_3$) irrigated every 8 days (8387.198 stoma. mm^{-2}). Significant differences were not observed in all other detected traits. Regression analysis showed that Mungbean plants were cubically correlated to irrigation frequencies in terms of stomata length, stomata aperture width and stomata population and other detected traits showed quadratic responses (Table 6). Stomata aperture length and stomata aperture width were linearly correlated to GA_3 rates. However, other detected characteristics were quadratically responded (Table 6).

These results are in accordance with those obtained by Abdel and Al-Hamadany (2010). Significant differences in stomata conductance between watered and stressed plants were detected in fababean cultivars after withholding water (Abdel, 1982). The relationship between photosynthetic rate and stomata conductance was linear during the stress period (Chartzoulakis et al., 2002). Stomata in plants regardless to their position on stem, flowers, fruits and leaves act as entrance gates for gaseous and vapour water exchange between internal tissues and micro environments. Therefore, they profoundly influence CO_2 availabilities in the mesophyll intercellular spaces (Abdel, 2009).

3.1.2 Stomata response to GA_3

In lentil results (Table 1; Figure 2), significant differences were not detected in the responses of stomata to varying GA_3 rates accept in stomata length of 100 and 200 mgl^{-1} GA_3 which showed reduction of 4.3%. All investigated parameters manifested linear relationship with GA_3 rates (Table 3).

Table 1. Stomata characters of Nineveh (N), Adlib (A), and Baraka (B) lentil cultivars irrigated whenever 25, 50, and 75% depleted from soil AWC to 0, 100, and 200 mg l^{-1} GA_3

Treatments		Stomata length (µm)	Stomata aperture length (µm)	Stomata width (µm)	Stomata aperture width (µm)	Stomata population ($µm^{-2}$)
AWC depletion (%)	25	5.92	3.09	2.73 a	0.93 a	2190.768b
	50	5.87	3.07	2.63 ab	0.93 a	2714.477a
	75	5.77	3.20	2.29 b	0.89 b	1740.977c
GA_3 mg l^{-1}	0	6.02 a	3.22	2.67	0.91	2170.323
	100	5.77 b	3.15	2.67	0.91	2266.258
	200	5.77 b	2.98	2.30	0.92	2201.777
Lentil Cultivars	N	5.91 a	3.25	2.65	0.90 b	2294.566
	A	5.71 b	3.12	2.51	0.89 b	2096.406
	B	5.94 a	2.98	2.49	0.95 a	2253.676
25% AWC depletion	0	6.30 a	3.42	2.96	0.88 d	1835.339d
	100	6.00 abc	3.10	3.06	0.94 b	2550.916a
	200	5.46 d	2.75	2.17	0.97 a	2184.477c
50% AWC depletion	0	6.10 ab	3.00	2.70	0.98 a	2953.527a
	100	5.56 bc	3.15	2.61	0.90 c	2410.946b
	200	5.95 abc	3.05	2.57	0.90 c	2778.957a
75 AWC depletion	0	5.65 bc	3.25	2.35	0.88 d	1747.267d
	100	5.75 abc	3.20	2.35	0.90 c	1835.339d
	200	5.90 abc	3.13	2.16	0.88 d	1641.897d
25% AWC depletion	N	6.14 abc	3.50	2.90	0.90 d	2027.208de
	A	5.46 d	2.95	2.61	0.86 f	2167.178cd
	B	6.17 ab	2.82	2.68	1.03 a	2376.347bc
50% AWC depletion	N	5.71 bcd	3.00	2.76	0.90 d	2988.126a
	A	6.26 a	3.30	2.82	0.88 e	2533.616b
	B	5.64 cd	2.90	2.30	1.00 b	2621.688b
75 AWC depletion	N	5.90 abc	3.25	2.28	0.90 d	1869.938def
	A	5.40 d	3.11	2.09	0.94 c	1589.998f
	B	6.00 abc	3.22	2.49	0.82 g	1764.567ef
Nineveh cv. (N)	0	6.30 a	3.40	2.55	0.92 d	2341.747a
	100	5.71 bc	3.20	2.46	0.86 g	2009.908b
	200	6.04 ab	3.07	3.00	0.96 b	2184.477ab

		0	5.46 c	3.15	2.90	0.90 e	2079.107ab
Adlib cv. (A)		100	5.95 abc	3.20	2.66	0.94 c	2253.676ab
		200	5.90 abc	3.10	2.46	0.90 e	2464.418a
Baraka cv. (B)		0	5.99 abc	3.20	2.49	0.88 f	2464.418a
		100	5.46 c	2.96	2.40	0.88 f	2027.208b
		200	5.87 abc	2.77	2.01	0.99 a	2113.706ab
25% AWC depletion	0	N	7.350 a	4.350 a	3.000	0.90 d	1624.597ij
		A	5.250 efg	3.10 abc	2.883	0.83 e	1835.339fj
		B	6.300 bc	2.760 bc	3.000	0.90 d	2044.507ei
	100	N	5.700 cf	3.000 bc	3.450	0.90 d	2410.946cde
		A	6.300 bc	3.45 abc	2.850	0.96 c	2464.418cde
		B	6.000 be	2.850 bc	2.883	0.96 c	2778.957bcd
	200	N	5.367 dg	3.15 abc	2.250	0.90 d	2044.507ei
		A	4.817 fg	2.250 c	2.100	0.78 f	2201.777eh
		B	6.20 bcd	2.850 bc	2.167	1.23 a	2307.148def
50% AWC depletion	0	N	6.15 bcd	2.550 bc	2.550	0.90 d	3197.295b
		A	6.333 b	3.000 bc	2.700	0.84 e	2778.957bcd
		B	5.817bf	3.45 abc	2.850	1.20 b	2882.755bc
	100	N	4.667 g	3.15 abc	2.700	0.90 d	2096.406ei
		A	6.300 bc	3.45 abc	2.883	0.90 d	2568.216cde
		B	5.700 cf	2.850 bc	2.250	0.90 d	2568.216cde
	200	N	6.300 bc	3.300 abc	3.033	0.90 d	3669.104a
		A	6.150 bcd	3.45 abc	2.883	0.90 d	2253.676dg
		B	5.400 dg	2.40 bc	1.800	0.90 d	2410.946cde
75% AWC depletion	0	N	5.400 dg	3.30 abc	2.100	0.96 c	2201.777eh
		A	5.550 dg	3.45 abc	1.800	0.90 d	1415.428j
		B	6.000 be	3.000 bc	3.150	0.78 f	1624.597ij
	100	N	6.000 be	3.30 abc	2.550	0.90 d	1729.968gj
		A	5.250 efg	2.700 bc	2.250	0.96 c	1729.968gj
		B	6.000 be	3.600 ab	2.250	0.84 e	2044.507ei
	200	N	6.300 bc	3.10 abc	2.183	0.84 e	1678.069hij
		A	5.400 dg	3.13 abc	2.217	0.96 c	1624.597ij
		B	6.000 be	3.060 bc	2.067	0.84 e	1624.597ij

Table 2. Regression analysis for the responses of Lentil stomata characters to irrigation frequencies and GA_3 rates

Character	Regression equation	(R^2)
Supplementary irrigation		
Stomata length (µm)	Y = 6.006 – 0.003 X	0.9
Stomata aperture length (µm)	Y = 3.013 + 0.002 X	0.4
Stomata width (µm)	Y = 2.994 – 0.009 X	8.4
Stomata aperture width (µm)	Y = 0.957 – 0.0009 X	1.8
Stomata population ($µm^{-2}$)	Y = 2663.88 – 8.97 X	9.9
GA_3 level		
Stomata length (µm)	Y = 5.975 – 0.001 X	2.2
Stomata aperture length (µm)	Y = 3.24 – 0.001 X	2.5
Stomata width (µm)	Y = 2.733 – 0.002 X	5.8
Stomata aperture width (µm)	Y = 0.914 + 0.00004 X	0.2
Stomata population ($µm^{-2}$)	Y = 2190.13 + 12.727 X	46.9

200 mgl^{-2} GA_3 Common Vetch treated plants exceeded that of untreated in term of stomata aperture length (22.4%), and stomata population (29.9%). In addition to that this treatment was superior over 100mgl^{-2} GA_3 in length of stomata (10%), length of stomata aperture (20%) and width of stomata aperture (14.9%). It is worthy to reveal that 100 mgl^{-2} GA_3 tended to decrease stomata population (27.5%), as compared to untreated. Regression analysis (Table 3 and Figure 1), showed that stomata length and stomata aperture width were quadratically related to GA_3 rates.

Mungbean irrigated results (Table 5) every six days and sprayed with 200 $mgl^{-1}GA_3$ gave the highest stomata length (5.4 micron). While the highest stomata aperture length (3.75 micron) was accompanied to non- treated mash plants irrigated every 2 days. However the highest population of stomata was found in plants treated by (100 $mgl^{-1}GA_3$) irrigated every 8 days (8387.198 stoma. mm^{-2}). Significant differences were not observed in all other detected traits. Naturally stomata closure occurs under water scarcities and thereafter, negatively reflected on photosynthesis. Stomata aperture length and stomata aperture width were linearly correlated to GA_3 rates. However, other detected characteristics were quadratically responded (Table 6).

Abdel and Al-Hamadany (2010) revealed that Aquadulce faba bean cultivar is the most drought resistance cultivar; this can be ascertained by its prominent yield of dry seeds (33.03 $gplant^{-1}$), stomata aperture length of upper and lower leaf surfaces (6.7 and 7.1µm, respectively), aperture width of upper and lower leaf surface stomata (1.4 and 1.9µm, respectively). Negligible differences were detected between Aquadulce and Local Syrian cultivars. Moreover, the latter cultivar revealed unequivocal yield of mature pods (91.33 $gplant^{-1}$). Exogenous ABA application highly improved the drought resistance capabilities of most cultivars, particularly interaction treatment of Aquadulce and $2x10^{-3}$ M ABA, which showed profound dry seeds yield per plant (42.93 g), stomata aperture length of upper and lower leaf surfaces (6.89 and 6.6µm, respectively), stomata aperture width of upper and lower leaf surfaces (1.8 and 2.1µm, respectively). ABA application slightly affected all detected traits. Significant differences in stomata conductance between watered and stressed plants were first detected at day 4 after withholding water in both cultivars. The relationship between photosynthetic rate and stomata conductance was linear during the stress period (Chartzoulakis et al., 2002). Stomata in plants regardless to their position on stem, flowers, fruits and leaves act as entrance gates for gaseous and vapour water exchange between internal tissues and micro environments. Therefore, they profoundly influence CO_2 availabilities in the mesophyll intercellular spaces (Abdel, 2010). Stomata of stressed plants are the main barrier for CO_2 entrance as, they prevail their closure. There was a strong inverse correlation r^2 ¼ 0:61 between photosynthesis of control and stressed plants and CO_2 draw-down. Photosynthesis as well as stomata conductance of cv. Fuerte fully recovered 2 days after re-watering, while in cv. Hass plants attained 20% lower values than that of the control (Chartzoulakis et al., 2002). There are indirect roles of gibberellins on stomata behaviours through improving expansion and division of leaf cells. In fact GA_3 experiences its roles after irrigation or considerable rainfalls where a mesophyll tissue possesses adequate moistures. Kaneko et al. (2003) determined the expression patterns of the GA-biosynthetic genes *OsGA20ox2* and *OsGA3ox2* and signaling component *SLR1* in elongating rice stems. Hedden and Thomas (2006) showed that Gibberellic acid also imparts its physiological roles through Abscisic acid antagonizing and thus promotes growth and sustaining open stomata apertures. This change may be related to an increase in GA_3 level at the 4th level of rolling or an antagonistic interaction between the GA_3 level and the high level of ABA at this rolling level (Kutlu et al., 2009).

3.1.3 Cultivar stomata responses

Baraka lentil cultivar revealed significant increases in stomata aperture 5.6% and 6.7%, as compared to Nineveh and Adlib, respectively. Baraka was also superior over Adlib stomata length (4%) (Table 1; Figure 2). Levitt (1980) categorized crops according to their stomata behaviour to spender plants that capable to maintain open stomata aperture, conservatives plants that show earlier stomata closure to sustain ample moisture in the internal tissues and semi conservatives where plants partially close their stomata in order to permit CO_2 influxes combined with transpiration reductions. Franks and Farquhar (2001) observed that ABA-treated plants had significantly smaller stomata and higher stomata density in their lower epidermis.

3.1.4 Stomata responses to irrigation and GA_3 interaction

GA_3 lentil treated plants seems to be highly improved plants resistance to water scarcity, since 200mgl^{-1} GA_3 applied on lentil grown under 75% level resulted in profound reduction in stomata population (1641.897 stomata mm^{-2}). Stomata population (2410.946 stomata mm^{-2}) was also reduced when lentil plants grown under 50% level sprayed by 100mgl^{-1} GA_3, as compared to untreated of the same irrigation level (18.78). Moreover, 200mgl^{-1} GA_3 under level 25% of irrigation tended to reveal significant stomata aperture width (0.97 micron), as compared to control of the same watering level (0.88 micron) (Table 1).

200 mgl^{-1}GA_3 treated Vetch plants grown at 50% level was the paramount treatment, it exhibited the highest length of stomata (6.15 µm) and length of stomata aperture (3.15µm). The highest stomata population was confined to 200mgl^{-2}GA_3 with 75% level of irrigation (5032.6 stomata mm^{-2}). However, (200mgl^{-2}GA_3) with 75% level was the most potent treatments, as it showed the highest aperture length and width of stomata 3.15 and 0.87µm, respectively (Table 3). 200 mgl^{-1}GA_3 Mungbean treated plant irrigated every 8days showed the lowest stomata population (7548.95 stomata mm^{-2}).

Table 3. Stomata characters of Common Vetch in response to three water depletion levels and three concentration levels of GA3

Treatments		Stomata length (μm)	Stomata aperture length (μm)	Stomata width (μm)	Stomata aperture width (μm)	Stomata population (μm^{-2})
AWC depletion (%)	25	4.25 c	2.35	1.85 c	0.64 c	2778.957c
	50	5.50 a	2.85	2.60 b	0.76 b	3529.134b
	75	5.05 b	2.75	3.50 a	0.86 a	4123.614a
GA3 mg l-1	0	5.35 a	2.45 b	2.45	0.82 a	2918.927b
	100	4.50 b	2.50 b	2.75	0.67 b	3722.576a
	200	4.95 a	3.00 a	2.75	0.77 a	3791.775a
25% AWC depletion	0	4.95 cd	2.40 bc	1.65 ef	0.69 c	2464.418e
	100	3.75 f	1.95 c	1.50 f	0.48 d	2673.587ed
	200	4.05 ef	2.70 ab	2.40 cde	0.75 bc	3197.295c
50% AWC depletion	0	5.70 ab	2.55 abc	2.10 def	0.90 a	3354.565c
	100	4.65 ed	2.85 ab	2.55 cd	0.69 c	4089.015b
	200	6.15 a	3.15 a	3.15 bc	0.69 c	3145.396c
75% AWC depletion	0	5.40 bc	2.40 bc	3.60 ab	0.87 a	2936.227cd
	100	5.10 bcd	2.70 ab	4.20 a	0.84 ab	4403.554b
	200	4.65 ed	3.15 a	2.70 cd	0.87 a	5032.633a

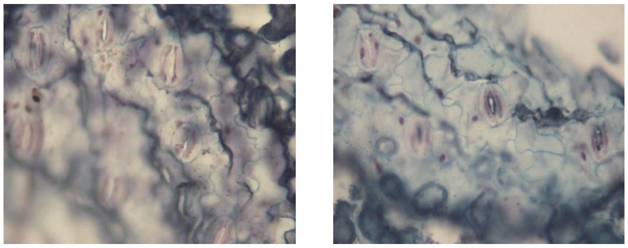


Fig. 1. Vetch stomata leaf upper surface (Type of stomata). (Magnification 7X40)

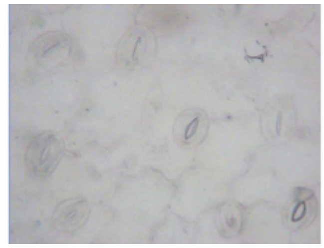


Fig. 2. Lentil stomata of leaf upper surface. Magnification 7X10

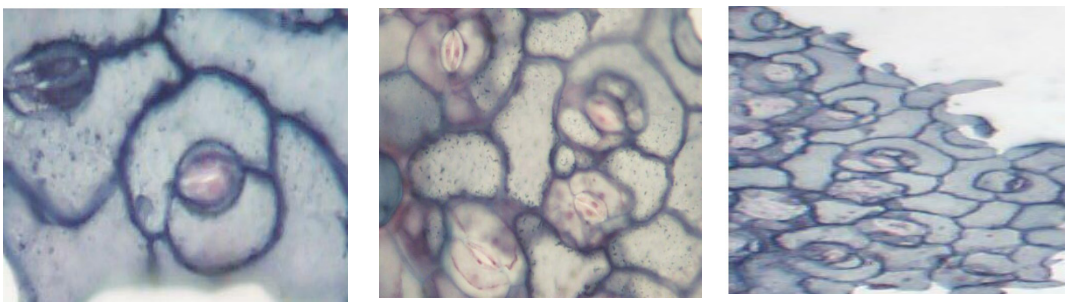


Fig. 3. Stomata type of Mungbeans

Table 4. Regression analysis for the responses of Common Vetch stomata characters to irrigation frequencies and GA_3 rates

Character	Regression equation	R^2
Supplementary irrigation		
Stomata length (μm)	$Y = 1.3 + 0.152 X – 0.001 X^2$	45.1
Stomata aperture length (μm)	$Y = 2.25 + 0.008 X$	12.4
Stomata width (μm)	$Y = 1 + 0.033 X$	53.7
Stomata aperture width (μm)	$Y = 0.533 + 0.004 X$	44.6
Stomata population (μm^{-2})	$Y = 1869.76 + 39.492 X – 0.126 X^2$	44.4
GA_3 level		
Stomata length (μm)	$Y = 5.35 – 0.015 X + 0.00007 X^2$	20.4
Stomata aperture length (μm)	$Y = 2.375 + 0.003 X$	23.4
Stomata width (μm)	$Y = 2.5 + 0.002 X$	1.8
Stomata aperture width (μm)	$Y = 0.82 – 0.003 X + 0.00001 X^2$	21.5
Stomata population (μm^{-2})	$Y = 2804.64 + 6.728 X$	44.2

Untreated Mungbean irrigated every 2 days gave the highest aperture length (3.75 μm) (Table 5). ABA imparted temporary elastic (not plastic) changes in stomata sizes, since ABA was sprayed immediately after re-watering. Besides ABA application and drought acquired the stomata degree of adaptation which enabled them to cope with further high leaf content of ABA (Davies, 1978). Active ABA undergoes a conversion into inactive ABA for instance Dihydrophaseic acid (Srivastava, 2002).

Table 5. Leaves and stomata characters of Mungbean in response to four irrigation types and three concentration levels of GA_3

Treatments		Stomata length (µm)	Stomata aperture length (µm)	Stomata width (µm)	Stomata aperture width (µm)	Stomata population (μm^{-2})
Irrigation Type	Every 2days	4.91	3.15	3.10	0.838	7898.089
	Every 4days	4.70	2.86	3.10	0.88	7898.089
	Every 6days	4.81	2.74	3.05	0.86	8038.059
	Every 8days	4.80	3.00	3.35	0.86	7846.190
GA_3 mg l^{-1}	0	4.88	3.08	3.26	0.84	7876.071
	100	4.73	2.95	3.04	0.87	7994.024
	200	4.80	2.82	3.15	0.87	7890.225
Every 2 days	0	5.1ab	3.75a	3.3	0.77	8072.658
	100	4.83ab	2.85b	3	0.84	7706.220
	200	4.8ab	2.85b	3	0.9	7915.389
Every 4 days	0	4.8ab	2.7b	3.3	0.9	7706.220
	100	5.1ab	3.3ab	3	0.9	7915.389
	200	4.2b	2.57b	3	0.84	8072.658
Every 6 days	0	4.83ab	2.7b	3.15	0.84	8178.029
	100	4.2b	2.67b	2.7	0.84	7968.860
	200	5.40a	2.85b	3.3	0.9	7968.860
Every 8 days	0	4.75ab	2.95ab	3.4	0.84	7706.220
	100	4.8ab	3ab	3.45	0.9	8387.198
	200	4.8ab	3ab	3.3	0.84	7548.950

Table 6. Regression analysis for the responses of Mungbean stomata characters to irrigation frequencies and GA_3 levels

Character	Regression equation	R^2
Supplementary irrigation		
Stomata length (µm)	$Y = 3.4 + 1.192\ X – 0.28\ X^2 + 0.019\ X^3$	4.2
Stomata aperture length (µm)	$Y = 2.384 + 0.231\ X – 0.023\ X^2$	4.9
Stomata width (µm)	$Y = 3.526 – 0.18\ x + 0.017\ X^2$	2.2
Stomata aperture width (µm)	$Y = 0.98 – 0.092\ X + 0.02\ X^2 – 0.001\ X^3$	1.5
Stomata population (μm^{-2})	$Y = 8000.7 – 1708.12X + 380.069\ X^2 – 25.12\ X^3$	19.6
GA_3 level		
Stomata length (µm)	$Y = 4.883 – 0.003\ X + 0.00001\ X^2$	1.2
Stomata aperture length (µm)	$Y = 3.047 – 0.001\ X$	3.4
Stomata width (µm)	$Y = 3.263 – 0.004\ X + 0.00002\ X^2$	4
Stomata aperture width (µm)	$Y = 0.844 + 0.0002\ X$	2.9
Stomata population (μm^{-2})	$Y = 50.083 + 0.015\ X – 0.00007\ X^2$	3

3.1.5 Cultivar stomata responses to irrigation

The highest stomata length was confined to Adlib lentil cultivar grown with 50% irrigation level and the highest stomata aperture width was accompanied by Baraka with 50% watering level. Moderate drought conditions were recommended by previous studies for instance Abdel and Al-Salem (2010). It may be referred to the mild drought ability to ensure better adaptation and aeration which is brought about by heavy watering and logging.

3.1.6 Cultivar stomata responses to GA_3

Significant differences were not observed between GA_3 treated and non-treated lentil plants. However, significant differences in stomata population between untreated and Nineveh treated by 100mgl^{-1} GA_3, and stomata aperture width was highly increased when Nineveh and Baraka treated with 200mgl^{-1} GA_3 and also when Adlib was treated by 100mgl^{-1} GA_3 (Table 1). Cultivar potencies in resisting drought mainly referred to the techniques that been applied by seed producing companies by which gene diversity can be preserved this idea was confirmed by Abdel and Al- Salem (2010). Under drought conditions GA_3 roles were found to be altered and this alteration was reflected on leaf morphology (Kutlu et al., 2009). They found that the amount of GA_3 increased at the 2nd level of leaf rolling but the increase was not significant.

3.1.7 Cultivar responses to irrigation and GA_3 interaction

The highest values of stomata length (7.35 micron) and stomata aperture length (4.35 micron) was found in Nineveh sprayed by distilled water grown under 25% irrigation level. While the highest stomata aperture was confined with Baraka sprayed by 200mgl^{-1} GA_3 and grown under 25% watering level. On the other hand the worst triple interaction was Nineveh grown under 50% and 200mgl^{-1} GA_3. Since, it gave the highest stomata population (23.33stomata mm^{-2}) (Table 1). Combinations among well irrigation, and GA_3 and the most potent cultivar resulted in the best growth and yield performance. These results might be attributed to the ability of GA_3 in ameliorating the adverse effects of drought and also due to highly preformed cultivars (Abdel and Al-Hamadany, 2010).

3.2 Anatomical Alteration in Leaf Tissues

3.2.1 Leaf tissues response to irrigation

Lentil plants irrigated by 50% level appeared to be the most effective treatment (Table 7; Figure 4). This treatment manifested the best results as it exceeded that of 75% level in terms of cuticle thickness (69.3%), epidermis thickness (12%), and spongy layer thickness (7%). Moreover, it also exceeded that of 25% level in epidermis thickness (22.6%), palisade thickness (2.8%), spongy layer thickness (21%), and thickness of lower epidermis (4.5%). Irrigation level of 75% exceeded that of 25% level in epidermis thickness (9.4%), palisade thickness (3.1%), spongy layer thickness (13.1%), and thickness of lower epidermis (13%). cross mid vein epidermis thickness, spongy layer thickness, out mid vein epidermis thickness, palisade thickness and thickness of lower epidermis were quadratically correlated to different supplementary irrigation levels (Table 8).

Common Vetch irrigated with 25% depletion level (Table 9; Figure 5, 5a) seems to be the best treatment. It substantially exceeded that of 75% level in terms of cuticle thickness (29.2%), epidermis thickness (92.2%), and collenchymas thickness (74.5%). Moreover, it also exceeded that of 50% level in cuticle thickness (48%), epidermis thickness (98.4%), collenchymas thickness (52.9%), and vessel number in main vein bundle (71.4%). Common Vetch irrigated by 25% level was the paramount treatment (Table 9). It exceeded that of 50% irrigation level in terms of cuticle thickness, epidermis thickness, palisade thickness, spongy layer thickness, thickness of lower epidermis, and thickness of lower epidermis cuticle by 54.9, 12, 13.3, 37.1, 37.9, and 71.9%, respectively. Furthermore, it surpassed that of 75% level in cuticle thickness, epidermis thickness, palisade thickness, spongy layer thickness, thickness of lower epidermis, and thickness of lower epidermis cuticle by 15.7, 9.1, 3.3, 31.1, 18.1, and 3.1%, respectively. However, 75% level exceeded that of 50% level in cuticle thickness, epidermis thickness, palisade thickness, spongy layer thickness, thickness of lower epidermis, and thickness of lower epidermis cuticle by 33.9, 2.7, 9.6, 4.5, 16.8, and 66%, respectively. Quadratic relation was found between supplementary irrigation levels and the responses of either cross mid vein cuticle thickness, vessel number in main vein bundle, thickness of lower epidermis, cuticle thickness out the mid vein, palisade thickness, lower epidermis thickness and cuticle thickness of lower epidermis. However, other detected parameters were linearly responded (Table 10).

Mungbean irrigated every 2 days level (Table 11, 11a; Figure 6, 6a, 6b), profoundly surpassed that irrigated every 8 days in terms of bundle sheath thickness (31.2%), vessel number in main vein bundle (16.1%), in highest vessel diameter (58.2%), and in narrowest vessel diameter (134.1%). It exceeded that of every 6 days irrigation in cuticle thickness (20.1%), epidermis thickness (63.2%), bundle sheath thickness (10.6%), vessel number in main vein bundle (6.7%), and narrowest vessel diameter (25.9%). Irrigation Mungbean every 2 days also exceeded that of 4 day frequency in epidermis thickness (18.6%), bundle sheath thickness (31.2%), widest vessel diameter (9.8%), and narrowest vessel diameter (16.7%). Mungbean irrigated every 2 days (Table 11a) exceeded that of Mungbean irrigated every 8 days in cuticle thickness (42.9%), epidermis thickness (22.2%), thickness of lower epidermis (5.1%), and thickness of lower epidermis cuticle (25%). It was exceeded that of every 6 days irrigation in cuticle thickness (42.9%), epidermis thickness (10%), thickness of lower epidermis (50%), and thickness of lower epidermis cuticle (45.8%). It highly surpassed that of 4 days in cuticle thickness (48.1%), epidermis thickness (15.9%), and thickness of lower epidermis (40%).

Table 7. Anatomical characters of cross section out of the main leaf vein of Nineveh, Adlib, and Baraka lentil cultivars irrigated whenever 25, 50, and 75% depleted from soil AWC and treated with 0, 100, and 200 mgl^{-1} GA_3

Treatments		Cuticle thickness µm	Epidermis thickness µm	Palisade thickness µm	Spongy layer thickness µm	Thickness of lower epidermis µm	Thickness of lower epidermis cuticle µm
AWC depletion (%)	25	0.285 a	2.346 c	27.914 b	33.967 c	2.53 c	0.258 a
	50	0.149 b	2.876 a	28.693 a	41.096 a	2.644 b	0.139 b
	75	0.088 c	2.567 b	28.789 a	38.411 b	2.86 a	0.118 b
GA_3 mg l^{-1}	0	0.267 a	2.485 c	27.37 c	40.607 a	2.684 b	0.228 a
	100	0.139 b	2.738 a	29.615 a	38.296 b	2.926 a	0.160 b
	200	0.117 c	2.566 b	28.411 b	34.570 c	2.424 c	0.127 b
Lentil Cultivars	N	0.178 b	2.503 b	30.607 a	41.496 a	2.759 b	0.169 a
	A	0.137 c	2.632 a	26.567 c	41.611 a	2.987 a	0.179 a
	B	0.208 a	2.654 a	28.222 b	30.367 b	2.287 c	0.167 a
25% AWC depletion	0	0.593 a	2.401 e	22.43 h	34.20 g	3.000 b	0.509 a
	100	0.150 c	2.249 f	28.27 e	32.99 h	2.539 f	0.151 bc
	200	0.112 d	2.389 e	33.04 a	34.71 f	2.051 i	0.114 bc
50% AWC depletion	0	0.120 d	2.538 d	29.08 d	45.91 a	2.111 h	0.087 c
	100	0.180 b	2.879 c	32.40 b	42.29 b	2.939 d	0.150 bc
	200	0.149 c	3.210 a	24.60 g	35.09 e	2.881 e	0.180 b
75% AWC depletion	0	0.088 e	2.516 d	30.60 c	41.71 c	2.940 c	0.088 c
	100	0.088 e	3.087 b	28.18 e	39.61 d	3.300 a	0.179 b
	200	0.089 e	2.100 g	27.59 f	33.91 g	2.340 g	0.088 c
25% AWC depletion	N	0.294 b	1.962 g	25.86 g	38.07 d	2.700 d	0.300 a
	A	0.176 c	2.689 d	28.70 d	35.73 f	3.1 ab	0.210 abc
	B	0.386 a	2.388 e	29.19 c	28.10 g	1.811 e	0.264 ab
50% AWC depletion	N	0.149 d	2.400 e	34.79 a	43.81 b	2.400 d	0.120 cd
	A	0.150 d	2.838 c	23.40 h	42.59 c	2.811 c	0.149 cd
	B	0.150 d	3.389 e	27.89 e	36.89 e	2.650 d	0.148 cd
75% AWC depletion	N	0.090 e	3.148 b	31.18 b	42.61 c	3.179 a	0.088 d
	A	0.087 e	2.369 e	27.60 f	46.51 a	3.001 bc	0.178 bcd
	B	0.088 e	2.186 f	27.59 f	26.11 h	2.400 d	0.089 d

		0	0.233 b	2.251 f	32.98 c	46.80 a	2.639 c	0.270 a
Nineveh cv. (N)		100	0.177 c	2.958 b	25.43 g	44.42 b	3.301 a	0.178 abc
		200	0.391 a	2.246 g	23.70 h	30.60 g	2.111 e	0.236 ab
		0	0.151 d	2.798 c	33.69 a	46.48 a	2.850 b	0.089 c
Adlib cv. (A)		100	0.118 ef	2.689 e	27.27 e	41.69 c	3.289 a	0.240 ab
		200	0.149 d	2.728 d	27.89 d	26.72 h	2.639 c	0.151 bc
		0	0.149 d	2.461 ef	25.16 g	31.21 f	2.790 b	0.149 bc
Baraka cv. (B)		100	0.118 de	2.249 f	27.00 f	38.72 d	2.371 d	0.119 bc
		200	0.083 e	2.989 a	33.08 b	33.78 e	2.111 e	0.114 c
		N	0.520 b	2.253 h	21.57 h	39.57 i	3.600 b	0.630 a
	0	A	0.357 c	2.700 f	20.50 i	32.43 m	3.600 b	0.360 b
		B	0.903 a	2.250 h	25.23 g	30.60 n	1.800 h	0.537 a
		N	0.183 d	1.833 i	28.20 e	38.63 j	2.250 f	0.090 c
25% AWC depletion	100	A	0.087 e	2.667 f	29.60 d	38.70 j	3.567 b	0.180 c
		B	0.180 d	2.247 h	27.00 f	21.63 q	1.800 h	0.183 c
		N	0.180d	1.800 i	27.80 e	36.00 l	2.250 f	0.180 c
	200	A	0.083 e	2.700 f	36.00 b	36.07 l	2.070 g	0.090 c
		B	0.073 f	2.667 f	35.33 c	32.07 m	1.833 h	0.073 c
		N	0.090 e	1.800 i	41.37 a	55.83 a	1.800 h	0.090 c
	0	A	0.087 e	3.567 c	27.00 f	49.50 d	2.700 d	0.087 c
		B	0.183 d	2.247 h	18.87 j	32.40 m	1.833 h	0.083 c
		N	0.180 d	2.517 g	36.00 b	46.80 e	1.800 h	0.090 c
50% AWC depletion	100	A	0.180 d	2.700 f	25.20 g	41.37 h	3.600 b	0.180 c
		B	0.180 d	3.420 d	36.00 b	38.70 j	3.417 c	0.180 c
		N	0.177 d	2.883 e	27.00 f	28.80 p	3.600 b	0.180 c
	200	A	0.183 d	2.247 h	18.00 j	36.90 k	2.343 e	0.180 c
		B	0.087e	4.50 a	28.80 e	39.57 i	2.700 d	0.180 c
		N	0.090 e	2.700 f	36.00 b	45.00 f	2.517 d	0.090 c
	0	A	0.087 e	2.607 f	28.80 e	51.33 c	3.603 b	0.087 c
		B	0.087 e	2.240 h	27.00 f	28.80 p	2.700 d	0.087 c
		N	0.090 e	4.043 b	36.87 a	54.00 b	4.500 a	0.087 c
75% AWC depletion	100	A	0.087 e	2.700 f	27.00 f	45.00 f	2.700 d	0.360 c
		B	0.090 e	2.517 g	20.67 i	19.83 r	2.700 d	0.090 c
		N	0.090 e	2.700 f	20.67 i	36.00 l	2.520 d	0.087 c
	200	A	0.087 e	1.800 i	27.00 f	43.20 g	2.700 d	0.087 c
		B	0.090 e	1.800 i	35.10 c	29.70 o	1.800 h	0.090 c

Cuticle thicknesses, epidermis thickness across mid vein, epidermis thickness, highest vessel diameter, spongy layer thickness were quadratically related to supplementary watering. While other detected traits were linearly responded except bundle sheath thickness which shoed cubic response (Table 12). These results suggested that water stress showed efficacies to modify plant tissues in order to facilitate water hydraulic conductivities. Lovisolo and Schubert (1998) found that vessels of water stressed plants had lower transactional areas.

3.2.2 Leaf tissues response to GA_3

Lentil treated by 100mgl^{-1} GA_3 was the best treatment (Table 7; Figure 4). It exceeded that of untreated in epidermis thickness (10.2%), palisade thickness (8.2%), and thickness of lower epidermis (9%). Additionally, it exceeded that of 100mgl^{-1} GA_3 in cuticle thickness (18.8%), epidermis thickness (6.7%), palisade thickness (8.2%), spongy layer thickness (10.8%) and thickness of lower epidermis (20.7%). Upper epidermis thickness, palisade thickness and thickness of lower epidermis manifested quadratic responses to varying GA_3 rates (Table 8).

Untreated Common Vetch plants (Table 9; Figure 5, 5a) were the best, since it highly exceeded that treated by 200mgl^{-1} in epidermis thickness (60.4%), collenchymas thickness (17.6%), vessel number in main vein bundle (104.9%), widest vessel diameter (59.9%), and narrowest vessel diameter (66.6%). Common Vetch treated with 100mgl^{-1} GA_3 exceeded that of untreated plants in terms of epidermis thickness (61.9%), and palisade thickness (47%). It also exceeded that of 200mgl^{-1} in epidermis thickness (55.1%), palisade thickness (5.4%), and thickness of lower epidermis (13.6%). Cuticle thickness across the mid vein, Collenchymas thickness, Cuticle thickness out the mid vein, epidermis thickness and thickness of lower epidermis cuticle were quadratically related to GA_3 rates (Table 10).

Untreated Mungbean (Table 11, 11a; Figure 6, 6a, 6b) exceeded that of 200mgl^{-1} in collenchymas thickness (5.4%), vessel number in main vein bundle (86.1%), widest vessel diameter (84.2%), and narrowest vessel diameter (80.1%). When this treatment was compared to that of 100mgl^{-1}, it showed higher collenchymas thickness, (14.8%), vessel number in main vein bundle (6.3%), widest vessel diameter (24.1%), and narrowest vessel diameter (60.7%). Cuticle thickness and spongy layer thickness were quadratically correlated to GA_3 rates. Mungbean plants treated by (200 mgl^{-1} GA_3) appeared to be the paramount treatment (Table 11, 11a). Since, it exceeded that untreated in terms of palisade thickness (3.3%), spongy layer thickness (33.4%), and thickness of lower epidermis (38.9%). It also exceeded that of (100 mgl^{-1} GA_3) in palisade thickness (8.6%), spongy layer thickness (4.6%), and thickness of lower epidermis (19.5%). However, other detected parameters were linearly responded (Table 12). Fan et al. (1994) suggested that plants with elastic cell walls have a high inherent drought tolerance.

3.2.3 Cultivar responses

Nineveh lentil cultivar appeared to be the most potent (Table 7; Figure 4). It exceeded Adlib in terms of palisade layer thickness (15.2%). It also surpassed Baraka in spongy layer thickness (36.6%). Adlib came next to Nineveh in superiority, as it exceeded Baraka in spongy layer thickness (37%), lower epidermis thickness (30.6%). Water influx to mesophyll facilitated by transfer cells, a short distance transport. Whereas, long distance transport of water is fulfilled by vessels. However, under drought conditions, water scarcity generating air bubbles which acts as water continuum barrier. Choat et al. (2003) proposed that larger pores, within only develop while the membrane is under mechanical stress.

3.2.4 Leaf tissue response to irrigation and GA_3 interaction

200mgl^{-1} GA_3 lentil treated plant irrigated by 25% depletion level gave the highest palisade layer thickness (33.04µm). Untreated irrigated by 50% level gave the highest spongy layer thickness (45.91µm) (Table 7; Figure 4).

Untreated Common Vetch irrigated by 75% possessed the highest value of widest vessel diameter (4.5µm), and narrowest vessel diameter (1.08µm). While, the highest cuticle thickness (1.07µm), epidermis thickness (9µm), and collenchymas thickness (38.6µm) were coincident to untreated with 25% irrigation level (Table 9, 9a). Common Vetch treated by 100mgl^{-1} GA_3 irrigated by 75% level gave the highest value of palisade thickness 37.73µm. Whereas, the highest spongy layer thickness (45%) was accompanied to 200mgl^{-1} GA_3 irrigated by 25% level. However, untreated plants grown under 25% level manifested superiority in terms of cuticle thickness, thickness of lower epidermis, and thickness of lower epidermis cuticle by 1.083, 3.567, and 0.99 µm, respectively (Figures 5, 5a).

Mungbean untreated plants with irrigated every 6 days (Table 11, 11a; Figure 6, 6a, 6b) gave the highest widest vessel diameter (18µm), and bundle sheath thickness (4.5 µm). 100mgl^{-1} GA_3 treated Mungbean irrigated ever 2 days gave the highest cuticle thickness (1.8µm), epidermis thickness (5.4µm), and bundle sheath thickness (5.4µm). Untreated Mungbean plants irrigated every 8 days gave the highest palisade layer thickness (63µm). Whereas the highest value for spongy layer thickness (63µm) was concomitant to treatment of 6 day frequency treated by 200mgl^{-1} GA_3. It is worthy to mention that Mungbean plants of 2days irrigation frequency sprayed by 100mgl^{-1} GA_3 gave the highest cuticle thickness (1.8µm), epidermis thickness (5.4µm), and thickness of lower epidermis (4.05µm) (Fig. 6, 6a, 6b). During cell expansion process, the cell wall must be made extensible in a finely tuned manner to allow it to yield to the turgour pressure while maintaining its integrity (Cosgrove, 2005). Garcia-Martinez et al. (1984) found that simultaneous application of IAA and GA_1 evoked continuous release of ethylene for at least 48 h after treatment and the yield of ethylene was the sum of amounts produced separately by IAA and $GA_{1.}$

Table 8. Regression analysis for the responses of lentil anatomical characters to supplementary irrigation and GA_3 levels

Character	Regression equation	R^2
Supplementary irrigation		
Cuticle thickness µm	Y = 0.371 – 0.004 X	21.8
Epidermis thickness µm	Y = 0.98 + 0.071 X – 0.0007 X^2	11.2
Palisade thickness µm	Y = 27.591 + 0.017 X	0.3
Spongy layer thickness µm	Y =17.022 + 0.874 X – 0.008 X^2	10.7
Thickness of lower epidermis µm	Y = 2.348 + 0.007 X	3.2
Thickness of lower epidermis cuticle µm	Y = 0.312 – 0.003 X	13.2
GA_3 level		
Cuticle thickness µm	Y = 0.25 – 0.0008 X	12.7
Upper Epidermis thickness µm	Y = 2.485 + 0.005 X – 0.00002 X^2	2.6
Palisade thickness µm	Y = 27.37 + 0.04 X – 0.0002 X^2	2.2
Spongy layer thickness µm	Y = 40.843 – 0.03 X	7.5
Thickness of lower epidermis µm	Y = 2.684 + 0.006 X – 0.00004 X^2	7.5
Thickness of lower epidermis cuticle µm	Y = 0.222 – 0.0005 X	6.8

Table 9. Anatomical characters of cross section-in of the main leaf vein of common vetch in response to three water depletion levels and three concentration levels of GA_3

Treatments		Cuticle thickness, µm	Epidermis thickness, µm	Collenchymas thickness, µm	Bundle sheath thickness	Vessel no. in main vein bundle, µm	Highest vessel diameter, µm	Lowest vessel diameter, µm
AWC depletion (%)	25	1.008 a	5.400 a	37.156 a	1.200 b	14.38 b	2.453 b	0.766 b
	50	0.681 c	2.722 b	24.300 b	1.197 b	8.389 c	3.451 a	0.849 a
	75	0.780 b	2.809 b	21.289 c	1.431 a	15.4a	0.824 c	0.733 b
GA_3 mg l^{-1}	0	0.836 b	4.811 a	32.067 a	1.171 b	17.14 a	2.860 a	0.981 a
	100	0.896 a	3.120 b	23.400 c	1.278 ab	12.66 b	2.080 b	0.778 b
	200	0.738 c	3.000 b	27.278 b	1.379 a	8.367 c	1.789 c	0.589 c
25% AWC depletion	0	1.077 a	9.000 a	38.600 a	1.350 bc	19.806b	3.147 b	0.963 b
	100	1.080 a	3.600 b	36.867 b	1.167 cd	13.47 d	2.247 d	0.767 d
	200	0.867 c	3.600 b	36.000 c	1.083 cd	9.867 e	1.967 e	0.567 f
50% AWC depletion	0	0.713 d	1.833 d	27.000 f	1.170 cd	9.967 e	4.500 a	1.080 a
	100	0.703 d	3.600 b	16.233 h	1.167 cd	8.067 f	3.153 b	0.833 cd
	200	0.627 e	2.733 c	29.677 e	1.253 bd	7.133 g	2.700 c	0.633 ef
75% AWC depletion	0	0.717 o	3.600 b	30.600 d	0.993 d	21.67 a	0.933 f	0.900 bc
	100	0.903 b	2.160 cd	17.100 g	1.500 b	16.433 c	0.840 f	0.733 de
	200	0.713 d	2.667 c	16.167 h	1.800 a	8.100 f	0.700 g	0.567 f

Table 9a. Anatomical characters of cross section out of the main leaf vein of common vetch in response to three water depletion levels and three concentration levels of GA_3

Treatments		Cuticle thickness, µm	Epidermis thickness µm	Palisade thickness µm	Spongy layer thickness µm	Thickness of lower epidermis µm	Thickness of lower epidermis cuticle µm
AWC depletion (%)	25	0.973 a	3.011 a	24.778 a	36.589a	2.689 a	0.930 a
	50	0.628 c	2.688 b	21.878 c	26.689 c	1.950 c	0.541
	75	0.841 b	2.761 b	23.989 b	27.900 b	2.277 b	0.902 b
GA_3 mg l^{-1}	0	0.871 b	2.310 c	18.278 c	24.611 c	2.406 a	0.841 a
	100	0.669 c	3.739 a	26.867 a	29.978 b	2.399 a	0.723 c
	200	0.903 a	2.411 b	25.500 b	36.589 a	2.111 b	0.809 b
25% AWC depletion	0	1.083 a	2.700 e	18.867 f	36.00 d	3.567 a	0.990 a
	100	0.930 c	4.500 a	26.667 d	28.767 e	2.700 b	0.900 b
	200	0.907 c	1.833 g	28.800 c	45.000 a	1.800 de	0.900 b
50% AWC depletion	0	0.537 e	1.350 h	19.767 e	20.733 g	1.767 e	0.633 c
	100	0.447 f	3.567 b	16.200 h	19.767 h	2.250 c	0.363 e
	200	0.903 c	3.147 c	29.667 b	39.567 c	1.833 de	0.627 d
75% AWC depletion	0	0.993 b	2.880 d	16.200 h	17.100 i	1.883 d	0.900 b
	100	0.630 d	3.150 c	37.733 a	41.40 b	2.247 c	0.907 b
	200	0.900 c	2.253 f	18.033 g	25.200 f	2.700 b	0.900 b

Table 10. Regression analysis for the responses of Common Vetch anatomical characters to supplementary irrigation and GA_3 levels

Character	Regression equation	R^2
Supplementary irrigation		
Cross section-in of the main leaf vein		
Cuticle thickness, μm	$Y = 1.76 – 0.039 X + 0.0003 X^2$	73.8
Epidermis thickness, μm	$Y = 6.235 – 0.051 X$	27.5
Collenchyma thickness, μm	$Y = 43.448 – 0.317 X$	57
Bundle sheath thickness, μm	$Y = 1.045 + 0.005 X$	13.1
Vessel no. in main vein bundle, μm	$Y = 33.367 – 1.02 X + 0.1 X^2$	36.7
Highest vessel diameter, μm	$Y = 2.169 + 0.257 X – 0.003 X^2$	80.4
Lowest vessel diameter, μm	$Y = 0.815 – 0.0006 X$	0.6
Cross section out of the main leaf vein		
Cuticle thickness, μm	$Y = 1.874 – 0.047 X + 0.0004 X^2$	46.5
Epidermis thickness, μm	$Y = 3.07 – 0.005 X$	1.3
Palisade thickness, μm	$Y = 32.689 – 0.417 X + 0.004 X^2$	3
Spongy layer thickness, μm	$Y = 39.082 – 0.174 X$	13.1
Thickness of lower epidermis, μm	$Y = 4.493 – 0.093 X + 0.0009 X^2$	28.7
Thickness of lower epidermis cuticle, μm	$Y = 2.069 – 0.061 X + 0.0006 X^2$	84.2
GA_3 levels		
Cross section-in of the main leaf vein		
Cuticle thickness, μm	$Y = 0.836 + 0.002 X – 0.00001X^2$	16.7
Epidermis thickness, μm	$Y = 4.549 – 0.009 X$	13.4
Collenchyma thickness, μm	$Y = 32.067 – 0.149 X + 0.0006 X^2$	17.1
Bundle sheath thickness, μm	$Y = 1.172 + 0.001 X$	10.6
Vessel no. in main vein bundle, μm	$Y = 17.111 – 0.044 X$	49.3
Highest vessel diameter, μm	$Y = 2.779 – 0.005 X$	13.1
Lowest vessel diameter, μm	$Y = 0.979 - 0.002 X$	83.1
Cross section out of the main leaf vein		
Cuticle thickness, μm	$Y = 0.871 – 0.004 X + 0.00002 X^2$	24.8
Epidermis thickness, μm	$Y = 2.31 + 0.028 X – 0.0001 X^2$	53.9
Palisade thickness, μm	$Y = 19.937 + 0.036 X$	17.4
Spongy layer thickness, μm	$Y = 24.404 + 0.06 X$	24.9
Thickness of lower epidermis, μm	$Y = 2.452 – 0.001 X$	4.5
Thickness of lower epidermis cuticle, μm	$Y = 0.841 – 0.002 X + 0.00001 X^2$	6.6

3.2.5 Cultivar leaf tissue responses to irrigation

Nineveh lentil cultivar irrigated by 50% level of irrigation gave the highest palisade thickness (34.79μm). While the thicker spongy layer (46.51μm) was confined to Adlib irrigated with 75% level (Table 7; Figure 4). Water stress induced embolisms result from heterogeneous nucleation of cavitations within xylem conduits (Pickard, 1981; Tyree et al., 1994).

Table 11. Anatomical characters of cross section-in of the main leaf vein of Mungbean in response to four irrigation types and three concentration levels of GA_3

Treatments		Cuticle thickness, µm	Epidermis thickness, µm	Collenchyma thickness, µm	Bundle sheath thickness, µm	Vessel no. in main vein bundle, µm	Highest vessel diameter, µm	Lowest vessel diameter, µm
Irrigation Type	2 days	1.100 b	3.900 a	45.00 3d	3.149 a	33.578 a	12.833 b	2.645 a
	4 days	1.079 b	3.289 b	51.30 c	2.400 c	33.567 a	11.678 c	2.267 b
	6 days	0.916 c	2.389 c	92.689 a	2.847 b	31.456 b	15.300 a	2.100 c
	8 days	1.321 a	3.900 a	74.111 b	2.400 c	28.922 c	8.111 d	1.130 d
GA_3 mg l^{-1}	0	0.991 c	3.375 b	69.967 a	2.587 c	41.017 a	15.300 a	2.804 a
	100	1.139 b	3.583 a	60.967 c	2.700 b	38.583 b	12.333 b	1.745 b
	200	1.182 a	2.700 c	66.392 b	2.810 a	22.042 c	8.308 c	1.557 c
Every 2days	0	0.633 g	2.700 d	36.00 i	2.247 d	46.33 a	16.200 b	4.467 a
	100	1.800 a	5.400 a	54.00 f	4.500 a	30.133 e	13.30 c	1.800 d
	200	0.867 e	3.600 c	45.00 h	2.700 c	24.267 g	9.00 f	1.667 d
Every 4 days	0	1.350 b	3.600 c	36.867 i	1.800 e	45.767 a	16.20 b	2.700 b
	100	0.807 f	3.567 c	54.00 f	1.800 e	39.60 b	10.80 e	2.300 c
	200	1.080 c	2.700 d	63.03 e	3.600 b	15.33 i	8.03 g	1.800 d
Every 6 days	0	0.900 e	2.700 d	99.00 b	4.500 a	37.80 c	18.00 a	2.700 b
	100	0.867 e	2.667 d	89.067 c	1.800 e	30.50 e	16.20 b	1.800 d
	200	0.980 d	1.800 e	90.00 c	2.240 d	26.07 f	11.70 d	1.800 d
Every 8 days	0	1.080 c	4.500 b	108.0 a	1.800 e	34.17 d	10.80 e	1.350 e
	100	1.083 c	2.700 d	46.80 g	2.700 c	30.10 e	9.033 f	1.080 f
	200	1.800 a	2.700 d	67.533 d	2.700 c	22.50 h	4.500 h	0.900 f

Table 11a. Anatomical characters of cross section out of the main leaf vein of Mungbean in response to four irrigation types and three concentration levels of GA_3

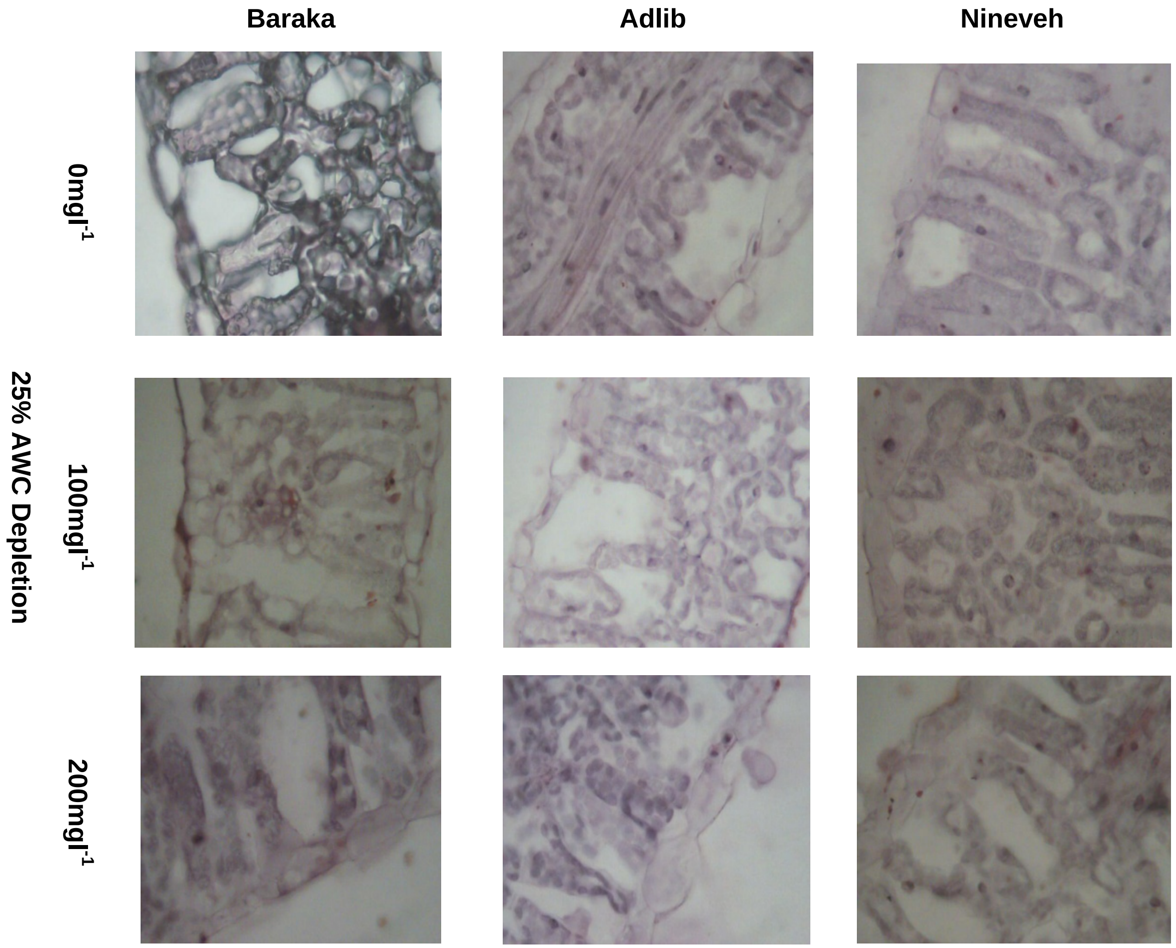
Treatments		Cuticle thickness µm	Epidermis thickness µm	Palisade thickness µm	Spongy layer thickness µm	Thickness of lower epidermis µm	Thickness of lower epidermis cuticle, µm
Irrigation Type	2 days	1.200 a	3.300 a	37.489 d	28.500 d	3.150 a	1.050 b
	4 days	0.810 b	2.847 c	38.100 c	43.200 b	2.250 b	1.110 a
	6 days	0.840 b	3.000 b	40.178 b	53.400 a	2.100 c	0.720 d
	8 days	0.840 b	2.700 d	48.000 a	40.478 c	2.089 c	0.840 c
GA_3 mg l^{-1}	0	0.855 b	2.925 b	41.175 b	34.408 c	2.025 c	1.035 a
	100	1.125 a	3.375 a	39.133 c	43.875 b	2.354 b	0.990 b
	200	0.788 c	2.585 c	42.517 a	45.900 a	2.813 a	0.765 c
Every 2 days	0	0.900 b	1.800 e	25.20 h	22.50 k	2.700 c	0.900 c
	100	1.800 a	5.400 a	36.00 g	31.50 j	4.05 a	1.35 b
	200	0.900 b	2.700 d	51.27 b	31.50 j	2.70 c	0.90 c
Every 4 days	0	0.900 b	2.700 d	40.50 e	33.30 i	1.80 d	1.80 a
	100	0.900 b	2.700 d	37.80 f	54.00 b	1.80 d	0.90 c
	200	0.630 d	3.140 c	36.00 g	42.30 f	3.15 b	0.63 f
Every 6 days	0	0.900 b	3.600 b	36.00 g	45.00 e	1.80 d	0.72 e
	100	0.900 b	2.700 d	37.73 f	52.20 c	1.80 d	0.81 d
	200	0..720 c	2.700 d	46.80 c	63.00 a	2.70 c	0.63 f
Every 8 days	0	0.720 c	3.600 b	63.00 a	36.83 h	1.80 d	0.72 e
	100	0.900 b	2.700 d	45.00 d	37.80 g	1.77 d	0.90 c
	200	0.900 b	1.800 e	36.00 g	46.80 d	2.70 c	0.90 c

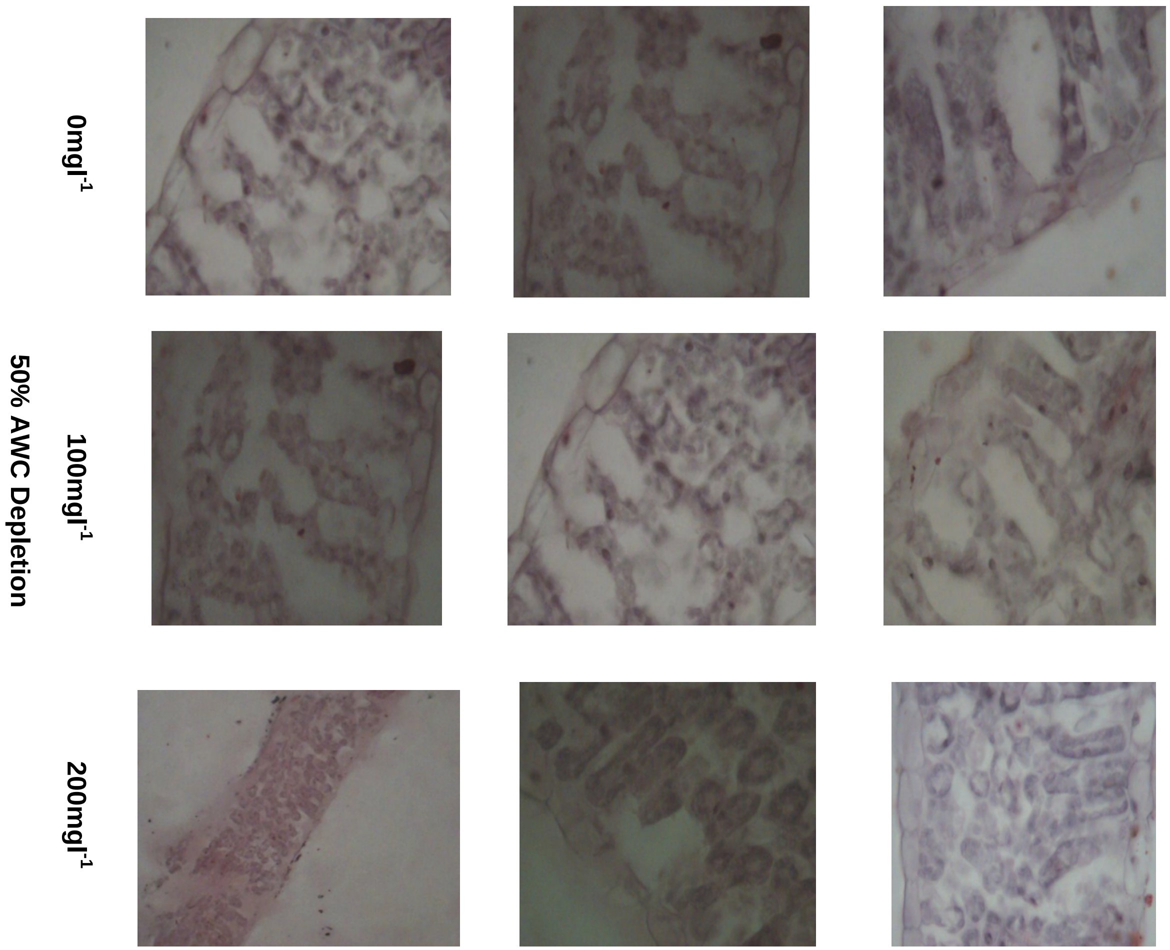
Table 12. Regression analysis for the responses of Mungbean anatomical characters to frequencies of irrigation and GA_3 levels

Character	Regression equation	R^2
Frequencies of irrigation		
Cross section-in of the main leaf vein		
Cuticle thickness, µm	$Y = 1.512 – 0.242 X + 0.027 X^2$	11.5
Epidermis thickness, µm	$Y = 5.797 – 1.086 X + 0.095 X^2$	26.9
Collenchyma thickness, µm	$Y = 33.594 + 6.436 X$	36.4
Bundle sheath thickness, µm	$Y = 7.182 – 3.186 X + 0.672 X2 – 0.044 X^3$	11
Vessel no. in main vein bundle, µm	$Y = 35.9 – 0.804 X$	4
Highest vessel diameter, µm	$Y = 7.075 + 3.244 X – 0.377 X^2$	43.5
Lowest vessel diameter, µm	$Y = 3.213 – 0.236 X$	33.9
Cross section out of the main leaf vein		
Cuticle thickness, µm	$Y = 1.185 – 0.053 X$	17.4
Epidermis thickness, µm	$Y = 3.373 – 0.082 X$	4.1
Palisade thickness, µm	$Y = 32.539 + 1.681 X$	16.7
Spongy layer thickness, µm	$Y = 4.667 + 19.571 X – 1.726 X^2$	62
Thickness of lower epidermis, µm	$Y = 3.231 – 0.167 X$	28.4
Thickness of lower epidermis cuticle, µm	$Y = 1.185 – 0.051 X$	12.8
GA_3 levels		
Cross section-in of the main leaf vein		
Cuticle thickness, µm	$Y = 1.163 – 0.005 X + 0.00003 X^2$	15.1
Epidermis thickness, µm	$Y = 3.669 – 0.005 X$	15.4
Collenchyma thickness, µm	$Y = 48.217 + 0.176 X$	36.2
Bundle sheath thickness, µm	$Y = 2.925 – 0.002 X$	3.7
Vessel no. in main vein bundle, µm	$Y = 36.089 – 0.042 X$	14.7
Highest vessel diameter, µm	$Y = 14.314 – 0.023 X$	23.6
Lowest vessel diameter, µm	$Y = 2.716 – 0.007 X$	37.8
Cross section out of the main leaf vein		
Cuticle thickness, µm	$Y = 1.08 – 0.002 X$	20.9
Epidermis thickness, µm	$Y = 3.187 – 0.002 X$	4.1
Palisade thickness, µm	$Y = 36.213 + 0.047 X$	17.6
Spongy layer thickness, µm	$Y = 29.7 + 0.291 X – 0.001 X^2$	57.7
Thickness of lower epidermis, µm	$Y = 2.683 – 0.003 X$	11.1
Thickness of lower epidermis cuticle, µm	$Y = 1.155 – 0.002 X$	33.2

3.2.6 Cultivar leaf tissue responses to GA_3

Untreated Adlib was superior it gave the highest palisade layer thickness (33.69µm), and untreated Nineveh possesses the thickest spongy layer (46.8) (Table 7; Figure 4). The latter cultivar was also revealed the thicket lower epidermis cuticle (0.27µm). GA_3 play important roles in delaying cell organelles senescence besides its role in cell divisions, cell expansions and dictysomes performance (Hopkins, 1999).


Baraka
Adlib
Nineveh
0mgl-1
100mgl-1
200mgl-1
25% AWC Depletion


50% AWC Depletion
0mgl-1
100mgl-1
200mgl-1

75% AWC Depletion

0mgl^{-1}

100mgl^{-1}

200mgl^{-1}

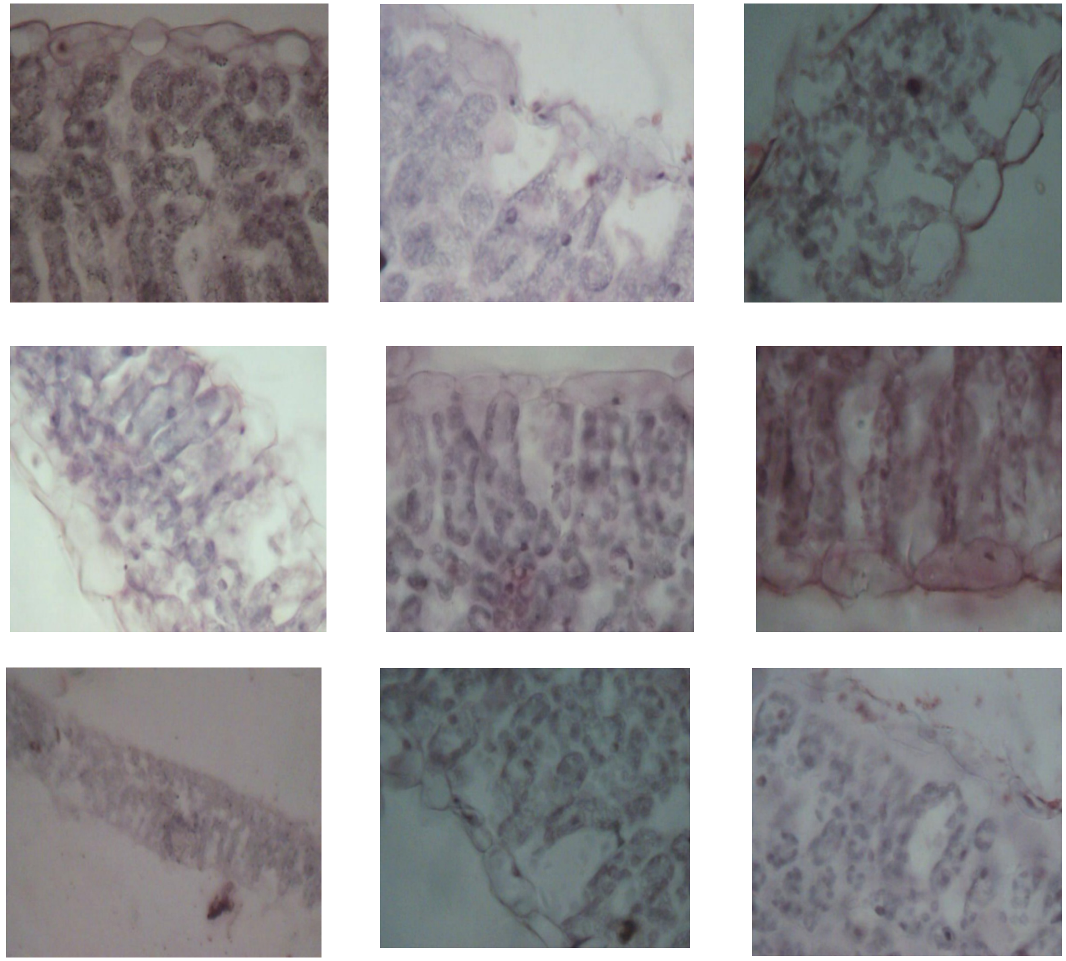


Fig. 4. The effects of GA_3 rates on the anatomical parameters of lentil leaves grown under 75% AWC depletion irrigation level (Magnification 7X40)

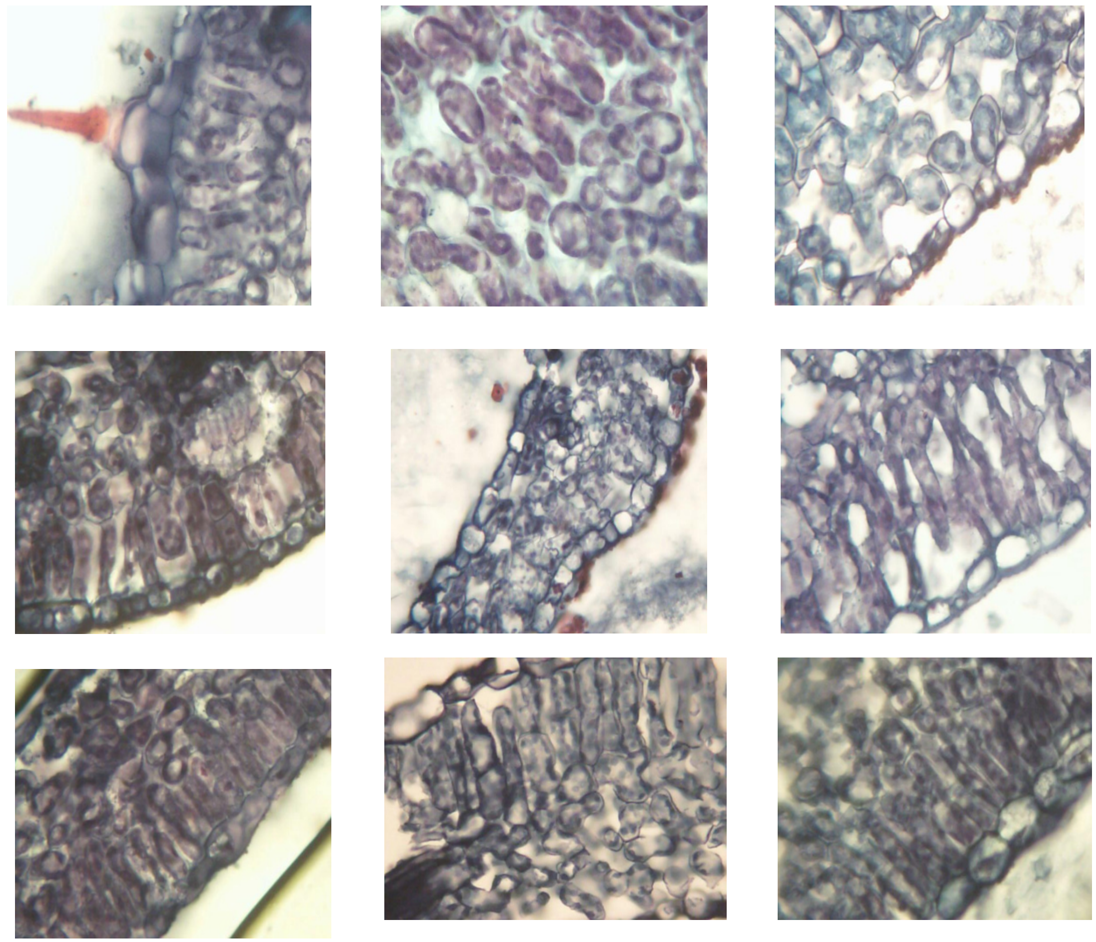


Fig. 5. The influence of different GA$_3$ rates on anatomical characteristics of Common Vetch leaf mesophyll grown under three supplementary irrigation levels. (Magnification 7X40)

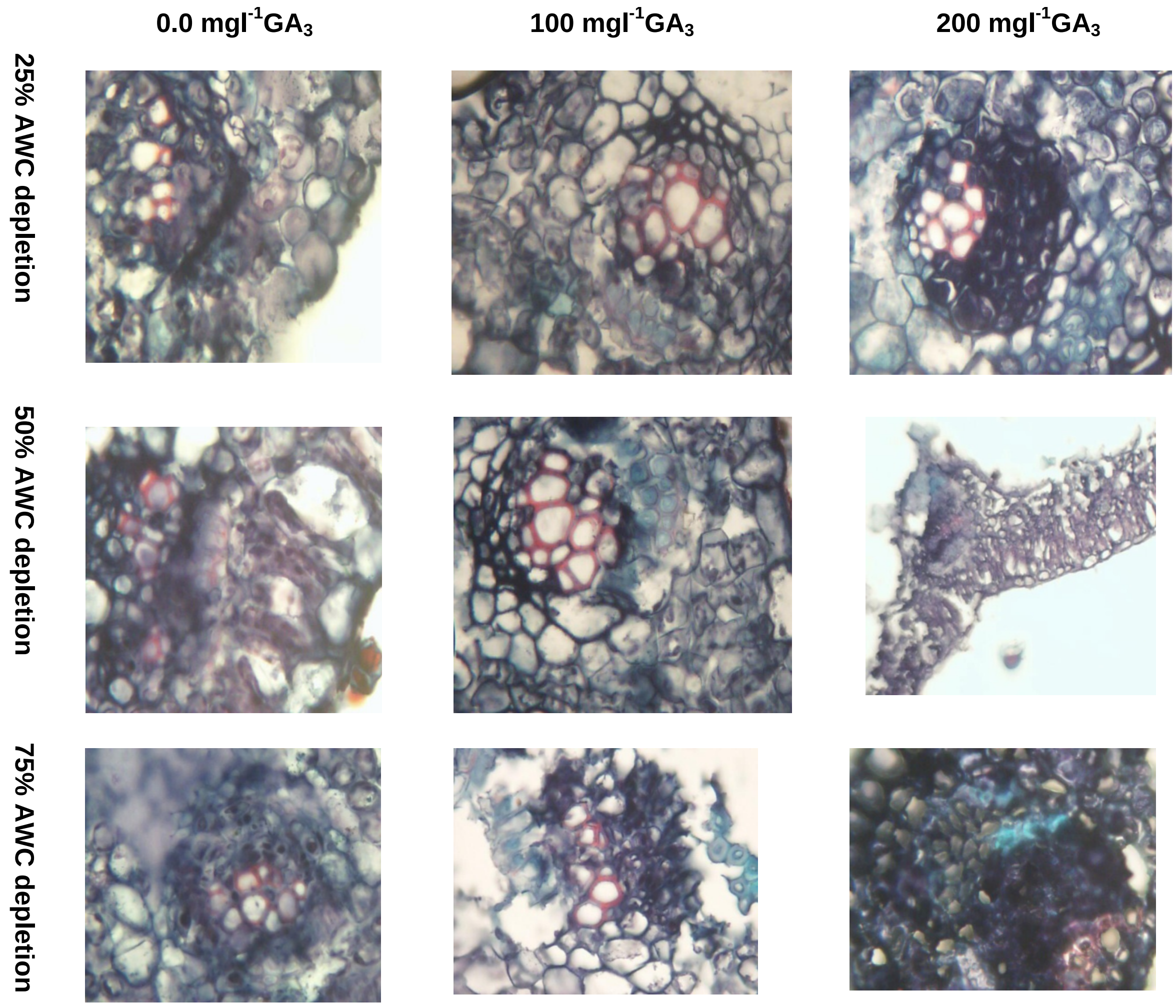
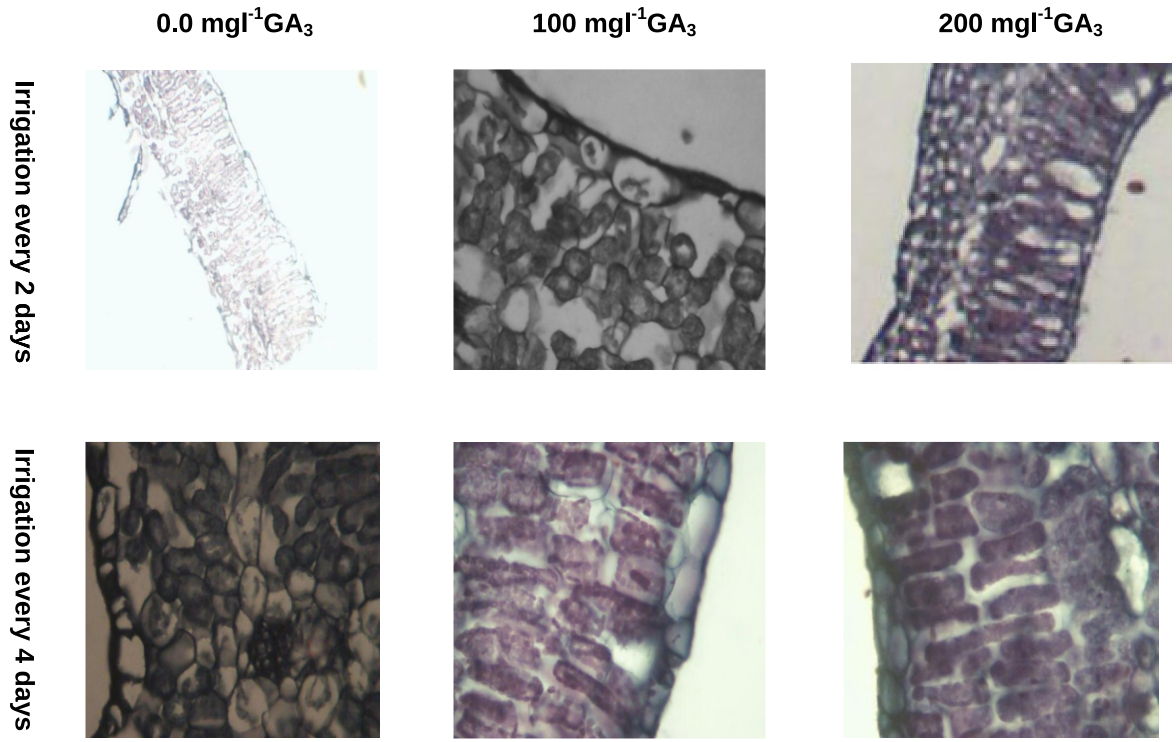


Fig. 5a. The influence of different GA_3 rates on anatomical characteristics of Common Vetch leaf mid vein grown under three supplementary irrigation levels (Magnification 7X40)


0.0 mgl^{-1}GA$_3$
100 mgl^{-1}GA$_3$
200 mgl^{-1}GA$_3$
Irrigation every 2 days
Irrigation every 4 days

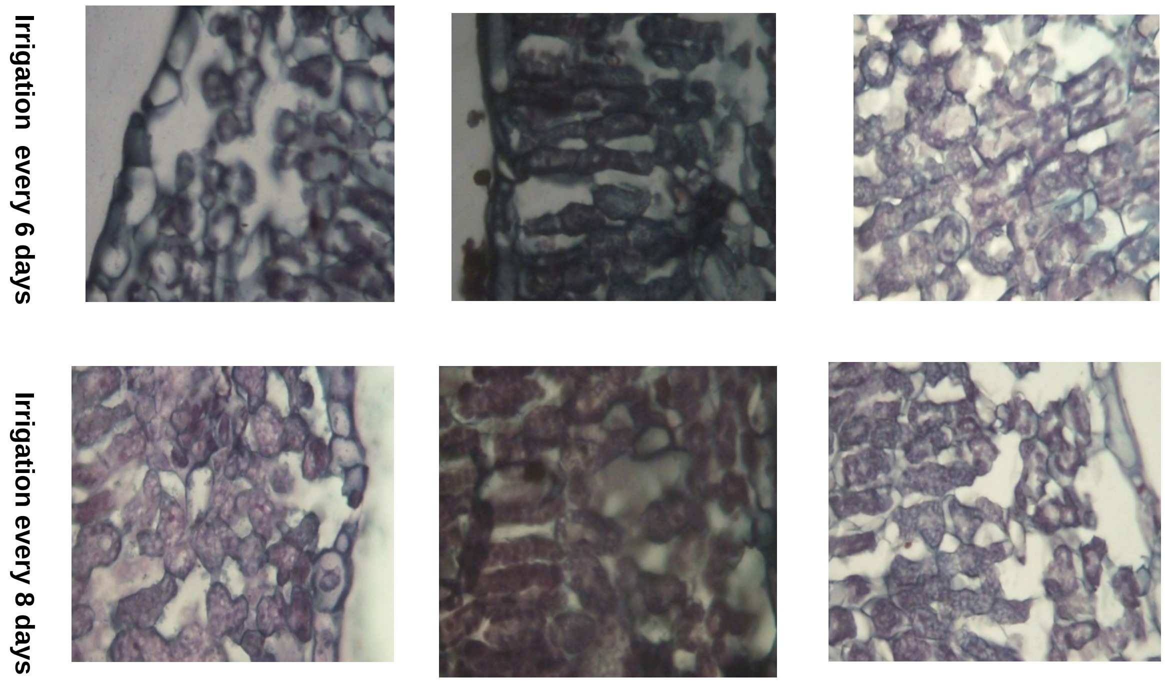
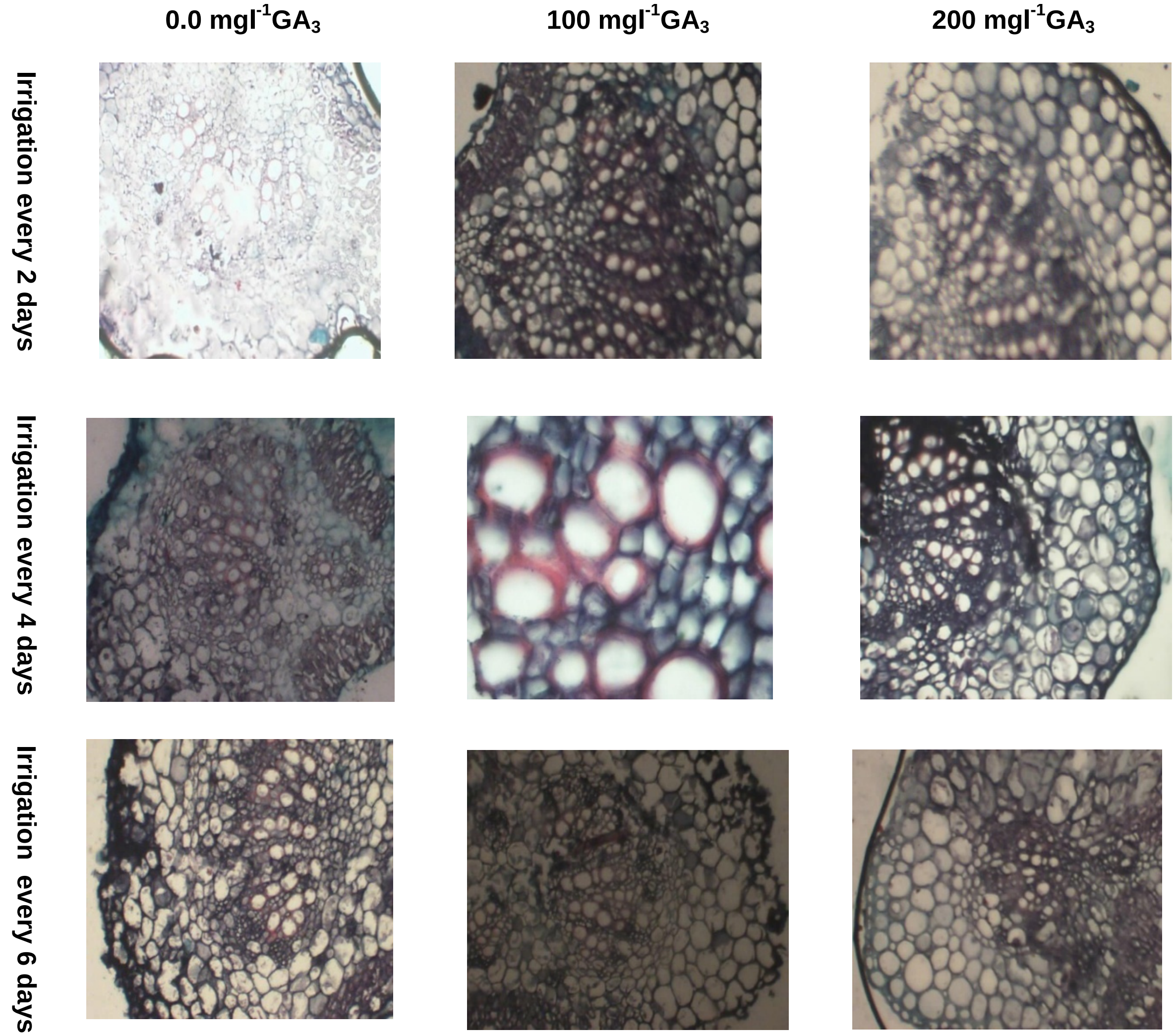


Fig. 6. The influence of different GA_3 rates on anatomical characteristics of Mungbean leaf mesophyll grown under three supplementary irrigation levels. (Magnification 7X40)


0.0 mgl^{-1}GA$_3$
100 mgl^{-1}GA$_3$
200 mgl^{-1}GA$_3$
Irrigation every 2 days
Irrigation every 4 days
Irrigation every 6 days

Irrigation every 8 days

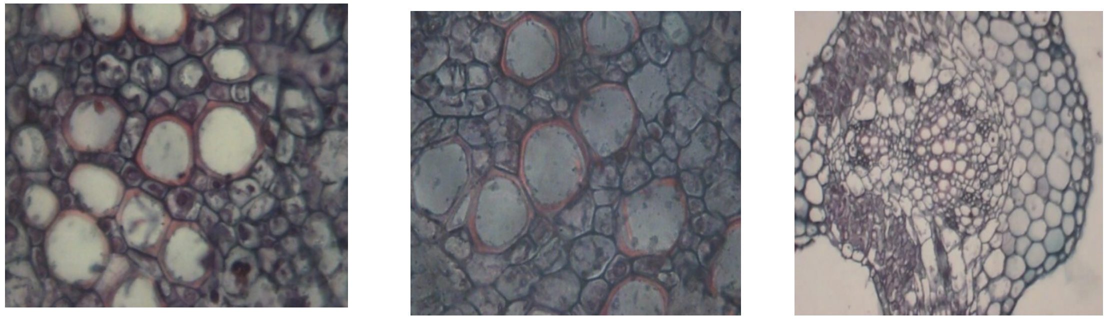


Fig. 6a. The influence of different GA_3 rates on anatomical characteristics of Mungbean leaf mid vein grown under three supplementary irrigation levels. (Magnification 7X40)

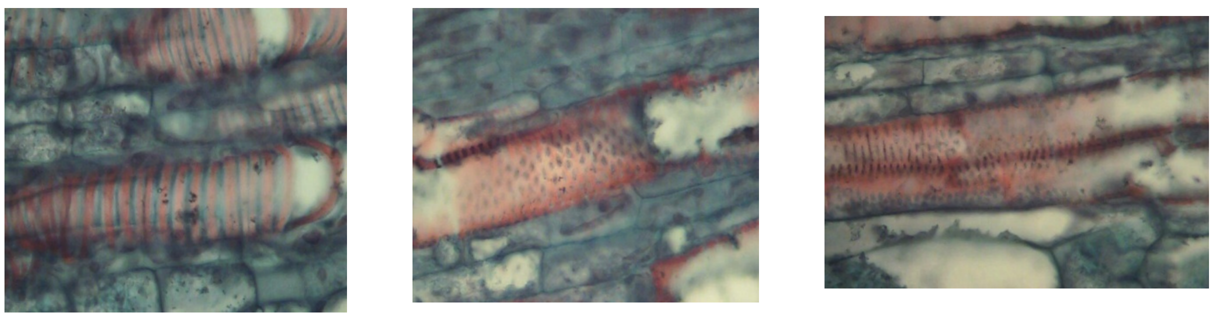


Fig. 6b. Angular thickening Vessels, Pitted thickening and sclariform thickening in Mungbean

3.2.7 Cultivars leaf tissue responses to irrigation and GA_3

Nineveh lentil cultivar untreated grown under (50% level) gave the thickest palisade layer (41.37µm) and spongy layer (55.83µm) (Table 7; Figure 4). Moreover untreated Baraka irrigated by 25% level showed the thickest cuticle (0.903µm) and thickest lower epidermis cuticle (0.537µm). Combinations among the best response cultivars to drought and ameliorating these negative effects by growth promoter such as GA_3 surely would be resulted in reasonable growth and yield owing to combination roles at the cell levels (Figure 4). Mozer (1980) suggested that GA_3 and ABA may affect the synthesis of mRNAs or proteins, at some stages or increase membrane permeability leading to the release of the performed enzymes.

4. CONCLUSION

Detection of drought extent through anatomical alterations is a supreme criterion over growth and yield parameters for discrimination drought susceptible and resistance cultivars. Therefore apparent variations among legume species and even between lentil cultivars were observed. Higher stomata population is confined to susceptible cultivars and vice versa. Higher aperture width and length dedicate to drought spender plant. Lower apertures are related to semi-conservative plant. However, closed stomata indicated that plant is a conservative type. Additionally, widest vessels can be referred to drought avoidance and or escape plants. Angular and coiled vessel thickenings related to high growth potentials. Thicker spongy and palisade layers are accompanied to drought resistance cultivars. Finally, widest bundle sheath is coincident with higher drought tolerance capability. Thicker cuticle devoted to plants underwent severe water and light stresses.

REFERENCES

Abdel, C.G. (1982). Drought resistance in (*Vicia faba* L.): A study of four cultivars. M. Sc. Thesis, Bath University, England, UK.

Abdel, C.G. (2006). Improving yield and yield quality of four faba bean cultivars grown under rainfalls: 2- Application of growth regulators. Mesopotamia J. of Agric., 34(4), 21-30.

Abdel, C.G. (2009). Stomata behavior of three irrigated and non-irrigated onion (*Alium cepa* L.), cultivars grown on polyethylene mulched soils. J. Dohuk Univ. Agric. Vet., 12(2), 1-11.

Abdel, C.G, Al-Salem, M.S.S.D. (2010). Influence of three irrigation levels on growth, stomata behavior and yield of cowpea (*Vigna unguiculata* L. Walp, cv. Ramshorn) produced by three varying seed companies. Tikrit J. of Agric. Sci., 10(2), 14.

Abdel, C.G, Al-Hamadany, S.H.Y. (2010). Resonse of five water stressd faba bean (*Vicia faba* L.) cultivars to exogenous abscisic acid application. Dohuck Univ. J. Agric. Vet., 13(1), 10-25.

Berlyn, G.P., Miksche, J.P. (1976). Botanical micro technique and cytochemistry. Ames Iowa Press.

Chartzoulakis, K., Patakas, A., Kofidis, G., Bosabalidis, A., Nastou, A. (2002). Water tress affects leaf anatomy, gas exchange,water relations and growth of two avocado cultivars. Scientia Horticulturae, 95, 39–50.

Chazen, O., Neumann, P.M. (1994). Hydraulic signals from the roots and rapid cell wall hardening in growing maize leaves are primary responses to PEG induced water deficit. Plant Physiol., 104, 1385-1392.

Choat, B., Ball, M., Luly, J., Holtum, J. (2003). Pit Membrane Porosity and Water Stress-Induced Cavitation in Four Co-Existing Dry Rainforest Tree Species. Plant Physiol., 131, 41–48.
Cosgrove, D.J. (2005). Growth of the plant cell wall. Nat. Rev. Mol. Cell Biol., 6, 850–861.
Dickson, W.C. (2000). Integrative Plant Anatomy. Academic Press, San Diego.
Fan, S., Blake, J.T., Blumwald, E. (1994). The relative contribution of elastic and osmotic adjustments to turgor maintenance in woody species. Physiol. Plant, 90, 408–413.
Franks, P.J., Farquhar, G.D. (2001). The effect of exogenous Abscisic acid on stomatal development, stomatal mechanics, and leaf gas exchange in *Tradescantia virginiana*. Plant Physiol., 125, 935–942.
Garcia-Martinez, J.L., Ben-Shalomand, N., Rappaport, L. (1984). Gibberellin-induce ethylene production and its effect on cowpea epicotyls elongation. Plant Growth Mills, H.A. (2001). Vegetable Crops. January 2000, Revised 2001. File://G:\ Vegetables.htm.
Harvey, H.P., van den Driessche, R. (1997). Nutrition, xylem cavitation and drought resistance in hybrid poplar. Tree Physiol., 17, 647–654.
Hedden, P, Thomas, S.G. (2006). Plant Hormone Signaling. Blackwell Publishing Ltd. 155-174.
Hopkins, W.G. (1999). Introduction to Plant Physiology. P 309-360, 2nd Ed., John Wiley & Sons, Inc.
Kaneko M., Itoh, H., Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Ashikari, M., Matsuoka, M. (2003). Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants? Plant J., 35, 104–115.
Kutlu, N., Terzi, R., Tekeli, I., Senel, G., Battal, P., Kadiglu, A. (2009). Changes in anatomical structure and levels of endogenous phytohormones during leaf rolling in *Ctenanthe setosa* under drought stress. Turk J. Biol., 33, 115-122.
Levitt, J. (1980). Responses of Plants to Environmental Stresses: Water, Radiation, Salt, and other stresses, Academic Press, New York, pp. 365-488.
Lovisolo, C, Schubert, A. (1998). Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. J. Experim. Bot., 49(321), 693–700.
Mozer, T.J. (1980). Control of protein synthesis in barley alurone layers by the plant hormones gibberellic acid and abscissic acid. Cell, 20, 379-485.
Pickard, W. F. (1981). The ascent of sap in plants. Prog. Biophys. Mol. Biol., 37, 181–229.
Pritchard, J.R., Wyn Jones, G., Tomos, A.D. (1991). Turgor, growth and rheological gradients of wheat roots following osmotic stress. J. Experim. Bot., 42, 1043–1049.
Shane, M.W., McCully, M.E., Canny, M.J. (2000). Architecture of branch root junctions in maize: structure of the connecting xylem and the porosity of pit membranes. Ann. Bot., 85, 613–624.
Silk, W.K. (1992). Steady form from changing cell. Int. J. Plant Sci., 153, 549-558.
Sperry, J.S., Tyree, M.T. (1988). Mechanism of water-stress induced embolism. Plant Physiol., 88, 581–587.
Srivastava, S.P., Bhandari, T.M.S., Yadav, C.R., Joshi, M., Erskine, W. (2000). Boron deficiency in lentil: Yield loss and geographic distribution in a germplasm collection. Plant Soil, 219, 147–151.
Tyree, M.T., Davis, S.D., Cochard, H. (1994). Biophysical perspectives of xylem evolution: Is there a trade off of hydraulic efficiency for vulnerability to dysfunction? IAWA J., 15, 335–360.
Zimmermann, M.H. (1983). Xylem structure and the ascent of sap. Berlin: Springer Verlag.