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Ultrastructural response of wheat (*Triticum aestivum* L.) lines to potential allelopathy of *Alstonia scholaris* (L.) R. Br. leaf extract

Mansoor HAMEED[1,*], Sana FATIMA[2], Syed Mohsan Raza SHAH[1], Farooq AHMAD[1], Muhammad ASHRAF[3], Maryam[2], Muhammad Sajid Aqeel AHMAD[1], Iftikhar AHMAD[4], Ummar IQBAL[1]

[1]Department of Botany, Faculty of Sciences, University of Agriculture Faisalabad, Faisalabad, Punjab, Pakistan
[2]Department of Botany, Government Sadiq College Women University, Bahawalpur, Punjab, Pakistan
[3]Vice Chancellor, University of Agriculture Faisalabad, Faisalabad, Punjab, Pakistan
[4]Department of Botany, University of Sargodha, Sargodha, Punjab, Pakistan

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Abstract: An investigation was carried out to evaluate the impact of the allelopathic effect of *Alstonia scholaris* leaf extract on morphoanatomical and physiological characteristics of 5 wheat lines. Three extract dilutions were prepared, viz. 0 (control), 15 and 30% of the aqueous extract and sprayed on wheat lines (3094, 5066, M5082, 7076 and A2011) at 15-day intervals until maturity from November 2017 to May 2018. All wheat lines responded differently to an allelochemical extract of *A. scholaris.* Disintegration of root parenchyma and transformation of stem chlorenchyma to sclerenchyma in stems were noted in Line 3094. Increased sclerification in the root cortex (141.7–212.5 µm) and stem hypodermal region (212.5–354.2 µm) was recorded in Line 5066. Line M5082 showed intensive sclerification in the root cortex and vascular region, and around stem vascular bundles along with a significant decrease in leaf thickness. Line 7076 was the most sensitive genotype showing significant reduction in the stem (1402.8–821.8 µm) and root area (963.5–791.9 µm) and leaf thickness (1346.2–396.8 µm). Line A2011 responded very differently by increasing proportion of parenchyma and sclerenchyma. A low concentration (15%) of allelochemical extract promoted growth and development and a high concentration (30%) was toxic, causing growth retardation and tissue damage.

Key words: Chlorenchyma, devil tree, leaf thickness, parenchyma, sclerenchyma

1. Introduction

Plants produce several allelochemicals for plant defense, especially to avoid herbivory, though these chemicals contain harmful allelopathic properties (Duke, 2003). Such allelochemicals are released into the environment, which suppress the growth of other plants (Wardle et al., 2011). They are generally secondary metabolites, mainly terpenoids, phenolics, cyanides, and fatty acids in nature (Bartwal et al., 2013). They alter the biochemical reaction and as a result, the physiological process undergoes modification (Olivoto et al., 2017).

Allelochemicals are produced in the tissue of plants and released in soil as leaf leachates, or by root exudation and tissue decomposition (Inderjit and Duke, 2005). Airborne allelopathy via volatile allelochemicals are reported by Matsuyama et al. (2000). They have a multitude of impacts on plant communities such as directly inducing the severe toxicity in physiological development of plant species (Bais et al., 2003). Indirectly, they change the environment by alteration in soil microorganisms, pH, and nutrient uptake (Blum et al., 1993).

Allelochemicals act like synthetic herbicides, so are considered bioherbicides for the control of weeds. The action of these compounds depends on the concentration, as they inhibit and promote the plant growth at higher and lower concentrations (Tamak, 1994). A well–known example of natural herbicides is the use of water extract of sorghum and sunflower for protection of plants without yield loss (Irshad and Cheema, 2005).

Alstonia scholaris L. (ditabark or devil tree) is a native tree of Pakistan and other regions of the Indian subcontinent. It can grow to 35 m in height, with a grimy and irregular bark. Large leaves arrange in a cluster; the inflorescence is strongly aromatic and pale green in color (Channa et al., 2005). The tree contains a pungent and sticky fluid. Secondary metabolites, e.g., indol alkaloids, are released by it (Thankamani et al., 2011).

A large number of chemical compounds have been reported in *Alstonia* spp. (Cai et al., 2008; Wang et al.,

* Correspondence: hameedmansoor@yahoo.com

2009; El-Askary et al., 2012). *Alstonia scholaris* extract is effectively used an herbicide as it impedes germination and growth in many weedy species (Shafique et al., 2007; Sultana and Saleem 2010). *A. scholaris* extract is used as potent herbicide for the invasive *Parthenium* weed (Javaid et al., 2010). Soil, leaves, and litter from *A. scholaris* inhibit the growth of *Bidens pilosa* (Wang et al., 2009; Sultana and Saleem, 2010). *A. scholaris* contains allelochemical pentacyclic triterpenoids including ursolic acid, oleanolic acid and betulinic acid (Wang et al., 2014). Ursolic acid abundantly accumulates in soil, which inhibits weed growth (Macias et al., 2007). The present study was focused on the impact of allelochemical extract of *A. scholaris* on bread wheat cultivars, expecting that a low dose may act as a growth promoter and a high dose suppresses growth and can be affected as an herbicide. Keeping in view past literature, it was hypothesized that allelochemicals in the leaf extract of *A. scholaris* might induce ultrastructural modifications when applied to wheat at the vegetative growth phase.

2. Materials and methods

An experiment was conducted at Wire House of the Old Botanic Garden, University of Agriculture, Faisalabad to investigate the allelopathic effects of leaf extracts of *Alstonia scholaris* on morphoanatomical and physiological characteristics of 5 wheat lines. The experiment was conducted in earthen pots (diameter 30 cm). Seeds of 5 wheat undertrial lines 3094, 5066, M5082, 7076 and A2011 were obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad. A pilot experiment containing 20 wheat lines was conducted to screen various doses of phytochemical extract (0, 10, 20, 30, 40, and 50% diluted solution; data not presented in this manuscript). Of these wheat lines, 3 resistant and 2 susceptible wheat lines were selected for further study. Similarly, 3 phytochemical levels, 0, 15, and 30% were selected. A 50% reduction in growth attributes was used as selection criteria for wheat lines.

Clayey loam soil with sufficient moisture content was taken from cultivated fields, and underside particles were cleaned from the soil. Fresh, young, but fully expanded leaves of *Alstonia scholaris* were taken from the Old Botanic Garden. The leaves were thoroughly washed with distilled water. Washed leaves were chopped and then soaked for 24 h in distilled water at 1:10 ratio to obtain the crude leaf extract. The leaf extract was filtered and then concentrated by continuous boiling to reduce the volume by 20 times. Three treatments were prepared from the concentrated extract, i.e. control (0% extract), 15% extract, and 30% extract in distilled water.

Seeds of all wheat lines were sown in the first week of November 2017 in pots, and all the pots were properly labeled. After germination, aqueous extracts were applied as a foliar spray by adding Tween 20 as surfactant at an interval of 15 days until the maturity of the crop. Control plants were covered with plastic sheets during spraying in order to avoid any exposure to the phytochemical extract; rather, they were sprayed by distilled water thereafter. A recommended dose of NPK fertilizers was applied to all pots including control. Data for morphological characteristics were taken at crop maturity until 4 months after germination. The samples were preserved in 70% ethanol for anatomical studies. Standard freehand sectioning was used for the preparation of permanent slides of fast green and safranin stains. Anatomical data were recorded by ocular micrometer under a camera–equipped compound microscope. Leaves were extracted in acetone for photosynthetic pigments (chlorophyll a and b) and readings were recorded in a spectrophotometer at wavelengths of 663 nm, 645 nm, and 480 nm. Data were subject to analysis of variance (ANOVA) using 2 factorial completely randomized designs with 3 replications using CoStat computer software, v. 6.303. The means were compared for significance using a least significant difference (LSD) test at 5% confidence level.

3. Results

3.1. Morphology

Plant height of wheat lines were generally not affected by *Alstonia scholaris* allelochemical extract (AsEx). Only wheat line A2011 responded to AsEx, where a significant increase was recorded along with an increase in the AsEx level (Table 1). Lines M5082 and 5066 increased significantly in plant height, but only at a 15% AsEx level. Lines 7076 and A2011 showed an increase in plant height at 15% and 30% levels, respectively. The highest level significantly decreased plant height in these wheat lines as compared to 15% level. The number of tillers per plant generally showed no significant response. Lines 3094 and 5066 showed an increase in this character at the 15% level only. Line 5066 showed a significant increase at the 30% level regarding tillers per plant (Table 1).

Leaf number, ear length, spikelet number, and grain yield in all lines of wheat showed a consistent decrease at both extract levels, i.e. 15% and 30% AsEx; however, the highest extract level proved to be more toxic. However, in wheat line 7076, 100–grain weight showed an increase at a smoderate level. All other wheat lines showed a significant decrease as the level of AsEx increased.

Shoot fresh weight increased significantly in 4 wheat lines (3094, 5066, M5082, and 7076) at 15% AsEx. Two lines, 3094 and 5066, showed an increased value of fresh weight at 30% AsEx level (Table 1). Root dry weight consistently decreased in 3 wheat lines (3094, 5066, and A2011) as the level of AsEx increased. This parameter was

Table 1. Morphological and physiological characteristics of wheat lines treated with *Alstonia scholaris* leaf extract.

	Levels	3094	5066	M5082	7076	A2011	F-ratio
Morphology							
Plant height (cm)	0%	55.4a	57.2a	55.3a	52.9a	51.9a	1.2^{NS}
	15%	56.3a	60.5b	57.3b	55.8b	55.1b	
	30%	55.2a	56.6a	54.8a	56.7b	58.0c	
Number of fertile tillers per plant	0%	2.3b	1.3a	2.3b	2.7b	3.0b	3.6^{NS}
	15%	2.7b	2.7b	1.5a	1.4a	2.6a	
	30%	1.4a	2.7b	1.3a	1.2a	2.3a	
Leaves per plant	0%	10.4b	11.5c	11.3b	10.4b	14.8b	4.7*
	15%	8.5b	9.3b	6.7a	6.7a	8.7a	
	30%	6.3a	6.3a	6.4a	6.3a	7.3a	
Spike length (cm)	0%	9.3a	11.bc	11.6c	10.3b	9.4b	3.1^{NS}
	15%	9.1a	9.4a	9.8b	9.6ab	8.9ab	
	30%	8.3a	8.4a	8.5a	8.9a	7.8a	
Spikelets per spike	0%	15.3a	17.4b	18.3b	18.7b	17.7b	2.2^{NS}
	15%	14.4a	15.1a	15.5a	18.3b	18.4b	
	30%	13.7a	13.5a	15.2a	16.4a	14.3a	
100 grain weight (g)	0%	3.4a	4.2b	2.6b	2.5b	2.2b	6.9*
	15%	3.2a	3.2a	3.0b	3.2c	1.8ab	
	30%	3.1a	2.9a	2.1a	2.0a	1.5a	
Yield per plant (g)	0%	6.2b	7.7c	7.3b	8.4c	6.1b	8.4**
	15%	6.2b	5.2b	3.6a	3.8b	2.0a	
	30%	3.7a	3.6a	3.5a	2.6a	1.7a	
Shoot fresh weight (g $plant^{-1}$)	0%	28.7a	29.9a	31.0a	28.9a	24.6a	4.6*
	15%	38.3b	41.3b	39.5b	39.2b	28.3a	
	30%	34.1b	36.5b	31.4a	30.7a	29.3a	
Shoot dry weight (g $plant^{-1}$)	0%	10.3b	3.8a	5.5a	5.8a	5.7a	6.2*
	15%	21.0c	12.1c	8.3b	10.1b	5.9a	
	30%	7.9a	8.5b	11.0c	12.3c	7.4b	
Root fresh weight (g $plant^{-1}$)	0%	5.6b	4.7b	5.0b	3.7b	7.3c	4.8*
	15%	3.2a	4.3ab	3.6a	3.4ab	5.7bc	
	30%	3.1a	3.3a	3.4a	2.5a	2.5a	
Root dry weight (g $plant^{-1}$)	0%	2.1b	1.9c	1.3b	1.3b	2.2c	6.3*
	15%	0.7a	1.0b	1.2b	1.2b	1.1b	
	30%	0.3a	0.3a	0.5a	0.5a	0.5a	
Chlorophyll pigments							
Chlorophyll a (mg g^{-1} f.wt.)	0%	2.4b	1.2a	0.9a	0.6a	1.3a	9.6**
	15%	1.6a	1.2a	1.0a	1.2b	1.5a	
	30%	1.2a	1.1a	1.1a	2.2c	1.3a	
Chlorophyll b (mg g^{-1} f.wt.)	0%	0.53b	0.65b	0.21a	0.74b	0.24a	5.2*
	15%	0.22ab	0.28a	0.30a	0.13a	0.23a	
	30%	0.16a	0.25a	0.26a	0.12a	0.18a	

Means sharing similar letters are statistically not significant at LSD = 5%.
* = significant at P > 0.05, ** = significant at P > 0.01, *** = significant at P > 0.001, NS = statistically not significant.

severely affected at 30% AsEx in 2 lines (M5082 and 7076), where a significant decrease was recorded (Table 1).

3.2. Chlorophyll pigments

Chlorophyll a was not altered by AsEx treatment in 3 wheat lines (5066, M5082 and A2011). This parameter decreased with increasing AsEx levels in wheat line 3094, but increased in 7076 (Table 1). Two wheat lines (5066 and 7076) showed a significant decrease in response to increasing AsEx levels. Chlorophyll a decreased significantly in line 3094 but only at 30% AsEx level.

3.3. Root anatomy

Root radius was invariably decreased in response to an increase in AsEx concentration. The highest concentration (30%) was more toxic, which showed a significant decline in most of the wheat lines as compared to that recorded at 15% AsEx. Epidermal cell area increased at 15% AsEx in 3 wheat lines (5066, M5082, and 7076), but this parameter significantly decreased at 30%. Lines 3094 and A2011 showed a consistent decrease along with an increasing concentration of AsEx (Table 2).

Sclerification invariably increased in all wheat lines, but the response of each line to AsEx was slightly different (Figure 1, Table 2). Sclerification gradually increased in 3 wheat lines (3094, M5082, and 7076) as the concentration of AsEx increased. The other 2 lines (5066 and A2011) showed a significant increase, but only at 15% AsEx.

Cortical region significantly decreased in all wheat lines except 5066 as the concentration of AsEx increased. The 30% AsEx was more toxic in all cases. This parameter in wheat line 5066 remained unchanged during all AsEx treatments (Figure 1, Table 2). Cortical cell area responded differently; it significantly decreased with an increase in AsEx concentration in 3 lines (3094, 5066, and M5082). Wheat line 7076 showed a significant decrease in cortical cell area, but only at the highest AsEx concentration (30% extract). This parameter was increased at 15% AsEx in line A2011, which thereafter decreased at the highest concentration (30% AsEx).

Endodermal cell area decreased significantly in all wheat lines, but their response to increasing AsEx concentration varied (Table 2). Three lines (M5082, 7076, and A2011) showed a significant reduction at 15% AsEx, but no further change was observed at the highest level. Endodermal cell area was not affected in 2 lines (3094 and 5066) at 15% AsEx concentration, but the highest concentration resulted in a significant decrease.

Vascular region thickness showed variable response to AsEx concentrations. It decreased consistently in 3 wheat lines (M5082, 7076 and A2011) along with an increase in AsEx concentration. Vascular region thickness increased significantly in line 3094 at both 15 and 30% AsEx concentrations. Line 5066 showed a significant increase, but only at 15% AsEx concentration.

Metaxylem area showed a variable response to different AsEx levels. It consistently decreased in 3 wheat lines (M5082, 7076, and Aa2011) with an increase in AsEx concentration levels. A significant increase in the metaxylem area was recorded in line 3094 but only at the 30% AsEx level. Line 5066 showed a significant increase at 15% AsEx concentration (Figure 1, Table 2).

Pith cell area consistently decreased in 2 wheat lines (7076 and A2011) with an increase in AsEx concentrations. Wheat line 5066 showed a significant decrease in this parameter, but only at the 30% AsEx level (Figure 2, Table 2). Pith area decreased consistently in 3 wheat lines (3094, M5082, and 7076) along with an increase in AsEx concentration. A significant increase was recorded in line A2011 at both 15 and 30% AsEx levels. Line 5066 showed a significant increase, but only at 30% AsEx concentration.

3.4. Stem anatomy

Stem cellular region thickness increased consistently in 3 wheat lines (3094, 5066, and A2011) along with an increase in AsEx concentration but decreased in 2 lines (M5082 and 7076). Epidermal thickness area decreased in wheat line 3094, while it increased in line M5082 at both AsEx concentrations. This parameter increased in line A2011 and decreased in line 7076 only at 30% AsEx concentration.

Chlorenchymatous area invariably decreased in all wheat lines, but the response of each line to AsEx was slightly different. Chlorenchymatous area decreased gradually in 3 wheat lines (5066, M5082 and 7076) as the concentration of AsEx increased. The other 2 lines (3094 and A2011) showed a significant decrease in this parameter, but only at 30% AsEx.

Sclerenchymatous thickness showed a variable response to AsEx concentrations. It increased in 3 wheat lines (3094, 5066 and A2011) only at 30% AsEx concentration. A significant decrease was recorded in 2 lines (M5082 and 7076) but only at 30% AsEx concentration. Cortical cell area increased significantly in all wheat lines, but only at 15% AsEx concentration (Figure 1, Table 3). Two lines (3094 and 5066) showed a significant decrease in this parameter at 30% AsEx concentration.

Vascular bundle area increased consistently in 2 wheat lines (5066 and 7076) along with increase in AsEx concentration. Line M5082 showed a significant decrease in this parameter only at 30% AsEx concentration. Two wheat lines (3094 and A2011) showed a consistent increase with increasing AsEx concentrations.

Metaxylem decreased consistently in 2 wheat lines (5066 and M5083) along with an increase in AsEx concentration. Line 7076 showed a significant decrease in this parameter but only at 30% AsEx concentration. Wheat line A2011 showed a consistent increase at both 15% and 30% AsEx concentrations. Phloem area responded

Table 2. Root anatomical characteristics of wheat lines treated with *Alstonia scholaris* leaf extract.

	Levels	3094	5066	M5082	7076	A2011	F-ratio
Root radius (μ)	0%	1105.2c	850.2c	963.5c	963.5b	1133.6c	17.1***
	15%	921.0b	793.5b	793.5b	793.5a	991.9b	
	30%	821.8a	751.0a	708.5a	791.9a	651.8a	
Epidermal area (μ^2)	0%	3155.3b	2524.2b	1880.4a	1419.8b	3155.3c	3.4^{NS}
	15%	2524.2a	3155.2c	2524.2b	1893.1c	1887.5b	
	30%	2519.4a	1893.1a	1895.2a	631.0a	946.6a	
Sclerenchyma thickness (μ)	0%	184.2a	141.7a	127.5a	85.0a	140.8a	6.8*
	15%	255.0b	212.5b	141.7b	99.1b	212.5b	
	30%	283.4c	139.1a	212.5c	226.7c	139.2a	
Cortex region thickness (μ^2)	0%	354.2c	283.4a	538.4c	495.9c	425.1b	12.8**
	15%	283.4b	287.1a	311.7b	325.9b	419.6b	
	30%	255.0a	289.2a	240.9a	283.4a	283.4a	
Cortex cell area (μ^2)	0%	11043.3c	6626.0c	7572.6c	3155.3b	9939.0b	8.1**
	15%	3944.0a	5679 .5b	3155.3a	3055.3b	11358.9c	
	30%	4198.6b	5521.7a	4732.9b	1893.2a	1419.9a	
Endodermal cell area (μ^2)	0%	1893.2b	1893.1b	1419.9b	1419.8b	1893.1b	7.6**
	15%	1884.9b	1889.4b	946.6a	625.4a	642.8a	
	30%	1419.8a	1419.8a	941.2a	631.1a	631.0a	
Vascular region thickness (μ)	0%	212.5a	141.7a	283.4c	212.5b	283.4c	9.2**
	15%	269.2b	212.5b	212.5b	141.7a	184.2b	
	30%	283.4c	139.2a	141.7a	139.1a	141.2a	
Metaxylem area (μ^2)	0%	7572.6a	6626.0a	15776.3c	8834.7c	14198.6c	8.2**
	15%	7593.2a	7572.6b	11358.9b	6626.0b	4732.9b	
	30%	8834.7b	6611.7a	5521.7a	4732.9a	3155.2a	
Pith cell area (μ^2)	0%	631.7a	1419.8b	631.0a	946.6c	1419.8c	12.2**
	15%	629.6a	1416.3b	628.7a	631.5b	946.5b	
	30%	621.5a	631.1a	626.4a	315.2a	631.0a	
Pith area (μ^2)	0%	85191.8c	63105.0a	69415.6c	56794.5c	37863.0a	7.1*
	15%	63105.0b	63098.5a	48275.4b	48275.4b	65313.7b	
	30%	48275.3a	76199.3b	31552.5a	44173.5a	93711.0c	

Means sharing similar letters are statistically not significant at LSD = 5%.
* = significant at P > 0.05, ** = significant at P > 0.01, *** = significant at P > 0.001, NS = statistically not significant.

differentially to AsEx concentrations. It decreased consistently in 2 wheat lines (3094 and 5066) along with an increase in AsEx concentration. Two lines (M5082 and 7076) showed a significant decrease in this parameter but only at 30% AsEx concentration. Line A2011 showed a significant increase in both at 15% and 30% AsEx levels.

3.5. Leaf anatomy

Midrib thickness decreased consistently in wheat line 3076 along with increase in AsEx concentration. Three wheat lines (3094, M5082 and A2011) also decreased significantly, but only at 30% level. Midrib thickness increased significantly in line 5066 only at the 15% AsEx level (Figure 2, Table 4). Lamina thickness decreased consistently in lines 5066 and 7076 but increased in 3094 as the concentration of AsEx increased. A significant decrease was observed in line M5082 but only at the 30% level (Table 4).

Abaxial epidermal thickness showed a significant increase at 15% AsEx concentration only (Table 3), while all other lines showed no significant change. Adaxial

Figure 1. Root and stem transverse sections of some wheat lines treated with allelochemical extract of *Alstonia scholaris.*

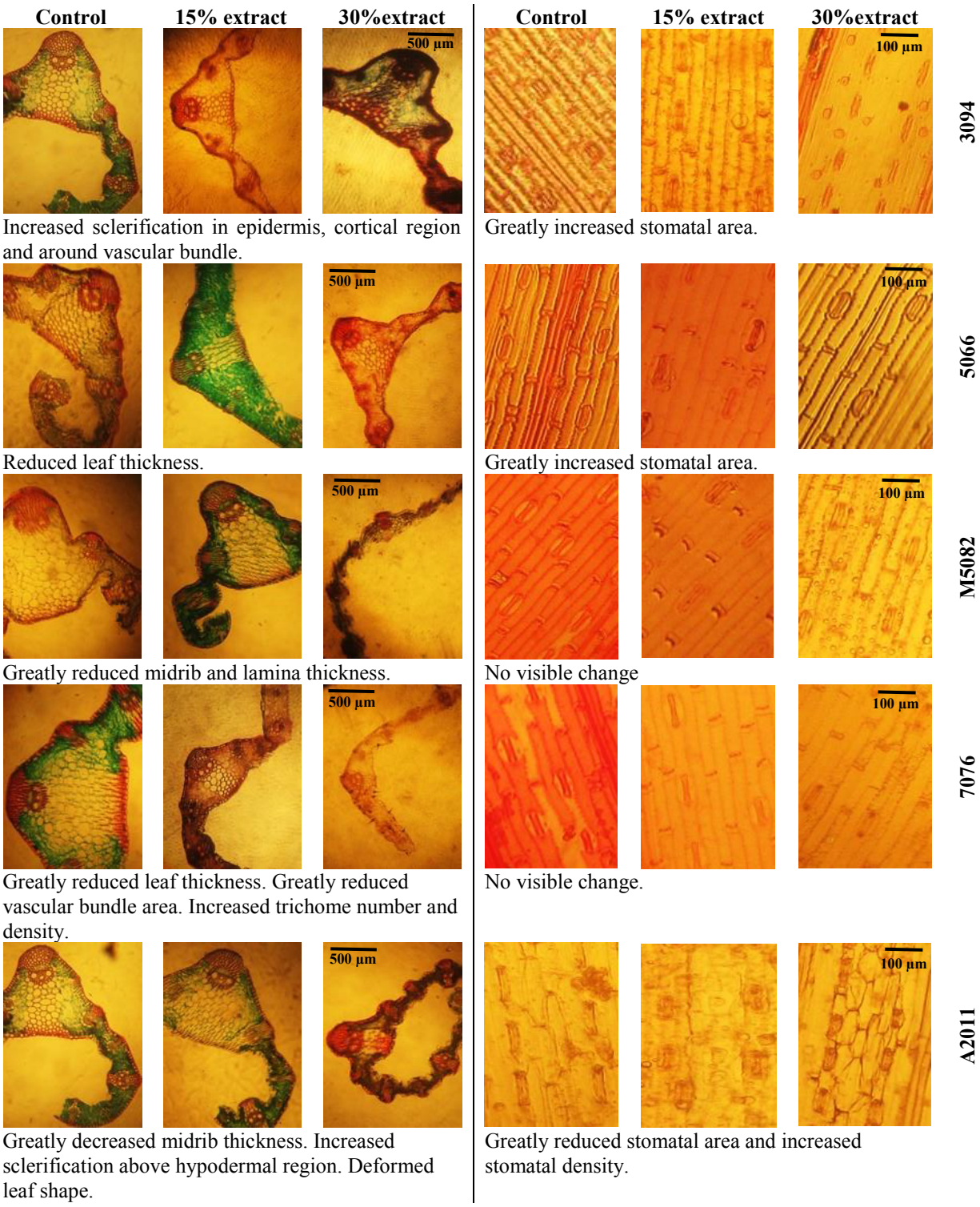

Figure 2. Leaf transverse sections and epidermal surface view of some wheat lines treated with allelochemical extract of *Alstonia scholaris*.

epidermal thickness, however, showed a variable response to AsEx concentrations. It increased consistently in wheat line M5082 along with an increase in AsEx levels. Line 3094 showed a significant increase but only at 15% concentration. Two wheat lines (5066 and 7076) showed a significant decrease only at the highest 30% AsEx level. Bulliform cell thickness decreased consistently in wheat line 5066 along with increasing AsEx levels. A significant

Table 3. Stem anatomical characteristics of wheat lines treated with *Alstonia scholaris* leaf extract.

	Levels	3094	5066	M5082	7076	A2011	F-ratio
Stem cellular region thickness (μm^2)	0%	779.3a	963.5a	1091.0c	1402.8c	850.2a	18.7***
	15%	991.9b	1402.8c	921.0b	991.9b	1275.3c	
	30%	991.9b	1133.6b	495.9a	821.8a	963.5b	
Epidermal thickness (μm)	0%	946.6b	946.6a	473.3a	946.6b	315.6a	19.9***
	0015%	631.0a	941.2a	1893.1c	943.8b	321.4a	
	30%	629.4a	940.9a	631.0b	631.0a	1893.1c	
Chlorenchymatous area (μm^2)	0%	47328.8b	41018.3b	30763.7b	53639.3c	15776.2b	15.9**
	15%	46328.8b	23664.4a	24610.9a	32814.6b	12305.4ab	
	30%	41018.3a	20509.1a	23197.4a	11043.3a	9465.7a	
Sclerenchymatous thickness (μm)	0%	283.4a	212.5a	240.8b	283.4b	170.0a	13.6**
	15%	290.7a	226.7a	212.5b	255.0b	177.3a	
	30%	354.2b	354.2b	70.8a	170.0a	325.9b	
Cortical cell area (μm^2)	0%	15776.2b	36916.4b	14198.6a	7572.6a	14198.6a	25.7***
	15%	20509.1c	59949.8c	11358.9b	15776.2b	42595.9b	
	30%	11043.4a	15776.2a	3944.0a	7891.5a	15776.3a	
Vascular bundle area (μm^2)	0%	59949.8a	141355.4c	63105.0b	94657.6c	47328.8a	23.2***
	15%	78881.3b	82036.6b	61685.2b	63105.0b	78881.3c	
	30%	75726.1b	72570.8a	37863.0a	50484.0a	53639.3b	
Metaxylem area (μm^2)	0%	3155.3a	8834.7c	4732.9c	3155.2b	2366.4a	7.0**
	15%	3118.2a	5521.7b	2366.5b	3097.6b	5521.7c	
	30%	2994.2a	4732.8a	1893.2a	1893.1a	4732.9b	
Phloem area (μm^2)	0%	8519.1c	13252.0c	7888.2b	6626.0b	4732.9a	14.9**
	15%	7888.1b	6626.0b	8519.2c	5634.1b	9167.6b	
	30%	7572.6a	5679.5a	2839.7a	3155.2a	11043.4b	

Means sharing similar letters are statistically not significant at LSD = 5%.
* = significant at P > 0.05, ** = significant at P > 0.01, *** = significant at P > 0.001, NS = statistically not significant.

decrease was recorded in 3 wheat lines (M5082, 7076 and A2011) but only at the 15% level. Line 3094 showed a significant increase at the 15% level only. Trichome length decreased in 2 wheat lines (5066 and 7076) and increased in line A2011 along with increase in AsEx levels. Line M5082 showed an increase at 15% but thereafter reduced at the highest level. Line 3094 showed a significant increase, but only at the 30% AsEx level.

Cortical thickness decreased significantly in all wheat lines at 30% concentration of AsEx (Figure 2), however these lines showed a differential response at 15% concentration. Two lines (M5082 and 7076) showed a decrease, whereas line 5066 showed a significant increase. Cortical cell area consistently decreased in 3 lines (3094, M5082, and 7076) and increased in line 5066 with an increase in AsEx concentrations. Line A2011 showed an increase at 15% but decreased at the 30% AsEx level. Mesophyll thickness showed a variable response to AsEx concentration. Two lines (3094 and A2011) showed a consistent increase along with an increase in AsEx concentration. Two other lines (5066 and M5082) showed different responses; they increased in this parameter but only at 15%, while these lines showed a decrease at the highest level 30% concentration. Line 5066 showed a consistent increase in sclerenchyma thickness while line M5082 showed a decrease with increase in AsEx concentration. Line 3094 showed an increase at the highest concentration 30% in this parameter. Line M5082 showed a consistent decrease in this parameter along with an increase in AsEx concentration. Line 7076 showed a decrease at the highest 30% concentration.

Two lines (7076 and A2011) showed a decrease in vascular bundle area, while line 5066 increased as AsEx concentrations increased. Line 3094 showed a decrease

Table 4. Leaf anatomical characteristics of wheat lines treated with *Alstonia scholaris* leaf extract.

Leaf anatomy	Levels	3094	5066	M5082	7076	A2011	F-ratio
Midrib thickness (µm)	0%	1048.6b	892.7a	1246.9b	1346.2b	1006.0b	42.2***
	15%	1034.4b	1544.5b	1046.9b	637.6a	1105.2b	
	30%	708.5a	793.5a	283.4a	396.8a	708.5a	
Lamina thickness (µm)	0%	212.5a	425.1c	425.1b	425.1b	283.4a	8.0**
	15%	311.7b	269.2b	410.9b	212.5a	203.5a	
	30%	425.1c	212.5a	226.7a	198.4a	183.0a	
Abaxial epidermal thickness (µm)	0%	28.4a	28.3a	28.3a	28.3a	42.5a	1.7^{NS}
	15%	42.5b	70.8b	20.5a	23.3a	32.0a	
	30%	28.3a	28.0a	18.1a	13.3a	22.3a	
Adaxial epidermal thickness (µm)	0%	28.3a	28.3b	14.2a	42.5b	42.5a	2.6^{NS}
	15%	42.5b	28.3b	28.2b	40.0b	34.1a	
	30%	28.3a	14.2a	24.3b	14.2a	31.0a	
Bulliform thickness	0%	2.4a	3.7b	4.2b	2.7b	2.9b	3.1^{NS}
	15%	3.8b	2.7a	4.8b	2.8b	2.1ab	
	30%	2.4a	2.5a	2.1a	1.6a	1.7a	
Trichome length	0%	1.2a	4.3b	1.7b	5.6c	1.5a	4.1*
	15%	1.6a	1.5a	5.2c	3.4b	2.4b	
	30%	3.5b	1.0a	1.0a	1.5a	2.1b	
Cortical thickness (µm)	0%	765.2b	680.2b	921.0c	850.2c	552.6b	17.5***
	15%	779.3b	1119.4c	751.0b	354.2b	637.6b	
	30%	566.8a	566.8a	141.7a	141.7a	340.0a	
Cortical cell area (μm^2)	0%	7572.6b	1893.2a	15776.3c	11043.3c	5521.7b	25.0***
	15%	3155.2a	4732.9b	11358.9b	3155.3b	7572.6c	
	30%	3075.2a	15776.3c	631.0a	315.6a	1893.2a	
Mesophyll thickness (µm)	0%	212.5a	354.3b	311.7b	396.7c	141.7a	9.3**
	15%	255.0b	566.8c	410.9c	283.4b	283.4c	
	30%	425.1c	255.0a	141.7a	155.8a	198.3b	
Sclerenchymatous thickness (µm)	0%	99.2a	141.7a	354.2c	212.5b	212.5a	15.5**
	15%	85.0a	255.0b	269.2b	203.5b	182.0a	
	30%	141.7b	283.4c	70.8a	99.2a	108.3a	
Vascular bundle area (μm^2)	0%	63105.0b	35496.6a	70993.2b	52061.7c	56794.5c	7.6**
	15%	60105.0b	36916.5b	78881.3c	28397.3b	53639.3b	
	30%	39756.2a	88347.1c	7572.6a	23664.4a	25242.0a	
Metaxylem area (μm^2)	0%	56.7a	56.7b	56.7b	56.7b	56.6b	4.4*
	15%	54.7a	70.8c	46.3b	51.0b	53.0b	
	30%	70.7b	42.5a	42.5a	42.5a	42.5a	
Phloem area (μm^2)	0%	7888.2a	4417.4a	9465.7b	7888.2b	7888.2b	12.5**
	15%	7134.1a	7572.6b	16565.0c	11043.4c	6547.1b	
	30%	6347.1a	23664.4c	3155.3a	4417.4a	6310.5a	
Abaxial stomatal density	0%	708.9c	667.2c	750.6c	625.5c	291.9a	18.1***
	15%	291.9a	293.4a	279.5a	291.9a	301.1a	
	30%	430.9b	429.6b	429.9b	426.4b	333.6a	

Table 4. (Continued).

Adaxial stomatal density	0%	903.5c	917.4c	875.7c	834.0c	889.6c	7.7**
	15%	208.5a	583.5a	708.6b	707.8b	542.1b	
	30%	708.9b	707.2b	250.2a	500.4a	250.2a	
Abaxial stomatal area (μm ^{2})	0%	23.1c	22.8b	27.2b	26.8b	24.9a	8.7**
	15%	19.0b	17.9ab	17.1a	23.2b	20.1a	
	30%	13.7a	13.7a	12.7a	16.3a	22.0a	
Adaxial stomatal area (μm ^{2})	0%	23.6b	16.2a	19.8a	14.1a	10.7a	4.8*
	15%	21.8b	23.1b	18.6a	22.2c	12.9a	
	30%	12.7a	20.9b	17.3a	18.5b	16.5b	

Means sharing similar letters are statistically not significant at LSD = 5%.
* = significant at P > 0.05, ** = significant at P > 0.01, *** = significant at P > 0.001, NS = statistically not significant.

at the highest 30% level only. Line M5082 showed an increase in this parameter at 15%, which thereafter decreased at the 30% level. Three lines (M5082, 7076, and A2011) showed a significant decrease in metaxylem area at the 30% AsEx level only. Metaxylem area increased at the 30% AsEx level in Line 3094, whereas in line 5066 it increased at the 15% level and then decreased at 30%. Phloem area consistently increased in line 5066 with an increase in AsEx concentration. Two lines (M5083 and 7076) responded very differently to allelochemical extract, showing an increase in phloem area at the 15% level, but there after decreased at the 30% level. Line A2011 showed a significant decrease at 30% AsEx concentration only.

Abaxial stomatal density in all wheat lines decreased consistently with an increase in AsEx levels (Figure 2, Table 4). Line A2011 responded otherwise, where no change in stomatal density was recorded after the application of AsEx. A similar trend was recorded in the case of adaxial stomatal density; all wheat lines showed a consistent decrease as AsEx levels increased. Abaxial stomatal area showed a consistent decrease in lines 3094 and M5082 with increasing AsEx levels. Lines 5066 and 7076 showed a significant decrease but only at the highest 30% AsEx concentration. Line A2011 showed no response to the application of AsEx concentrations. Adaxial stomatal area in 2 wheat lines (5066 and 7076) consistently increased along with an increase in AsEx concentrations. Line A2011 showed significant increase only at 30% AsEx concentration, whereas line 3094 showed a significant decrease only at 30% AsEx concentration.

4. Discussion

Research on the application of allelochemical extract is gaining great interest to investigate its stimulatory as well as inhibitory effects on plants (Macias et al., 2003; Zeng et al., 2008). For this reason, allelochemical extract can be used with great efficacy for controlling weeds of agricultural crops, and at the same time (especially in lower doses) as a growth promoter (Bhadoria, 2011). *Alstonia scholaris* contains many active ingredients that are used in folk medicines in southeast Asian countries. Important among them are iridoids, coumarins, terpenoids, alkaloids, steroids, and simple phenolics (Khyade et al., 2014; Arulmozhi, 2007). These allelochemicals can impose an inhibitory effect on other plant species, particularly at high doses (Yang et al., 2005). In contrast, there are many reports on the growth promoting role of allelochemicals at low doses as reported by Albuquerque et al. (2011), Maqbool et al. (2013), and Uddin et al. (2014). In the present investigation, 5 different wheat genotypes (undertrial lines) were used to evaluate the structural and functional response of allelochemical extract of this plant at low (15%) and high (30%) leaf extract.

All wheat lines responded differently to different allelochemical levels, as in some cases growth promotion was recorded at the 15% level while others showed growth promotion at the 30% level. Other morphological parameters generally decreased at both levels of *Alstonia scholaris* allelochemical extract. Morphological characteristics like overall plant growth (height) and biomass (fresh and dry weights) production are the indicators of physical health under normal or stressful conditions (Salim et al., 2004; Macabeo et al., 2005). Tillering capacity as well as leaf numbers were severely affected by allelochemical application in almost all wheat lines. This indicates the inhibitory effect of AsEx, as has been reported in earlier studies (Batish et al., 2006; Shahid et al., 2006).

Yield parameters were more responsive to AsEx, where characteristics like ear length, grain weight, and yield per plant were more adversely affected at both AsEx levels in nearly all wheat lines. Growth parameters, in particular, and leaves per plant directly influence yield and grain weight (Naseem et al., 2009) mainly because

of low photosynthetic activity (Benyas et al., 2010). As a result, reduction in yield potential in response to AsEx is quite understandable, as has been reported (Banerjee and Pandey, 2015; Aulya et al., 2018)

Many researchers reported a negative impact of allelochemicals on chlorophyll pigments. Abu-Romman (2011) reported a decrease of chlorophyll pigments by *Achillea biebersteinii* extract in *Capsicum annuum,* while Singh et al. (2009) reported similar findings in *Zea mays* in response to *Nicotiana plumbaginifolia* extract. Wheat lines in our case responded otherwise, as only one line showed a decrease in chlorophyll a under AsEx treatment, while in others chlorophyll was not altered by allelochemicals. Chlorophyll pigments may directly be related to the growth and development of plants; therefore, any increase under stressful environments is critical. Line 7076 showed an increase in chlorophyll a under both AsEx levels, and hence can be rated better among all lines.

Root radius significantly reduced in all wheat lines, mainly due to the reduction in cortical parenchyma, and in some cases due to vascular tissue and pith parenchyma. Pina et al. (2009) also reported similar findings with allelopathic extract of *Thevetia peruviana* on wheat. Cruz-Ortega (1998) related such structural changes of roots in response to allelochemicals to inhibition of growth.

The most striking feature in response to allelochemical extract was the increase in sclerification, which was either at the outer cortical region or in the stellar region. Dos Santos (2008) reported increased lignification in response to allelochemical application in roots of *Glycine max*. Sclerification is the unique response of many plant species when exposed to stressful environments, particularly when water is a limited commodity (Christensen et al., 1998). Allelochemical application severely damages water storing tissues, hence plants may face serious water deficiency (Weir et al., 2004).

Water storage cortical parenchyma is an important tissue that can store water for unfavorable environments (Gatti et al., 2010). Allelochemical extract of *A. scholaris* resulted in a decrease in cortical cell size and cortical region thickness, hence significantly decreasing storage capacity of the wheat lines. This increases the sensitivity level of wheat lines, and may cause severe tissue damage (Burgos et al., 2004).

Endodermal layer is a barrier to radial water movement in the root. Water has to move through this layer via symplastic movement, and in case of any decrease in its thickness, water movement may face less resistance (Burgos et al., 2004). Vascular tissue and metaxylem area showed a complex behavior as it increased in few cases, but decreased in others when the allelochemical extract of *A. scholaris* was applied. Increased vascular bundle along with broader metaxylem vessels positively correlated with water movement through roots (Chon et al., 2002). Wheat lines that showed increased vascular tissue can survive better under stressful conditions (Donaldson, 2001)

Pith parenchyma along with cortical parenchyma are water storing tissues in roots (Pereira at al., 2017). Allelochemicals result in severe water storage (Elansary et al., 2016) and as a result, plants go for structural modification (Feitoza et al., 2018). The most prominent is lignin deposition, which is either in the outer cortical region or the central pith region (Dos Santos et al., 2004). All wheat lines except A2011 responded similarly by increasing sclerification, more pronounced in stellar region and central pith, providing mechanical strength to the roots (Arioli et al., 2015). Line A2011 responded very differently at high allelochemical concentration. Storage parenchyma formation in the central region by pushing apart the vascular region is the unique development in this wheat line; it will significantly increase water storage capacity and succulence, which is extremely helpful under water deficit conditions.

All wheat lines responded differently to allelochemical extract, showing some drastic amendments in their tissue system. Transformation of chlorenchymatous tissues into sclerenchyma is an important modification in line 3094 that leads to a shift from photosynthate products to survival under harsh environmental condition. Chlorenchyma is a parenchymatous tissue that is more prone to damages under drought (Reddy, 2003). Sclerification in such conditions protects delicate photosynthetic tissues from collapse, also reported by Terzi (2008). Another modification is the increased storage parenchyma. Lines 5066 and A2011 depicted significant increases in proportion of storage parenchyma (cortex) in response to allelochemical application. This is again a critical feature for water storage capacity, which also increased along with it (El-Sahhar et al., 2011). Increased succulence under water deficit conditions has been related to drought tolerance by many researchers (Mancuso et al., 2006; Xu and Leskovar, 2015; Elansary et al., 2016).

Increased sclerification in hypodermal region is a xeromorphic character (Teriz et al., 2003) prominent sclerification in the hypodermal region, especially around peripheral vascular bundles of stem, as was observed in wheat lines 5066 and M5082. These wheat lines responded very strongly to allelochemical stress vital for the survival. These tissues not only resist collapse, but also offer resistance to water loss from the wheat stem. Line 5082 is apparently more sensitive to high concentration of allelochemical showing a decrease in stem cellular thickness. It severely affects proportion of parenchymatous, mechanical and vascular tissue systems.

Leaves are more responsive to an allelochemical extract of *A. scholaris*. Each wheat line showed different

	3094	5066	M5082	7076	A2011
Root anatomy	Conducting tissue-Broader metaxylem vessels. Mechanical tissue-Sclerification at outer cortical region.	Mechanical tissue-Intensive sclerification at outer cortical region and in vascular region.	Mechanical tissue-Sclerification in vascular region.	Mechanical tissue-Increased sclerification at outer cortical region. Conducting tissue-Increased metaxylem vessel number and decreased metaxylem area	Mechanical tissue-Increased sclerification at outer cortical region.
Stem anatomy	Photosynthetic tissue-Increased chlorenchymatous area. Mechanical tissue-Sclerification outside vascular bundles and around chlorenchyma.	Conducting tissue-Increasing vascular bundle area.	Mechanical tissue-Intensive sclerification inside epidermis.	Storage tissue-Reduced cellular area thickness. Conducting tissue-Vascular bundles deeper, away from stem periphery.	Storage tissue-Increased stem cellular area. Vascular tissue-Increased vascular bundle area.
Leaf anatomy	Leaf thickness-Reduced midrib thickness. Storage parenchyma-Reduced storage parenchyma.	Leaf thickness-Reduced midrib thickness but increased lamina thickness. Conducting tissue-Reduced vascular bundle area.	Mechanical tissue-Increased sclerification in hypodermis and epidermis.	Leaf thickness-Greatly reduced leaf thickness.	Storage tissue-Increased cortical parenchyma.
Stomata	Greatly increased stomatal area.	Greatly increased stomatal area.	Decreased stomatal density.	No visible change.	Decreased stomatal area.

Figure 3. Block diagram showing overall impact of 15% *Alstonia scholaris* allelochemical extract on anatomical features of five wheat lines.

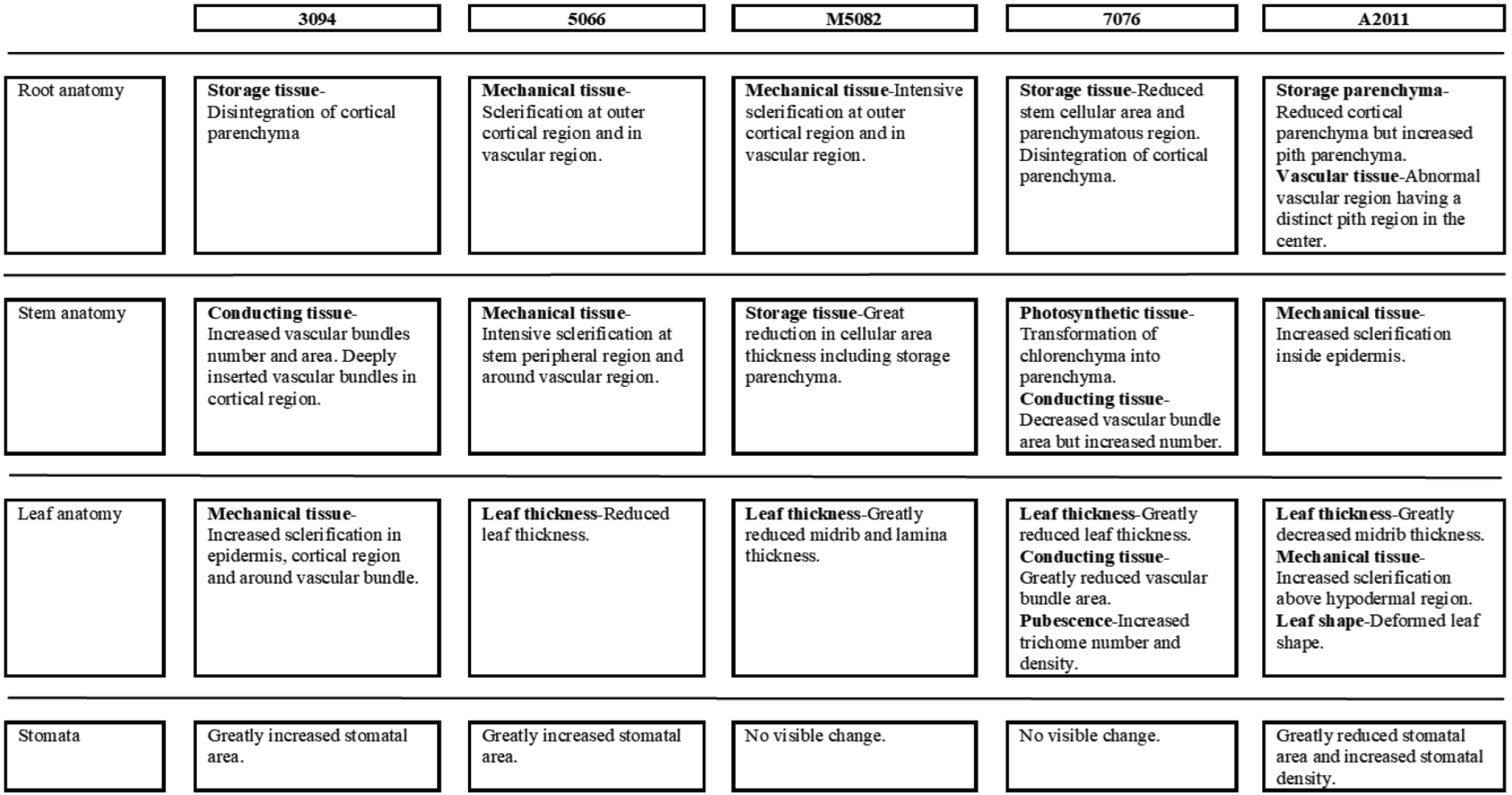

Figure 4. Block diagram showing overall impact of 30% *Alstonia scholaris* allelochemical extract on five wheat genotypes.

behavior regarding leaf thickness, tissue modification and distribution, intensity of sclerification, and storage parenchyma. Line 3094 relied on intensive sclerification, not only at abaxial and adaxial epidermis but also around vascular bundles and in the cortical region. This kind of modification primarily reduces risk of tissue collapse of cortical parenchyma (Bughio et al., 2013) and additionally water loss from leaf surface by sclerification of epidermal layer (Lam et al., 2001; Yamauchi and Fukushima, 2004).

Line 5066 showed a decrease in leaf thickness but there was still large proportion of cortical parenchyma. It indicates more tolerance to allelochemical extract as there was minimum damage to metabolically active tissues. Barkosky et al., (2000), Chai et al. (2013) and Novaes et al. (2013) reported similar findings.

M5082 showed a storage response to allelochemical extract, especially at high concentration. Leaf thickness enormously reduced making leaf more fibrous. The leaf can easily fold to minimize transpiration rate when environmental conditions are unfavorable by minimizing transpiration (Wang et al., 2016). Moreover, these sclerified leaves can survive better under stressful environment (Emamjamaati et al., 2016).

Leaf thickness drastically reduced in line 7076, but in this case leaf modification was not towards xerophily. In such conditions metabolic activity may reduce significantly due to low proportion of parenchymatous tissues. The plant may spend more energy on survival instead of normal growth, and the development process has been reported by Hussain et al. (2011), Gniazdowska et al. (2015), and Coelho et al. (2017). Leaf thickness in line A2011 was not altered at low allelochemical levels. High concentration, however, showed a strong influence that reduces leaf thickness, inducing sclerification and deformed leaf shape. All such changes indicate the toxic impact of high concentrations of allelochemical application. Such changes have also been reported by Sodaeizadeh et al. (2010) and Rouphael et al. (2017).

Stomata size, density, and regulation control the transpiration rate in plants, and thus is directly involved in water conservation (Yu et al., 2003). Stomatal density invariably decreased in all wheat lines, and this feature is critical under stressful environments where a plant encounters water deficit (Rashke and Hedrich, 1985). Low stomatal density lowers transpiration rate significantly, which enables a plant to survive more successfully. Stomatal area on adaxial leaf surface also decreased in most of the cases, but increased on abaxial side. Abaxial surface is generally more exposed to sun in wheat (Bouchagier et al., 2008). A decrease in stomatal size and number on adaxial side is more beneficial for a plant for regulation of transpiration rate (Jose and Gillespie, 1998).

All wheat lines showed different response to encounter allelochemical effects. Line 3094 showed cortical tissue disintegration in root, increased chlorenchymatous region at low concentrations and sclerification at higher levels in the stem, and sclerification of cortical parenchyma in leaves (Figure 3). Intensive sclerification of outer cortical region and stellar region of the root, and of hypodermal region in the stem, was more prominent in Line 5066. Reduced leaf thickness was also noted in this wheat line.

Increased sclerification of cortical parenchyma and stele at the root level, and outside vascular bundles at the stem level (at low concentrations) was observed in Line M5082. High concentrations of AsEx resulted in a significant reduction of stem cellular thickness (Figure 4). Leaf thickness drastically decreased in this line along with increased sclerification (sclerophily) while in an important modification. Line 7076 was more sensitive to allelochemical extract of *A. scholaris* showing significant reduction in thicknesses of root, stem and leaves. However, there was no visible change in stomatal size and area.

Line A2011 responded very differently by increasing storage parenchyma in roots, intensive sclerification in stem that replaced stem chlorenchyma, sclerification of leaf tissues, and decreased stomatal area. All these contributed to water conservation, either by minimizing water loss from the plant surface or by increased water storage capacity.

5. Conclusions

All wheat lines responded differently to leaf extract containing allelochemical compounds. Low concentrations of allelochemical extract promoted growth and development in most cases, while high concentrations were toxic, showing growth retardation, tissue damage, and inducing sclerification. High concentrations of allelochemical extract of *A. scholaris* can be considered as a potential herbicide, however this needs further investigation.

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