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Allelochemicals extract of star fruit (*Averrhoa carambola* L.) modulates wheat growth through alterations in anatomical architecture

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ABSTRACT

Context. Allelopathy is recognised as a potential technology to control weeds, and could also be a suitable approach for enhancement of crop yield. **Aims.** The study was conducted to investigate the allelopathic role of *Averrhoa carambola* leaf extract on growth, anatomical and physiological features of three wheat lines, namely 3094, 7076 and A2011. **Methods.** Three dilutions i.e. 0% (control), 15% and 30% of the leaf aqueous extract were applied at 15 day intervals (total five applications) on wheat lines until maturity. **Key results.** All wheat lines showed differential behaviour to allelochemicals of *A. carambola* leaf extract. Increased root area was accompanied by a high proportion of storage parenchyma tissues and enlarged vascular bundles in line 3094. Disintegration of root cortical parenchyma and complete transformation of chlorenchyma into sclerenchyma in stem was recorded in all wheat lines, particularly at the highest concentration of leaf extract. Line 7076 showed very different behaviour, as it possessed a proportionally enlarged root cortex, enlarged stem vascular bundles and increased leaf thickness, primarily at the highest concentration of leaf extract. Line A2011 was relatively more sensitive, indicating a significant reduction ($P < 0.05$) in root and stem area and deformed leaves. **Conclusions.** A low concentration (15%) of leaf extract promoted growth and development, whereas a higher concentration caused significant reduction in growth and anatomical attributes. **Implications.** A lower dose of *Averrhoa* leaf extract promoted growth and development in all wheat lines, and hence can be used as a growth promoter. A higher concentration is important for eradicating unwanted plants.

Keywords: allelochemicals, anatomical architecture, chlorenchyma, growth promotion, leaf thickness, parenchyma, sclerenchyma, storage tissue.

Introduction

Allelopathy is the release of phytochemicals that influence growth and development of other plants. It enhances the competitive success of the plant invaders, since the release of these phytotoxins in the environment may affect the growth and life processes of other species (Ferreira and Reinhardt 2016; Mirmostafaee *et al.* 2020). Allelopathy plays an important role in the productivity of agro-ecosystems (Abbas and Hussain 2020). Mostly allelopathic interactions are detrimental, but they are occasionally useful, so that allelopathy has gained attention in natural and agricultural ecosystems. Allelopathy is recognised as a potential technology to control weeds using chemicals released from plant parts of various species (Kato-Noguchi and Kurniadie 2020). For agriculture, an allelopathic environment could be suitable and a good approach for enhancement of yield in varying environments (Bilas *et al.* 2021).

Nowadays, allelopathy is understood to be one of the foremost ecological processes influencing plant growth within crops. It shows the effect of different plant species on each other that may be stimulatory or inhibitory. Different environmental conditions directly or indirectly affect the allelopathic performance, and allelopathic interactions between crops, weeds and shrubs surviving together (Medina-Villar *et al.* 2020). Allelochemicals are released as residues, exudates, and leachates by many plants from leaves, stems, roots, fruit and seeds. These have been reported to mostly interfere and hinder the growth of other plants. These chemical products mainly affect plants at seed emergence and seedling stages (Mushtaq *et al.* 2020).

Many plants naturally produce secondary active metabolites that are not only crucial for their biological and ecological functioning but are also involved in interactions among plant communities and environment (Cheng and Cheng 2015). Such interactions are more complex, vital, and advantageous for plants secreting those allelochemicals (Reigosa *et al.* 2013). Sometimes, allelochemicals in low quantities act as growth promoters and show positive impacts on receiver plants (Duke 2015) through enhancing seed germination, and growth and development (Mali and Kanade 2014). The stimulatory or inhibitory effects of allelopathic chemicals depend on the concentration and composition of the released compounds in the environment (AL-Jobori and Ali 2014). Allelochemicals with harmful allelopathic properties are a vital component of plant resistance against herbivory (Mali *et al.* 2021). Allelopathy has also been effective in controlling stored grain and field crops from pests and several pathogens. For example, foliar application of canola, sorghum, sunflower, and *Moringa* extracts has been found to be effective in this regard. More recently, allelopathic extracts are being evaluated for their potential role in improving resistance against abiotic stresses in cereals as well.

Averrhoa carambola L. (star fruit) is one of the tree species showing allelopathic effects on different crops. It grows in tropical Asian countries (Lakmal *et al.* 2021), exhibiting a perennial habit with medium-green compound leaves (Ferrara 2018). *Averrhoa carambola* contains phyto-constituents like steroids, flavonoids, alkaloids, carbohydrates, glycosides, saponins, and polyphenols, i.e. L-ascorbic acid, gallic acid, epicatechin, gallotannin, proanthocyanidins, 5-hydroxymethyl-2-furfural, and dihydroabscisic alcohol (Saikia *et al.* 2015). It was hypothesised that these allelochemicals present in the leaf extract of *A. carambola* might induce modifications on growth, structural and functional features when applied at an early growth stage. Based on this information, the current study aimed to investigate the impact of leaf extract of *A. carambola* on bread wheat lines by keeping in mind that a low level of extract may impose a stimulatory effect on growth, whereas a high level may have an antagonistic effect, and can effectively be used as an herbicide for other plants.

Materials and methods

Selection of plant material

In a pilot experiment, as already published in our earlier paper by Hameed *et al.* (2020), the allelochemical effects of *Alstonia scholaris* leaf extract were tested on five wheat lines (3094, 7076, A2011 3094 and M5082). Based on the findings of this experiment, three wheat lines, 3094, 7076 and A2011 were selected for testing their response to *A. carambola* L. (star fruit) leaf extract applied as foliar spray.

Research plan

Seeds of the wheat line were obtained from the cereal section, Ayub Agricultural Research Institute, Faisalabad. These lines were under trial for recommendation as varieties before commercial scale sowing in farmers' fields. Five seeds were sown in earthen pots (30 cm dia.), filled with equal quantities of sand, loamy soil and leaf manure and watered regularly throughout the course of experiment.

Preparation of *A. carambola* allelochemical extract

Young leaves from the new branches were taken and washed with distilled water. Ten gram fresh leaves were chopped and dipped in 100 mL distilled water for 24 h. The leaf extract was then boiled to reduce the volume by 20 times to get a concentrated solution. Two concentrations (i.e. 15% and 30% in distilled water) were then prepared from the concentrated solution. Leaf extract was applied as foliar spray after germination at intervals of 15 days until the maturity of crop (November–March 2019). Control plants were sprayed with distilled water at each cycle of extract application.

Morphological attributes

Morphological data were recorded after the crop reached maturity. Total plant height (including the spike) and spike length were measured by a scale. Fertile tillers per plant, leaves per plant and spikelets per spike were counted. The 100-grain weight, yield per plant, shoot and root fresh and dry weights were measured on a portable digital balance. Plants were immediately weighed after uprooting, and thereafter oven-dried at 65°C for 1 week for the dry weight.

Anatomical attributes

A 2 cm piece of the thickest adventitious root for root anatomy, 2 cm piece of the topmost internode of the longest tiller for stem anatomy, and 2 cm piece of the 3rd leaf of the longest stem for leaf anatomy were selected. The material was fixed in formalin acetic alcohol solution for 48 h and then transferred to acetic alcohol for preservation. Permanent slides were prepared by freehand sectioning, dehydrated by ethanol grades, and stained by safranin and fast green dyes following Ruzin (1999). Safranin was prepared in 70% ethanol, and fast green in 90% ethanol. Measurements were taken by ocular micrometre and photographs of the transverse sections were taken by a compound microscope (Nikon 104, Japan). Size of dermal, parenchymatous, mechanical, and vascular tissues were recorded.

Photosynthetic pigments

Chlorophyll *a* and *b* were analysed following Arnon (1949). Fresh leaf samples were dipped in pre-cooled 80% acetone solution for chlorophyll extraction. The extract was centrifuged at 1000*g*, and the optical density of supernatant was measured on a spectrophotometer (Hitachi-220 Japan) at wavelength of 663 and 645 nm.

$$\text{Chl. } a\ (\text{mg g}^{-1}\ \text{F. wt.}) = \left[12.7(\text{OD663}) - 2.69(\text{OD645})\right] \times \frac{V}{1000} \times W\Big]$$

$$\text{Chl. } b\ (\text{mg g}^{-1}\ \text{F. wt.}) = \left[22.9(\text{OD645}) - 4.68(\text{OD663})\right] \times \frac{V}{1000} \times W\Big]$$

$$\text{Carotenoids}\ (\text{mg g}^{-1}\ \text{F. wt.}) = \frac{A^{\text{car}}}{\text{Em}} \times 100$$

where F. wt. is the fresh weight, OD is optical density at the wavelength shown, *V* is the volume of sample, *W* weight of fresh tissue

$$A^{\text{car}} = \text{OD480} + 0.114(\text{OD663}) - 0.638(\text{OD645}),\ \text{Em} = 2500$$

A^{car} is the absorbance of carotenoids and Em is the beta carotene extinction coefficient.

Experimental design and statistical analysis

The experiment was designed in 2-factor completely randomised design with three replications. Analysis of variance was calculated using Co-Stat software (ver. 6.303). The means were compared by least significant difference (l.s.d.) test at $P < 0.05$ significance level. Relationships among various morpho-anatomical and physiological parameters were calculated by principal component analysis, heatmaps, ternary plots and estimated response (linear model, lm) using R statistical software for windows (ver. 1.1.463).

Ethical approval

The study does not include any animal or human subjects and no specific ethical approval is needed. Other necessary guidelines set by University of Agriculture, Faisalabad for handling of plant material during conduction of laboratory work were followed. All sampling was done with the least possible disturbances to plant communities and environment. After completion of the study, all experimental materials were properly discarded/incinerated in a controlled environment to avoid bio-contamination.

Results

Morphological parameters

The number of leaves per plant decreased significantly only in line 7076 at both concentrations of leaf extract, whereas the other two lines showed decreases at 30% extract (Fig. 1). Root fresh weight decreased significantly in lines 3094 and 7076 at both leaf extract concentrations. In line A2011, root fresh weight increased at 30% extract. Shoot fresh weight increased significantly in lines 3094 and 7076 at both leaf extract concentrations; however the 15% concentration, where the maximum increase in shoot fresh was observed, was more effective. Line A2011 was not affected by leaf extract application of *A. carambola* regarding shoot fresh weight.

Yield per plant decreased in all wheat lines treated with 30% leaf extract of *A. carambola*, however this trait was not altered in line 3094 and increased significantly in line A2011 with 15% extract application. Grain yield consistently decreased in line 7076 with an increase in concentration of *Averrhoa* leaf extract.

Chlorophyll pigments

Chlorophyll *a* decreased significantly with *Averrhoa* leaf extract concentrations in line 3094, whereas it increased in line 7076. This trait was not affected by 15% leaf extract in line A2011 but increased significantly with 30% extract. Chlorophyll *b* decreased significantly under both concentrations of *Averrhoa* leaf extract in lines 3094 and 7076. For the 7076 and A2011 lines, a significant increase in chlorophyll *b* was noted with 15% leaf extract, whereas it decreased significantly with 30% extract (Fig. 1).

Root anatomical parameters

Root area in line 3094 increased significantly by application of 15% and 30% leaf extracts. In the other two lines, 7076 and A2011, this trait decreased consistently with concentration of leaf extract (Figs 1, 2). Sclerenchyma thickness in the outer cortical region increased significantly only with 15% leaf extract in line 3094, but increased consistently with increase in leaf extract concentration in line 7076. In line A2011, this trait was unaltered by application of leaf extract. Cortical region thickness consistently decreased in line 7076 and A2011 treated with both concentrations of leaf extract, whereas it increased significantly in line 3094. Endodermal

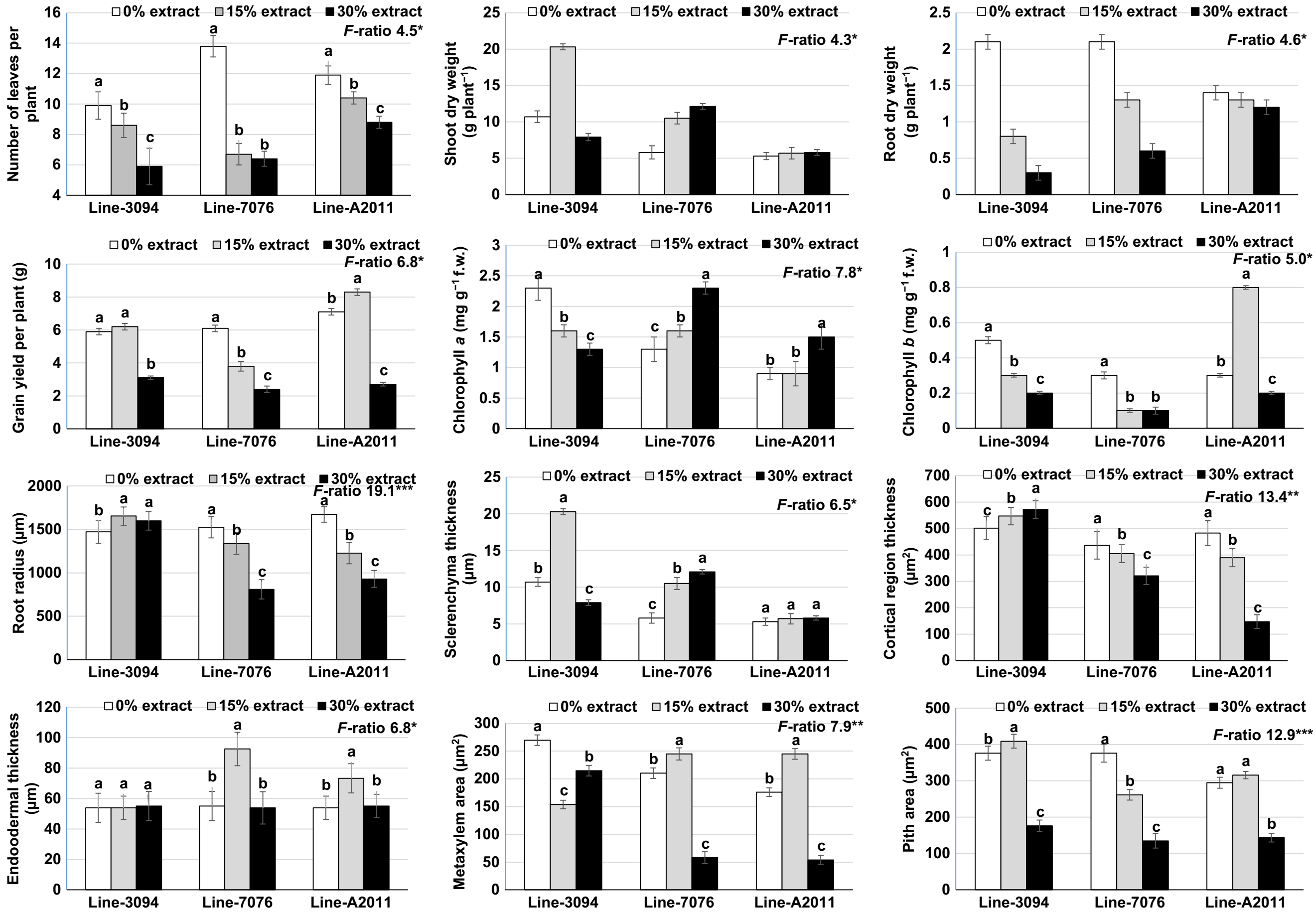

Fig. 1. Morphological, photosynthetic and root anatomical traits of wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. Only selected data is presented in bar diagrams; the complete data is presented in Supplementary Tables S1, S2. Means sharing different letters (a, b or c) are statistically significant at $P < 0.05$. F-ratio: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

thickness increased significantly in lines 7076 and A2011 with 15% leaf extract but the 30% level did not show any effect. Line 3094 showed no change in endodermal thickness at either leaf extract concentration. Metaxylem area decreased in line 3094 at both leaf extract concentrations. It increased significantly with 15% leaf extract in lines 7076 and A2011 but decreased with 30% leaf extract. A slight increase in pith area was recorded in wheat lines 3094 and A2011 at 15% leaf extract application but this decreased at the higher concentration. Line 7076 exhibited a continuous decrease in pith area as the concentration of leaf extract increased.

Stem anatomical parameters

Stem cellular region thickness increased in line 7076 at 15% but decreased at 30% leaf extract concentrations. In line 3094 and A2011, this parameter increased at both leaf extract applications (Supplementary Tables S3, S4). Epidermal thickness significantly increased in lines 3094 and 7076 with 15% leaf extract whereas it was reduced with 30% leaf extract. Line 7076 showed a consistent increase in epidermal thickness with an increase in concentration of leaf extract. Chlorenchymatous area decreased significantly in line 3094 with leaf extract applications, whereas in line 7076 this tissue was recorded only using 30% leaf extract. Cortical cell area showed a significant decrease with 30% extract in line A2011. Itecreased in line 7076 at both leaf extract concentration levels, whereas it increased significantly at 15% extract concentration in line 3094. Vascular bundle area increased in all wheat lines with 15% leaf extract. A significant decrease was recorded in lines 3094 and A2011 with 30% leaf extract. The metaxylem area increased significantly in all wheat lines treated with *A. carambola* leaf extract.

Leaf anatomical parameters

A significant decrease in midrib, lamina thickness, vascular bundle area and metaxylem area with increasing concentration of leaf extract was seen in all wheat lines (Figs 3, 4, 5). Parenchymatous cell area decreased consistently with increase in leaf extract concentrations in line 3094 and A2011. Line 7076 was not affected with 15% extract, but

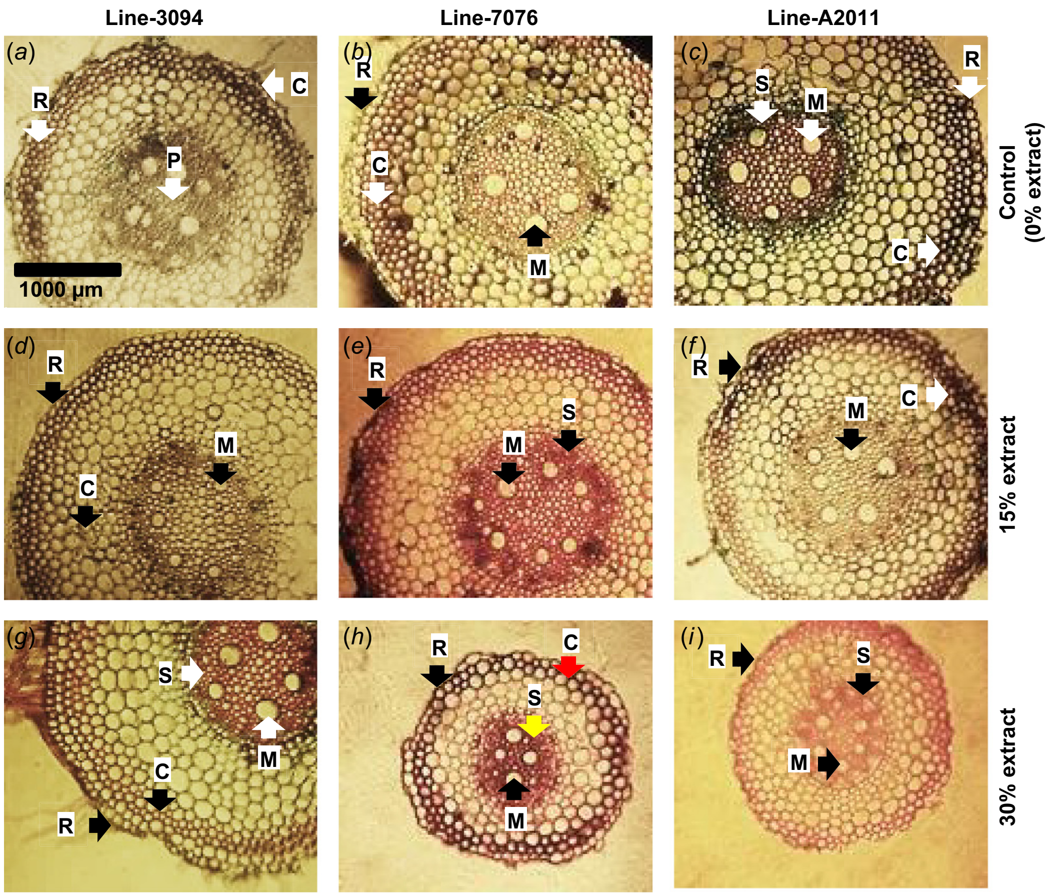

Fig. 2. Root transverse sections of some wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. (*a*) Root radius (R) was the minimum observed; pith (P) parenchyma reduced; outer cortical region (C) sclerified. (*b*) Root radius (R) was the maximum observed; slight sclerification at outer cortical region (C); outer cortex comprised of small closely-packed cells; metaxylem vessels (M) broad. (*c*) Root radius (R) was the maximum observed; sclerification at outer cortical region (C) and stele region (S); metaxylem vessels (M) broad. (*d*) Root radius (R) was the maximum observed; increased proportion of cortical parenchyma (C); reduced metaxylem vessels (M). (*e*) Slightly reduced root radius (R); increased sclerification in the stelar region (S); reduced metaxylem area (M). (*f*) Reduced root radius (R); increased sclerification at outer cortical region (C); slightly reduced metaxylem area (M). (*g*) Root radius (R) was the maximum observed; sclerification at outer cortical (C) and stelar regions (S); increased metaxylem area (M). (*h*) Greatly reduced root radius (R); increased sclerification at outer cortical (C) and stelar regions (S); greatly reduced metaxylem vessel (M) number. (*i*) Reduced root radius (R); increased sclerification at the stelar region (S); reduced metaxylem (M) area.

parenchymatous cell area decreased significantly with 30% leaf extract. Sclerenchymatous thickness decreased constantly with leaf extract concentrations in wheat lines 7076 and A2011. In line 3094, this parameter was not affected by 15% leaf extract. The phloem area consistently decreased in line 3094 with an increase in leaf extract concentration. It increased with 15% leaf extract in lines 7076 and A2011 but decreased significantly with 30% leaf extract. Stomatal density decreased at both concentrations of *A. carambola* leaf extract in wheat lines 3094 and A2011. The only exception was wheat line 7076, where an increase in stomatal density was observed with 15% leaf extract. Stomatal area decreased significantly in line 3094 and A2011 at both concentrations of *A. carambola* leaf extract (Fig. 6). Line 7076 exhibited an increase in this parameter with 15% and 30% leaf extracts.

Relationship among structural and functional traits

Principal component analysis (PCA) showed three different groups of traits, where each leaf extract concentration influenced morpho-anatomical traits of three wheat lines (Fig. 7). At 0% leaf extract concentration, line 3094 showed

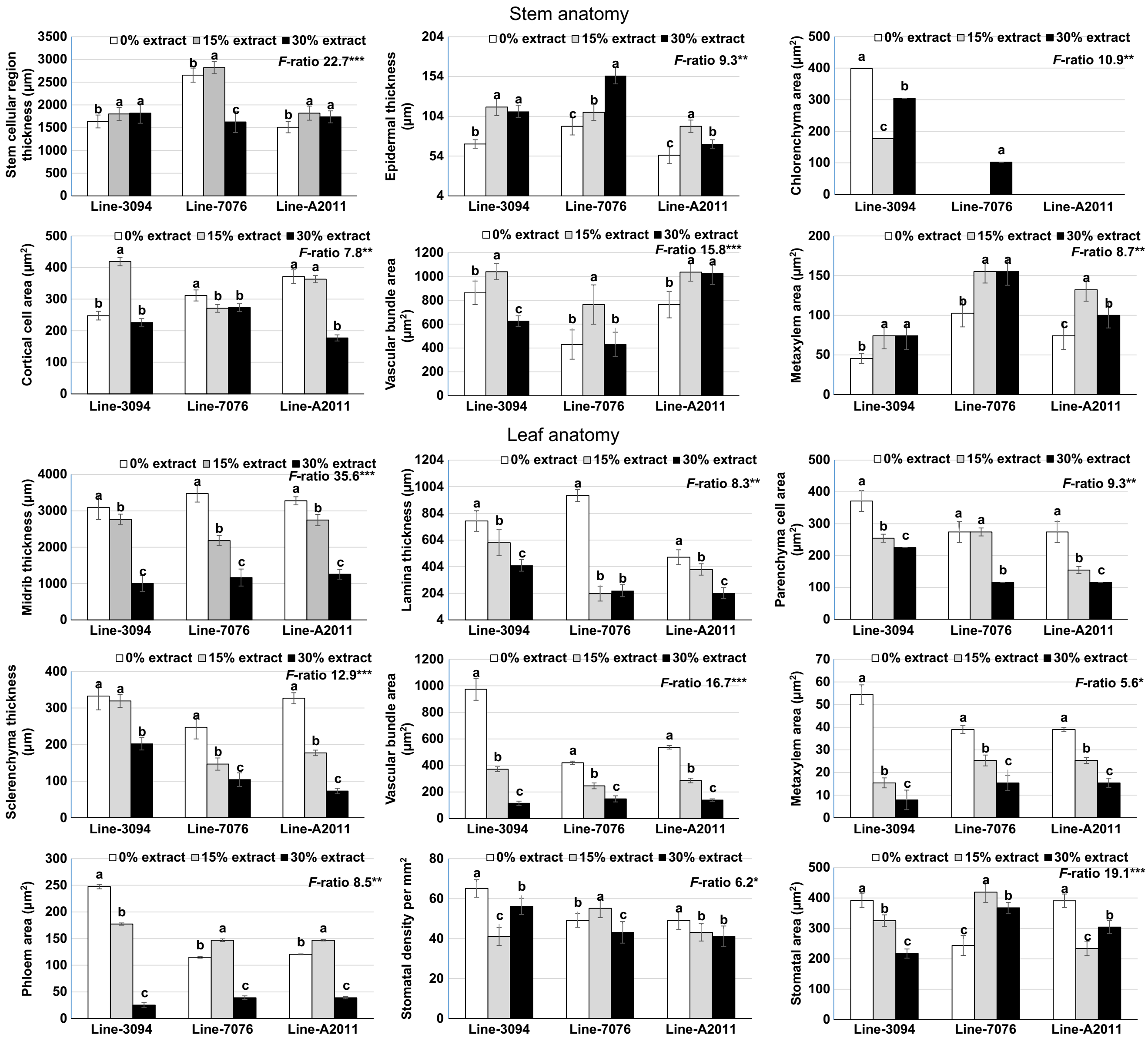

Fig. 3. Stem and leaf anatomical traits of wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. Only selected data is presented in bar diagrams; the complete data is presented in Tables S3, S4. Means sharing different letters (a, b or c) are statistically significant at $P < 0.05$. *F*-ratio: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

close association with leaf traits like parenchymatous cell area, sclerenchymatous thickness, stomatal density and root traits like cortical region thickness and root radius. Line A2011 was associated with leaf metaxylem area, midrib thickness and yield per plant, whereas line 7076 had association with root dry weight and chlorophyll *b*.

Line 3094 showed an association with chlorophyll *a*, shoot dry weight, stem chlorenchymatous area, root sclerenchymatous thickness and root cortical region thickness at 15% leaf extract concentration. Line 7976 was weakly associated with stem metaxylem area, whereas line A2011 was related to root endodermal thickness and stem cellular region thickness. At 30% leaf extract concentration, line 3094 was strongly associated with stem epidermal thickness, and line A2011 was associated with stem metaxylem area.

Ternary plots

Ternary plots displayed the distributional pattern of morpho-anatomical traits in three wheat lines at various concentrations of *A. carambola* leaf extract (Fig. 8). The morpho-anatomical traits, especially growth and root

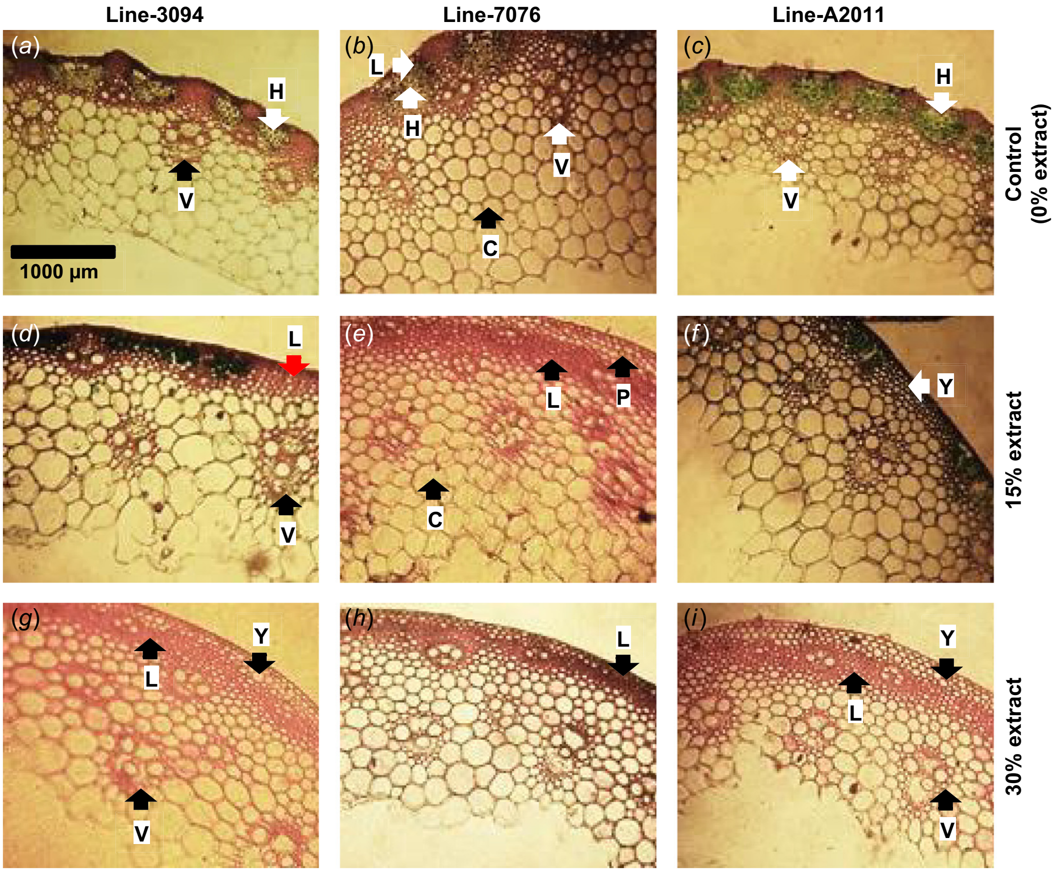

Fig. 4. Stem transverse sections of some wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. (*a*) Large chlorenchyma (H) inside epidermis between peripheral vascular bundles; vascular bundles (V) close to the stem periphery. (*b*) Distinct chlorenchyma region (H) inside epidermis; intensive sclerification (L) inside chlorenchyma; vascular bundle (V) large; greatly increased proportion of cortical parenchyma (C). (*c*) Large chlorenchyma (H) inside epidermis between peripheral vascular bundles; vascular bundles (V) oriented near stem periphery. (*d*) Sclerification (L) was replaced by chlorenchyma near stem periphery; vascular bundles (V) large in size and more elongated; oriented deeper in the cortical region. (*e*) Increased cortical parenchymatous (C) proportion; increased sclerification (L) inside hypodermis; hypodermis parenchymatous (P). (*f*) Hypodermal region (Y) extensively sclerified. (*g*) Thick layer of sclerenchyma (L) separating parenchymatous hypodermis (Y) and cortical region; vascular bundles (V) deeply oriented, broader than long. (*h*) Extensive sclerification (L) inside epidermis. (*i*) Hypodermis (Y) parenchymatous; extensive sclerification (L) inside hypodermis; increased vascular bundle (V) area.

anatomical traits, in line 3094 were more inclined towards 0% leaf extract. Few growth and leaf anatomical parameters deviated towards 15% and rarely towards 30% leaf extract. In wheat line 7076, morpho-anatomical features were not clearly diverged towards any particular leaf extract concentration; a few root anatomical traits, however, were skewed towards 15% leaf extract. Growth parameters were more tilted towards 0% leaf extract. In line A2011, leaf extract concentrations were generally not related with the distributional pattern of morpho-anatomical traits. Few root anatomical traits were tilted towards 30% leaf extract, whereas few growth and leaf anatomical traits tended towards 15% leaf extract.

Clustered heatmaps with soil factors

A clustered heatmap showed two distinct clusters of morpho-anatomical traits in wheat lines treated with different concentrations of *A. carambola* leaf extract. The larger cluster possessed two sub-clusters, which further divided into smaller clusters (Fig. 9). Among wheat lines, all wheat lines at 30% leaf extract concentration were grouped in close association, whereas wheat lines with 0% and 15% leaf extract collectively clustered. Line 7076 at 30% leaf extract concentration was strongly but negatively correlated with morphological traits like yield per plant, root anatomical traits like root radius, metaxylem area and pith area, stem vascular bundle area and leaf traits like

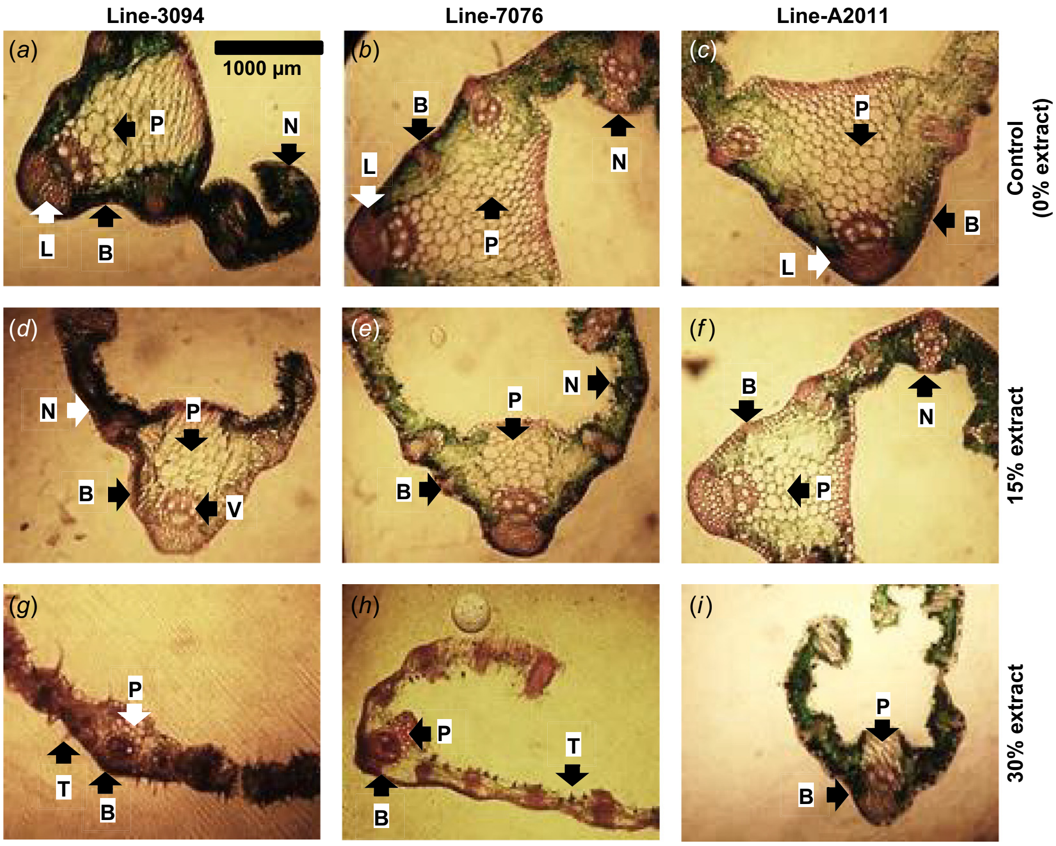

Fig. 5. Leaf transverse sections of some wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. (*a*) Thick midrib (B) and lamina (N); high proportion of parenchyma (P) in midrib; extensive sclerification (L) outside vascular bundle. (*b*) Thick midrib (B) and lamina (N); high proportion of parenchyma (P) in midrib; extensive sclerification (L) outside vascular bundle. (*c*) Increased midrib (B) thickness; high proportion of parenchyma (P) in midrib; extensive sclerification (L) outside vascular bundle. (*d*) Slightly decreased midrib (B) and lamina (N) thicknesses; decreased vascular bundle area (V) and parenchymatous (P) cell area. (*e*) Slightly decreased midrib (B) and lamina (N) thicknesses; decreased parenchymatous (P) cell area. (*f*) Slightly decreased midrib (B) and lamina (N) thicknesses; decreased parenchymatous (P) cell area. (*g*) Greatly reduced midrib thickness; proportion of parenchyma greatly reduced; increased trichome (T) density on both leaf surfaces. (*h*) Greatly reduced midrib (B) thickness; proportion of parenchyma (P) greatly reduced; increased trichome (T) density on adaxial leaf surfaces. (*i*) Midrib (B) shape slightly disorganised; greatly reduced midrib thickness; proportion of parenchyma (P) greatly reduced.

parenchymatous cell area. A strong positive association was recorded with stem epidermal cell area and chlorophyll *a*. With 30% leaf extract, line A2011 was negatively and strongly correlated with leaf chlorenchymatous area, root metaxylem area and cortical region thickness, and leaf sclerenchymatous thickness. At 30% extract concentration, line 3094 was negatively correlated with stomatal density and area, leaf metaxylem area and root dry weight, while positively correlated with stem cortical cell area.

At 15% leaf extract, line 7076 positively correlated with root endodermal thickness and stem radius. Line A2011 positively correlated with chlorophyll *b*, and negatively correlated with stem cortical cell area, chlorophyll *a*, and stomatal area. Line 3094 showed a strong positive correlation with stomatal density and shoot dry weight. At 0% leaf extract concentration, line 7076 positively correlated with number of leaves per plant and lamina thickness, and negatively correlated with stem vascular bundle area. Line A2011 showed a negative correlation with stem epidermal thickness. Line 3094 was negatively correlated with stem metaxylem area, whereas it was positively correlated with leaf vascular bundle area, stomatal density, and stem chlorenchymatous area.

Response curves

Estimated response curves of morphological traits in wheat line A2011 showed slight deviation from mean data; line 7076 had the minimum deviation (Fig. 10). Variation in

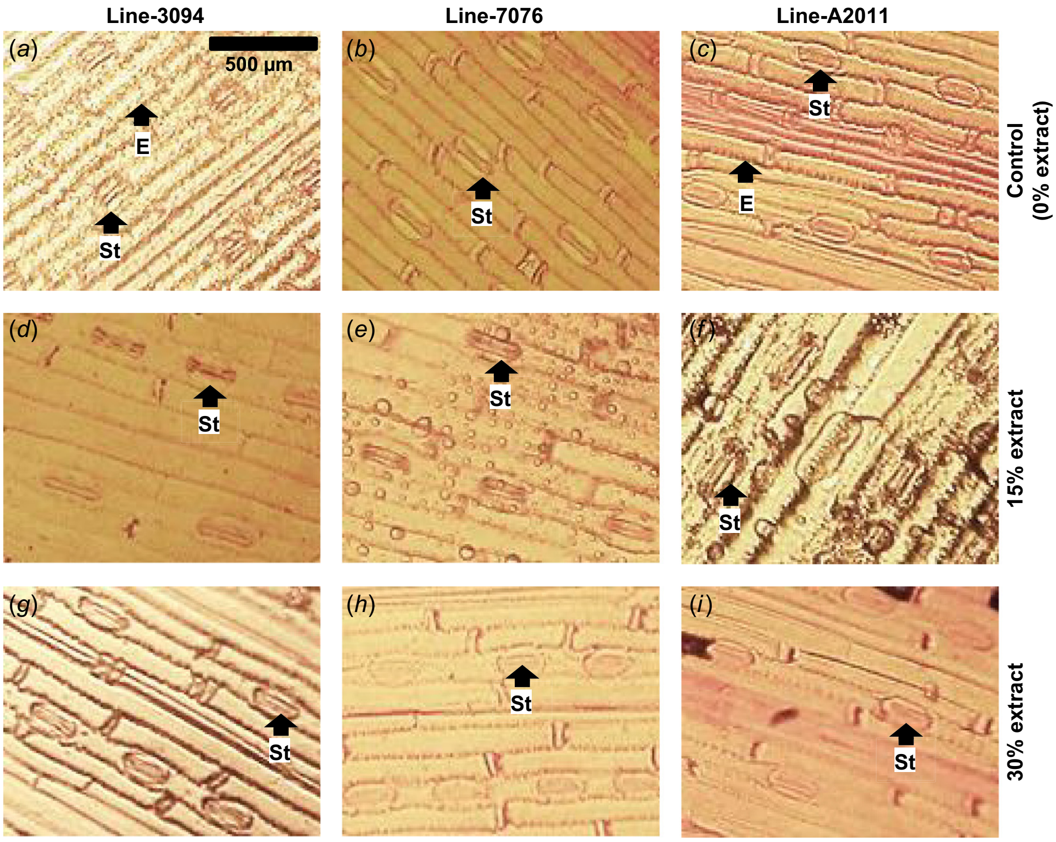

Fig. 6. Abaxial epidermal surface of some wheat lines treated with star fruit (*Averrhoa carambola* L.) leaf extract. (*a*) Stomata (St) more or less rectangular; epidermis (E) wall wavy. (*b*) Stomata (St) numerous; narrowly ovate; large. (*c*) Stomata (St) small; ovate; epidermal (E) wall wavy. (*d*) Stomatal (St) area greatly increased, broadly ovate. (e) Stomata (St) narrowly ovate; stomatal density greatly reduced. (*f*) Stomata (St) large, narrowly ovate. (*g*) Stomata (St) narrowly ovate; stomatal area reduced. (*h*) Stomata (St) ovate. (*i*) Stomata (St) ovate; stomatal density decreased.

response curve of root anatomical traits were relatively high, where line 7076 varied maximally. Line 7076 deviated more from the mean value regarding stem anatomical traits, whereas line A2011 deviated more regarding leaf anatomical traits. The response of stem and leaf anatomical traits was relatively linear.

Discussion

All the wheat lines showed very different behaviour for growth attributes in response to applied allelochemical extracts. Growth in terms of plant height and shoot fresh and dry weights increased when treated with *A. carambola* leaf extract, particularly at 15% concentration. Other growth parameters like root fresh and dry weights, and yield contributing parameters generally decreased at both concentrations of *A. carambola* leaf extract. Overall plant growth (plant height and shoot biomass) is the sign of plants' physical health under normal and stressed environmental conditions. Other morphological traits like root fresh and dry weight, fertile tillers per plant, leaves per plant and yield per plant were sensitive to *A. carambola* leaf extract. This is because of the toxic effect of allelochemicals even in minor concentrations, as reported by Hassan *et al.* (2012) and Sowiński *et al.* (2020). Yield contributing parameters like spike length, spikelets per spike and 100-grain weight were insensitive to allelochemical leaf extract, but the reduction in yield was primarily due to a significant decrease in fertile tillers per plant and leaves per plant (Tunio *et al.* 2018; Vaishali and Alka 2019).

Various authors have indicated the negative effects of allelochemicals on photosynthetic pigments (Hussain *et al.* 2010 in *Lactuca sativa*; Sadeghi *et al.* 2010 in *Triticum aestivum*). In the present study, very opposite responses of wheat lines were observed in wheat lines treated with *A. carambola* leaf extract. No significant change was observed in chlorophyll content in the studied wheat lines, instead an increase in chlorophyll *a* was observed in line 7076 with 30% extract and in 3094 at both extract levels. Increased

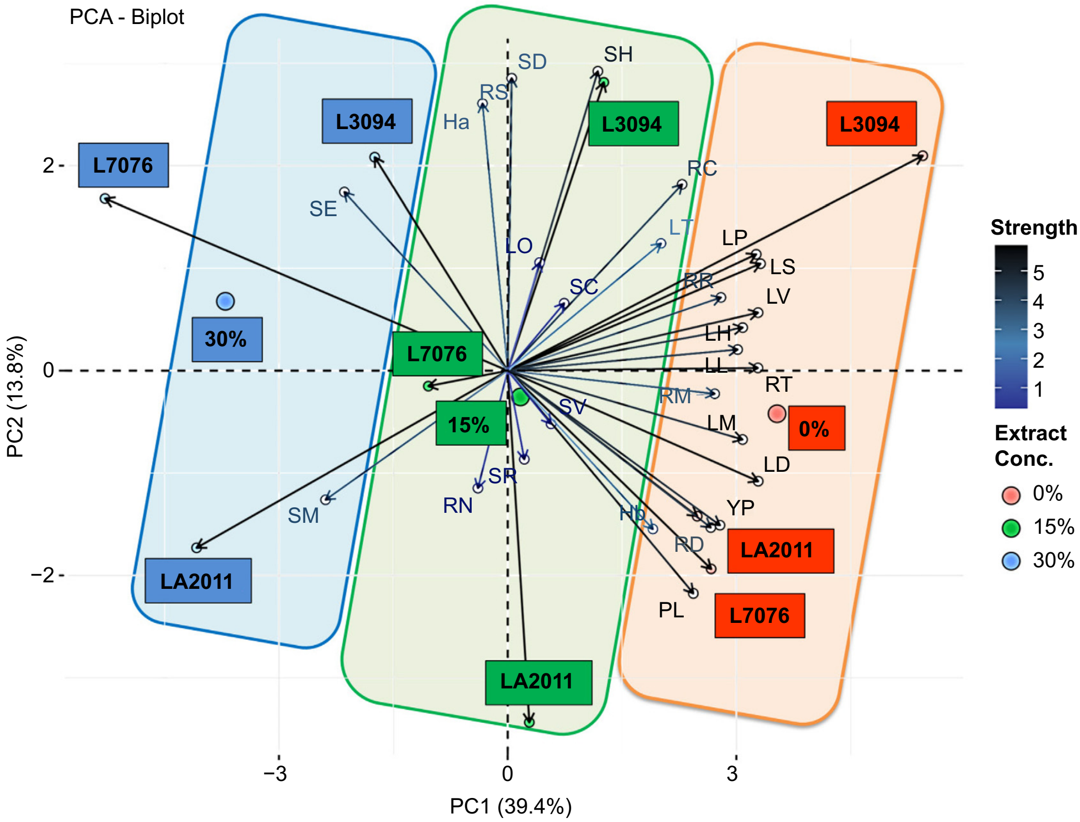

Fig. 7. Relationship among morpho-anatomical and physiological characteristics of some wheat lines treated with leaf extract of *Averrhoa carambola. Morphology:* PL, leaves per plant; SD, shoot dry weight; RF, root fresh weight; RD, root dry weight; YP, yield per plant. *Physiology:* Ha, chlorophyll *a*; Hb, chlorophyll *b. Root anatomy:* RR, root radius; RS, sclerenchymatous thickness; RC, cortical region thickness; RN, endodermal thickness; RM, metaxylem area; RT, pith area. *Stem anatomy:* SR, stem cellular region thickness; SE, epidermal thickness; SH, chlorenchymatous area; SC, cortical cell area; SV, vascular bundle area; SM, metaxylem area. *Leaf anatomy:* LD, midrib thickness; LL, lamina thickness; LP, parenchymatous cell area; LS, sclerenchymatous thickness; LV, vascular bundle area; LM, metaxylem area; LH, phloem area; LT, stomatal density; LO, stomatal area.

photosynthetic pigments relate to better growth of plants, hence lines 7076 and A2011 rated better regarding tolerance of chlorophyll pigments to applied extract.

Root radius decreased in lines 7076 and A2011 with increasing levels of allelochemical extract, which is mainly due to a significant reduction in the proportion of cortical parenchyma. Nouri *et al.* (2012) found similar results by the application of sorghum extract on mungbean and wheat, respectively. Line 3094 responded otherwise to allelochemical extracts, where root radius and cortical thickness increased significantly. This is a critical amendment for enhancing water storage capacity (i.e. succulence) (Feitoza *et al.* 2018). Pawlowski *et al.* (2012) reported an increase in number and size of vacuoles in response to allelochemical application. Larger cortical cells generally possess larger vacuoles (Barrera *et al.* 2019) hence provided more space for the accumulation of allelochemicals (Döll *et al.* 2021), and therefore, regions with larger cortical cell area in line 3094 possessed larger vacuoles. Cortical cell area increased only in line 7076 with 15% leaf extract, which is an indication of tolerance to allelochemical treatment. Endodermal thickness was another prominent feature of wheat lines 7076 and A2011, which increased with 15% leaf extract. This tissue acted as a barrier for radial water flow in roots, allowing water to move via a symplastic pathway (Cai and Mu 2012).

The lower concentration of allelochemical extract (15%) improved the size of metaxylem vessels in lines 7076 and A2011. Increased vascular bundles accompanied by broadened metaxylem vessels positively correlate with water movements via roots, hence improve tolerance to allelochemical extracts (Hameed *et al.* 2020). Wheat line 7076 showed very indifferent behaviour at 15% extract level in form of increased cortical region and metaxylem vessels number. Pith cell area was negatively affected due to allelochemical extract, whereas total pith area increased at 15% concentration in wheat lines

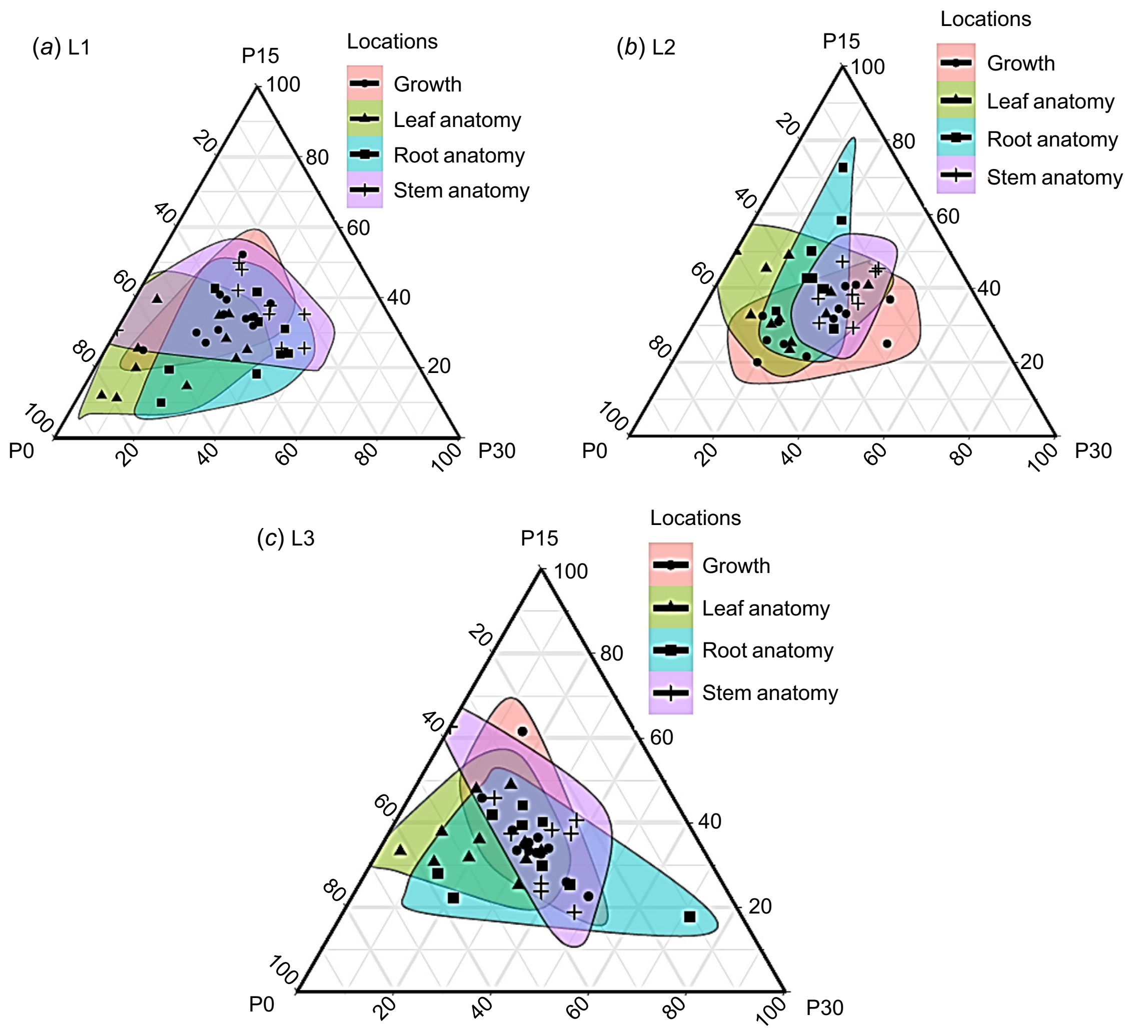

Fig. 8. Ternary plots [(*a*) Line 3094, (*b*) Line 7076, (*c*) Line A2011] of three wheat lines treated with leaf extract of *Averrhoa carambola*. L1, Line 3094; L2, Line 7076; L3, Line A2011, P0, 0% extract; P15, 15% extract; P30, 30% extract.

3094 and A2011. Pith is generally parenchymatous in nature, and is involved in storage of solutes and water, as was observed in our case too (Hameed *et al.* 2020).

All wheat lines showed very specific responses to allelochemical extract, showing some major adjustments at tissue level. Transformation of chlorenchymatous tissues into sclerenchyma in stems of wheat lines is a strategy for survival rather than photosynthetic activity under severely stressful environments (Golzardi *et al.* 2015). Chlorenchyma is more likely prone to damage under water deficit conditions (Souza *et al.* 2018). Sclerification of these tissues is vital for protection from collapse, as also indicated by Fatima *et al.* (2018). Increased cortical cell area in response to allelochemicals application is vital to enhance water storage, particularly in the parenchymatous tissue (Xu and Leskovar 2015; Elansary *et al.* 2016). An increased vascular region along with xylem and phloem area is a critical modification, as recorded in almost all wheat lines, which not only increases the stem cellular region, but in addition, allows the maximum translocation of assimilates and water content.

Leaves are sensitive to allelochemicals due to direct exposure to external environments (Rizzi *et al.* 2016). In the present study, a unique response was noted at leaf level, which included increased leaf thickness, extensive sclerification and a high proportion of storage parenchyma when treated with 15% leaf extract concentration. The highest concentration proved toxic, at least at leaf level. In many cases, such retarded growth is a survival strategy of stressed plants rather than normal growth (Mominul Islam and Kato-Noguchi 2013; Krumsri *et al.* 2019). The decreased leaf thickness with 30% leaf extract increased the fibrous nature of the leaves, and as a result, leaves could fold more easily. This will reduce water loss through transpiration and be extremely beneficial for survival when water is a limited commodity (Schuster *et al.* 2016). A significant decrease in size and density of stomata was

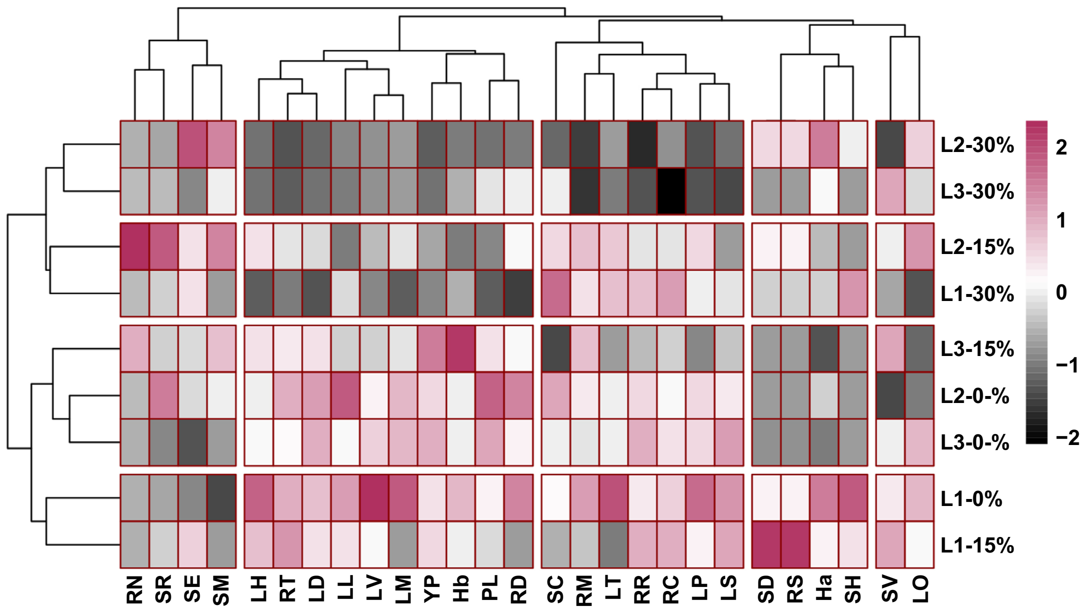

Fig. 9. Clustered heatmaps showing relationship between morphological and anatomical traits under various *Averrhoa carambola* leaf extract concentrations. L1, Line 3094; L2, Line 7076; L3; Line A2011. *Morphology:* PL, leaves per plant; SD, shoot dry weight; RF, root fresh weight; RD, root dry weight; YP, yield per plant. *Physiology:* Ha, chlorophyll *a*; Hb, chlorophyll *b*. *Root anatomy:* RR, root radius; RS, sclerenchymatous thickness; RC, cortical region thickness; RN, endodermal thickness; RM, metaxylem area; RT, pith area. *Stem anatomy:* SR, stem cellular region thickness; SE, epidermal thickness; SH, chlorenchymatous area; SC, cortical cell area; SV, vascular bundle area; SM, metaxylem area. *Leaf anatomy:* LD, midrib thickness; LL, lamina thickness; LP, parenchymatous cell area; LS, sclerenchymatous thickness; LV, vascular bundle area; LM, metaxylem area; LH, phloem area; LT, stomatal density; LO, stomatal area.

observed in lines 3094 and A2011 indicating more tolerance, because it regulates transpiration more easily (Hussain and Reigosa 2017). This will ensure the success of plants by lowering the transpiration rate (Zheng *et al.* 2021).

Line 3094 showed peculiar modifications in leaf extract-treated plants like enhanced shoot fresh and dry weights, root radius, root epidermal thickness, root sclerification region (at 30% only), root cortical region thickness, root pith area and pith cell area (at 15% only). Stem modifications in this line were increased stem cellular region thickness, epidermal thickness, vascular bundle area, phloem area and cortical cell area (at 15% only), sclerenchymatous thickness and vascular bundle number (at 30% only), and metaxylem area. All leaf anatomical traits negatively responded to *Averrhoa* leaf extract. Line 7076 showed increased root fresh and dry weights in plants treated with leaf extract. The 100-grain weight increased with 15% extract; chlorophyll *a* increased with 30% extract. In roots, epidermal thickness increased, and cortical cell area, endodermal thickness and metaxylem area increased when treated at the 15% level. Stem modifications were increased vascular bundle number and metaxylem area, whereas stem cellular region thickness, vascular bundle area and phloem area increased with 15% extract. Leaf modifications were increased phloem area, stomatal density, and area with 15% extract. Line A2011 showed an increase in chlorophyll *a* with 30% extract and chlorophyll *b* with 15% extract. Roots showed modifications like increased epidermal thickness and sclerenchymatous thickness with 30% extract; and endodermal thickness, metaxylem area and pith area with 15% extract. Stem modifications were increased stem cellular region thickness and vascular bundle area; whereas vascular bundle number increased with 30% extract and metaxylem area with 15% extract. Leaves showed increases in bulliform cell area and phloem area only with 15% extract.

The relationship among structural and functional traits indicated variable responses, not only of different wheat lines but also at different levels of *Averrhoa* leaf extract. Stem trait chlorenchymatous area, root traits like sclerenchymatous thickness and chlorophyll *a* significantly contributed to shoot dry weight at lower allelochemical level. Stem chlorenchyma can significantly increase photosynthetic rate which in turn can significantly increase yield and biomass production (Ávila *et al.* 2014). Ternary plots indicated the collective response of various morpho-anatomical or physiological traits in different wheat lines. Growth parameters generally inclined towards 0% leaf extract, however, a few growth traits deviated towards 15% leaf extract.

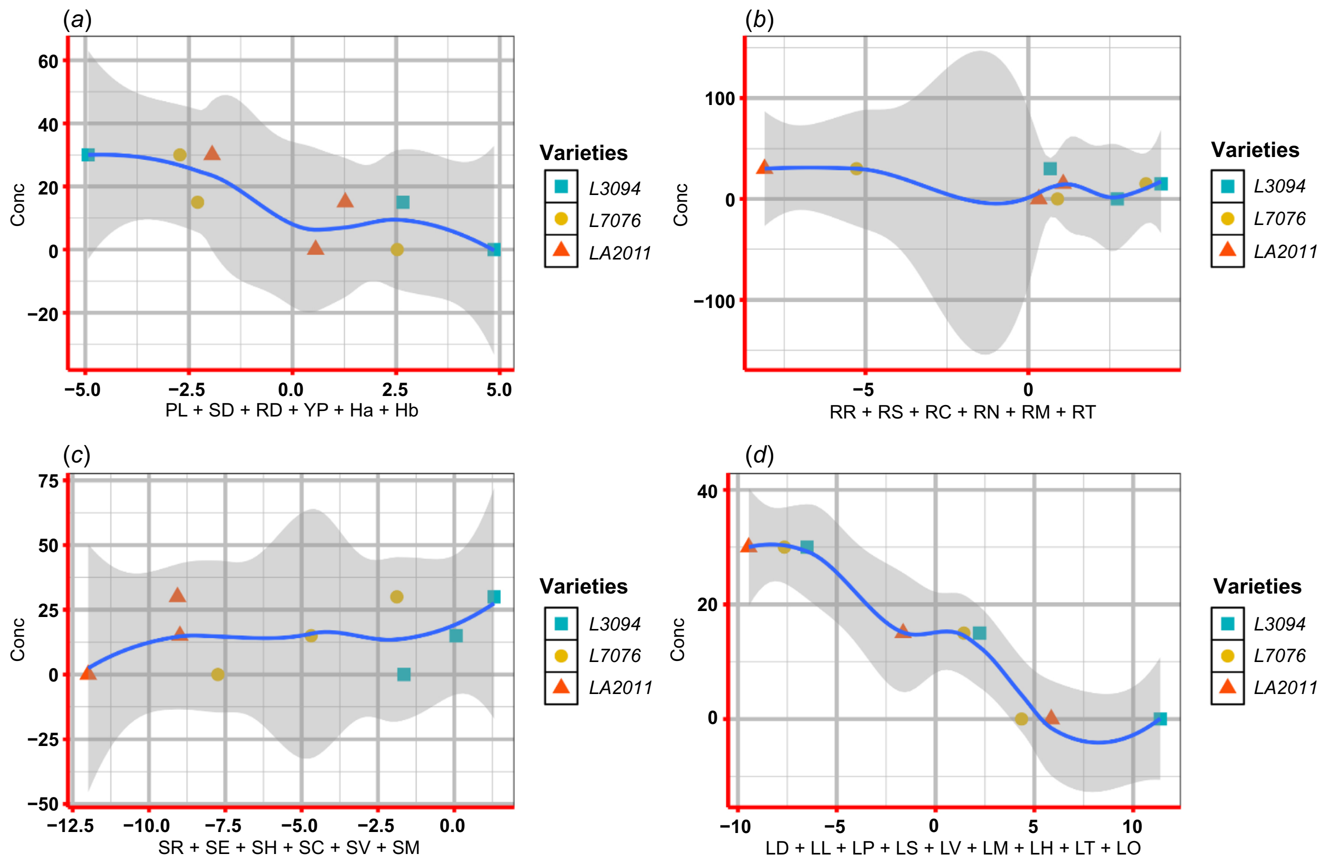

Fig. 10. Estimated responses of different traits [(*a*) morphology and physiology, (*b*) root anatomy, (*c*) stem anatomy and (*d*) leaf anatomy] of three wheat lines to leaf extract of *Averrhoa carambola*. *Morphology:* PL, leaves per plant; SD, shoot dry weight; RF, root fresh weight; RD, root dry weight; YP, yield per plant. *Physiology:* Ha, chlorophyll *a*; Hb, chlorophyll *b*. *Root anatomy:* RR, root radius; RS, sclerenchymatous thickness; RC, cortical region thickness; RN, endodermal thickness; RM, metaxylem area; RT, pith area. *Stem anatomy:* SR, stem cellular region thickness; SE, epidermal thickness; SH, chlorenchymatous area; SC, cortical cell area; SV, vascular bundle area; SM, metaxylem area. *Leaf anatomy:* LD, midrib thickness; LL, lamina thickness; LP, parenchymatous cell area; LS, sclerenchymatous thickness; LV, vascular bundle area; LM, metaxylem area; LH, phloem area; LT, stomatal density; LO, stomatal area.

Allelochemicals can enhance growth in lower concentrations (Ambika 2013), as was observed in the present study. The clustered heatmap indicated close clustering of all wheat lines at 30% leaf extract, whereas clustering at lower concentration was somewhat unpredictable. The 30% leaf extract severely hampered most of the growth, anatomical and physiological traits. Similar findings have earlier been reported by Nouri *et al.* (2012) and Sowiński *et al.* (2020). The decrease in growth traits were mainly due to a decrease in storage tissue (cortical parenchyma), mechanical tissue (sclerenchyma), vascular tissue (xylem and phloem) and dermal tissue (epidermis), as reported by Golzardi *et al.* (2015), Feitoza *et al.* (2018) and Hameed *et al.* (2020). Estimated response curves showed high plasticity of morphological, anatomical and physiological traits in response to different concentrations of allelochemicals. Overall, the lower concentration of allelochemical promoted growth and anatomical parameters, whereas the higher concentration proved toxic, retarding structural and functional traits of selected wheat lines.

Conclusion

All bread wheat lines responded very differently to an allelochemical extract of *A. carambola* leaves. Biomass production generally increased with leaf extract application in wheat lines. The major contributors for increased root area were proportion of cortical parenchyma, stelar area and pith area. This will enhance storage capacity and better translocation of solutes. Epidermis acts as a barrier to water movement across the roots. Increased thickness of root epidermis in leaf extract-treated plants will certainly be beneficial. Stem cellular region generally increased in leaf extract-treated plants, which is linked to a high proportion of storage parenchyma. Metaxylem are the main conducting tissue in xylem, broad metaxylem vessels are directly associated with the amount of water conduction. Well-developed bulliform cells are a xeric characteristic involved in leaf rolling, which is extremely helpful under limited water environments. A high density of large stomata is related to better energy production and gaseous exchange. The lower dose of *Averrhoa* leaf extract promoted growth and development in all wheat lines, hence can be used as growth promoter, whereas higher concentrations are important for eradicating unwanted plants.

Supplementary material

Supplementary material is available online.

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Data availability. The voucher specimens used for plant identification are deposited in the herbarium facility of the Department of Botany, University of Agriculture, Faisalabad, and are available for verification on request. Anatomical slides, photographs and raw data calculated from these photographs are available from the primary author and can be requested if needed.

Code availability. R codes and modelling details are available with author(s) listed as bio-statisticians under author's contribution section of Declarations and can be requested if needed to reproduce the data visualisation or other results.

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