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African Journal of Plant Science

Full Length Research Paper

Testing vertical distance and growth medium for vertically farming bush beans (*Phaseolus vulgaris* L.) and tomatoes (*Solanum lycopersicum* L.) in urban agriculture

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Vertical farming implies growing crops in superimposed growth beds. This study assessed the effects of vertical distance between growth beds and growth medium on beans and tomatoes. Three levels of vertical spacing and three levels of growth medium were studied in a split plot design and three replicates. The vertical distance was tested in main plots while the growth medium was tested in sub-plots. The main plot was a wood-made vertical structure with the ground growth bed and the top bed vertically separated by either 80, 120 or 160 cm. The subplot was a bottom-holed plastic bucket containing a thorough growth mix of loam soil and 0, 40, or 60% added manure (volume/volume). Three buckets were used per growth medium and growth bed, making 6 observations for each factorial combination. Tomato was cropped after bush bean harvest. Maximum bean grain yields were projected for 130 cm vertically spaced beds and for 30% added manure growth medium. Maximum tomato fruit yields should be expected for growth medium containing more than 60% manure and for spacing distance above 160 cm. Vertical farming appeared highly productive with 0.86 kg m^{-2} of total dry bean grain yield and 14.1 kg m^{-2} of total tomato fruits from the two beds.

Key words: Vertical farming, vertical spacing distance, growth beds, growth medium, tomatoes, bush beans.

INTRODUCTION

Urban agriculture is a new concept of agriculture that implies producing food in urban and peri-urban areas where the land has already been transformed into human settlements (Tornaghi, 2014). The practice balances a bit

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the reduction of the arable land while minimizing the distance from food production areas to consumers (Al-Kodmany, 2018; Molin and Martin, 2018; Birachi et al., 2011). Urban agriculture simply relates to the production of plants- and animal-related food within urban dwellings through home gardening or specifically designated zones for vegetable production (Tornaghi, 2014; Zezza and Tasciotti, 2010).

Within urban agriculture, vertical farming is the practice of vertically expanding the productive space above the ground surface (Vyas, 2018; Despommier, 2009) with the view to producing more food or medicine. Vertical farming can also be practiced on simple wood or metallic poles (pole farming) or constructed growth beds and sacks (Vyas, 2018; Peprah et al., 2014). As urban population grows and arable land declines, vertical farming can locally provide an interesting approach for sustainably growing fresh produces for the family and the community (Kalantari et al., 2017; Banerjee and Adenaeuer, 2014). However, the profitability of the technology is still debated although recent research findings validate its viability (Eaves and Eaves, 2018; Shao et al., 2016).

With the development of vertical farming technology, the range of crops that can be grown also expands (Cornia, 2014). Bush beans is the most commonly consummed legume while tomato is the most commonly consummed vegetable in many urban and rural areas of the African Great Lakes Region (Larochelle et al., 2014; Sonko et al., 2005). For instance, the annual consumption of beans is around 29 and 11 kg per capita in Rwanda and Uganda, respectively (SPIA, 2014). Tomatoes represent up to 28.4% of total volume of sales of horticultural produces in Rwanda (Clay et al., 2014). Bush beans are a major source of proteins while both bush beans and tomatoes supply vitamins and minerals such as iron and zinc (Takusewanya et al., 2017; Bizimana et al., 2013).

Home gardening of vegetables in small land plots or in containers has been promoted in the African Great Lakes Region notably for tomatoes, onions, and cabbages. In countries like Rwanda, home gardening is known as “kitchen gardening” and it is mainly promoted in rural areas as part of a national strategy to improve human nutrition. However, home gardening of beans is quite inexistent although the crop easily grows and the market of greens and grains is huge. Moreover, given the land scarcity and long distances from the production areas, backyards of urban residences could contribute fresh beans to urban consummers. However, very little is known about the productivity of both bush beans and tomatoes in a sunlight-dependent vertical farming technology. In addition, the appropriate growth medium based on locally available growth medium has not yet been documented for such a system. Therefore, this study aimed at testing the productivity of bush beans and tomatoes in a two-level vertical farming structure using growth medium made of mixes of locally available loam soil and cow manure.

MATERIALS AND METHODS

Experimental site

The study was conducted on the main Campus of the University of Kibungo, at Karenge (Kibungo town), southeastern Rwanda, at 1646 m above sea level, -2.149672° South, and 30.546533° East. The region is under tropical conditions with averages of 20°C of annual temperature and 1200 mm of rain precipitation. The climate follows a bimodal pattern with heavy rain precipitations extending from March to May and from October to December, alternating with two dry seasons.

Factorial treatments

Treatments composed of two factors: Vertical spacing and growth medium. Vertical spacing characterized the distance separating two growth beds or growth platforms, one on the ground level and the second at a variable distance above the ground. Three distances/levels of vertical spacing were studied: 80 cm (VS080), 120 cm (VS120), and 160 cm (VS160) between the two growth beds. Growth medium represented the second factor with three levels: a 0% dairy cow manure + 100% loam soil (GM00), a mix of 40% dairy cow manure + 60% loam soil (GM40), and a mix of 60% dairy cow manure + 40% loam soil (GM60). These mixes were made volume by volume. Therefore, 9 factorial combinations of the two treatments were generated for the study.

Experimental design

White plastic buckets (12-L), 25 cm mouth width, 27 cm depth, and 17 cm bottom width, were individually filled with thoroughly mixed growth medium to 2 cm below the top. Three buckets were used for each level of growth medium and for each growth bed (ground and top beds) by vertical spacing level. Each bucket was bottom-perforated to drain excess water.

A wood-made structure was built such that top growth beds are located at either 80, 120, or 160 cm distances above ground (Figure 1). Each individual structure was 1 m × 1 m framed so as to contain only nine (9) buckets by growth bed or 18 plants over the two superposed beds. An iron sheet beneath each top platform diverted the drainage water out of the ground bed.

A split plot design and three replicates were used for this study. The vertical spacing factor was randomly distributed in the main plots (wood structures) while the growth medium was randomly tested in the subplots (on the growth beds).

Agronomy

Bush beans

Two grains of RWA2245 bush bean variety were seeded in each bottom-holed bucket on 14 July, 2014 and watered throughout the dry season to maturity. A compound fertilizer (200 g of 17-17-17) was mixed with each growth substratum level before planting. This was equivalent to banding 400 kg 17-17-17 ha^{-1} in the soil having 1.4 kg dm^{-3} specific gravity.

Tomatoes

After bush beans, containers were emptied and the growth medium

Figure 1. Wood-made experimental structures with growth beds and sprouting beans emerging from containers.

re-worked by level to make it suitable for receiving tomato seeds. Additional 200 g of 17-17-17 were incorporated. Four grains of Better Boy tomato variety were seeded by container on December 16th 2014 and later thinned to the best two plantlets. Watering was done throughout the January - February dry season to the rainy season.

Recommended plant spacing was controlled by adjusting the distance between containers. In addition, weeding was done manually by picking and removing undesired plant species from containers. A mosquito net wrapped the site up to protect sprouting plants, particularly bush beans, against birds and insects. Dithan M-45 fungicide was applied a few times on tomatoes during the rainy season in late February 2015.

Data collection

Bush beans growth was monitored 14, 21, 28, and 35 days after planting by measuring each plant length. Plant diameter was measured at 2 cm above ground 45 days after planting. Flower counts and maturity pods were monitored 45 and 60 days after planting, respectively. Bean grain yield and biomass were monitored at harvest time. The grains and biomass were dried under atmospheric conditions until constant weight.

Tomato growth was monitored 40 and 55 days after planting by measuring the length of the plants. Collar shoot diameter was monitored 55 days after planting and fruits set monitored 60 and 75 days after planting. Harvesting of mature fruits was done three times, at 90, 97, and 104 days after planting. All the parameters were collected from every container. Few containers had missing data because of various damages due to birds and insects for beans and diseases for tomatoes.

Statistical analyses

The effects of treatments on monitored parameters were analyzed using the analysis of variance (ANOVA) with multiple observations in three different but complementary tests:

i) Tests on the effects of treatments considering the data from the two growth beds as continuous sets of observations; consequently, six obsevations were tested for each factorial combination;
ii) Tests on the effects of treatments using the sums of values of parameters from the two growth beds; consequently, three observations were tested by factorial combination;
iii) Comparison of the performance of the two growth beds by running separate T-tests with paired observations for specific parameters such as flowers and pods counts or tomato fruits, bean grain yield and biomass.

Adjustments for missing data were made based on Steel and Torrie (1980). All of the statistical analyses were performed using NCSS Computer Package, 2004 version (Hintze, 2004). Bartlet's Chi Square tests were run on all the data, and when the homogeneity of variances was not valid, ANOVA was performed on log-transformed data. Duncan multiple range test was performed for multiple means comparison. A 5% probability level was set for the significance of tests.

RESULTS

Effects of vertical spacing on yields and yield components of bush beans and tomatoes

The vertical spacing effects on the yields and yield components of the bush beans and tomatoes are presented in Table 1. These results are mean values of continuous sets of 6 observations monitored from the two growth beds. Repeated measures ANOVA with multiple observations detected no significant differences between vertical spacing distances with regard to the growth rates for each crop. In addition, no differences were detected with regard to the bush beans stem diameter, flowers counts, pod counts, and total aerial biomass. The same was true for the basal diameter of tomato shoots. However, significant differences ($P \leq 0.05$) between the

Table 1. Effects of vertical spacing distance of growth beds on yield and yield components of bush beans and tomatoes

Crop type	Monitored parameter	Vertical spacing distance		
		VS80	VS120	VS160
Bush beans	Plant growth rate (cm)	23.0^a	20.7^a	22.9^a
	Stem diameter (mm)	5.8^a	6.0^a	6.2^a
	Flowers counts /plant	13.0^a	12.0^a	11.9^a
	Maturity pods /plant	8.9^a	8.8^a	8.1^a
	Aerial biomass (g /m^2)	680^a	750^a	750^a
	Grain yields (g /m^2)	370^a	480^b	440^{ab}
Tomatoes	Growth rate (cm)	30.3^a	32.0^a	32.6^a
	Stem diameter (mm)	3.70^a	3.94^a	4.10^a
	Fruit counts by plant	6.0^a	6.8^a	8.2^b
	Fruit yields (g /plant)	274.0^a	418.8^b	468.0^c

Numbers sufficed with different letters in same rows are significantly different.

Table 2. Effects of the growth medium on yields and yield components of bush beans and tomatoes.

Crop type	Monitored parameter	Growth medium type		
		GM00	GM40	GM60
Bush beans	Plant growth rate (cm)	21.4^a	21.1^a	24.2^b
	Stem diameter (mm)	5.8^a	6.2^a	6.1^a
	Flowers counts /plant	11.4^a	12.9^b	11.6^a
	Maturity pods /plant	8.6^a	9.0^a	8.1^a
	Aerial biomass (g /m^2)	650^a	800^b	720^a
	Grain yields (g /m^2)	410^a	480^a	400^a
Tomatoes	Growth rate (cm)	29.9^a	32.2^{ab}	32.9^b
	Stem diameter (mm)	3.67^a	3.93^{ab}	4.13^b
	Fruit counts by plant	5.7^a	7.4^b	7.8^b
	Fruit yields (g /plant)	272.1^a	413.5^b	475.0^c

Numbers sufficed with different letters in same rows are significantly different.

three levels of vertical spacing existed for the bush bean grain yields and tomato fruit counts and yields. In that respect, VS120 yielded higher quantity of grains (480 g/m^2) than any other spacing distance, whether significantly or in actual value.

For tomatoes, VS160 significantly generated higher fruit counts (8.2 fruits/plant) and fruit yields (468g/plant) than lower spacing distances.

Effects of growth medium on yields and yield components of bush beans and tomatoes

The results on the growth medium effects were given in Table 2 for both bush beans and tomatoes. With regard to bush beans, significant differences between the three types of growth substratum were found in relation to the plant growth ($P < 0.05$).

Very significant differences also existed with regard to flower counts per plant ($P < 0.001$) and total biomass ($P < 0.001$) per m^2 of land cropped to bush beans. However, no statistical differences were found between GM00, GM40, and GM60 with regard to basal shoot diameter, maturity pod counts by bush bean plant, and grain yields. GM40 resulted in higher number of flowers and aerial biomass weight than the two other levels. In addition, with regard to bean grain yields, although statistically unsignificant, GM40 yielded 70 and 80 g higher than GM00 and GM60, respectively. Such yield differences were not negligible since they were significant at 6.8% probability level.

For tomatoes, significant differences between GM00, GM40, and GM60 were found for plant growth ($P \leq 0.05$), stem diameter ($P \leq 0.05$), fruit counts ($P \leq 0.01$), and fruit yields ($P \leq 0.01$). Again, GM40 resulted in equal or higher tomato stem diameter and fruit counts than GM60. This latter, however achieved significantly higher tomato fruit yields with 475 g/plant than the former with 413.5 g/plant.

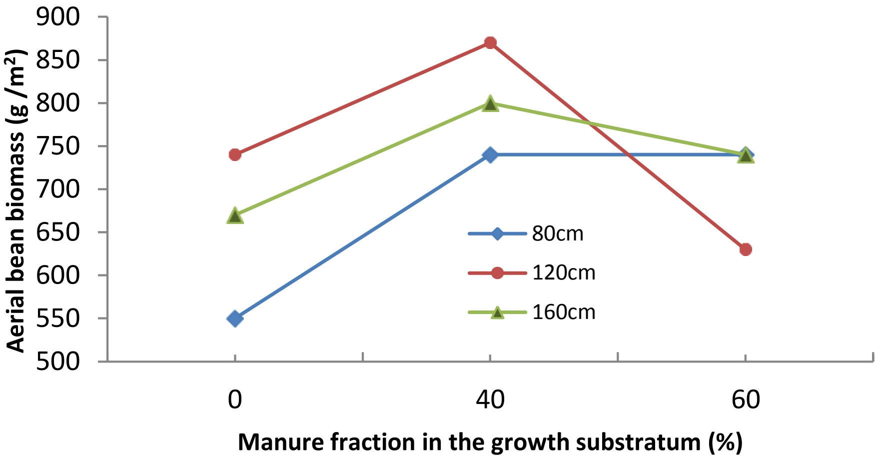


Figure 2. Interaction effects of added-manure growth medium and vertical spacing distance on the aerial biomass of bush beans.

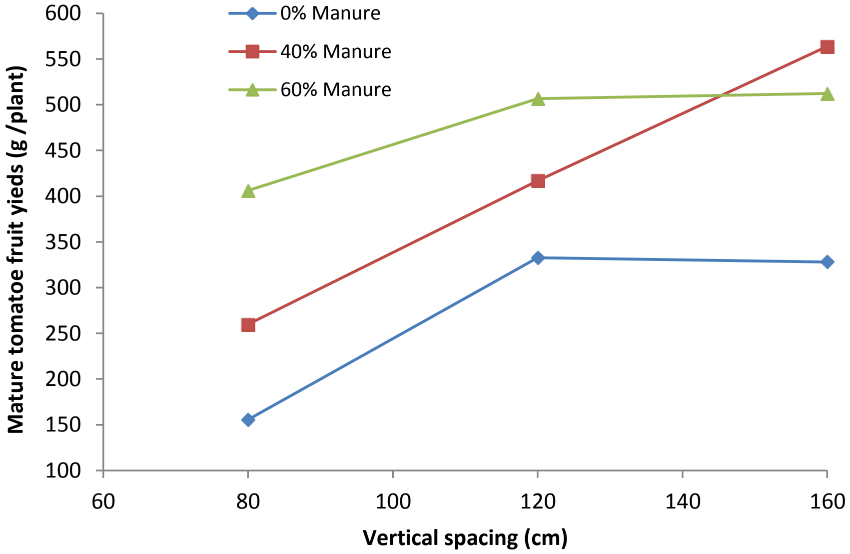


Figure 3. Interaction effects of vertical spacing and growth medium on tomatoe fruit yields.

Also, significant interaction effects ($P < 0.05$) between the growth rate monitoring time and the growth medium were observed for bush beans. The plant grew longer under GM60, reaching 45.1 cm after 35 days while the plant length under GM00 and GM40 were 6.8 cm and 5.9 lower at the same period, respectively.

Interaction effects of vertical spacing and growth medium on yields and yield components of bush beans and tomatoes

Significant interaction effects ($P \leq 0.05$) between the two factors were only observed for the aerial bush bean biomass and tomato fruit yields. Maximum bush beans biomass (846 g/m^2) was produced when GM40 was combined with VS120 (Figure 2).

For tomatoes (Figure 3), combining GM40 and VS160 or GM60 and VS160 statistically produced equal fruit yields of 564 g/plant and 522 g/plant for the first and second combinations, respectively. However, tomato plants did not achieve their maximum potential under these interactions, thus pointing to different combinations that might be more suitable for full production potential of tomato plants.

Yield comparisons from the two growth beds

The analysis results using T-tests with paired

Table 3. Comparisons of yields and yield components of Bush Beans and Tomatoes from the ground and top growth beds.

Monitored parameter	Bush bean		Tomatoes	
	Ground bed	Top bed	Ground bed	Top bed
Plant height (cm)	23.7^a	20.7^b	33.0^a	30.4^b
Basal stem diameter (mm)	5.5^a	6.5^b	3.40^a	4.42^b
Flowers' counts by plant	9.0^a	14.9^b	N/^{A}	N/^{A}
Pods /Fruits' counts by plant	6.9^a	10.2^b	3.2^a	10.8^b
Aerial biomass (g/m^2)	610^a	840^b	N/^{A}	N/^{A}
Bean grains (g/m^2)	310^a	550^b	-	-
Tomato fruits (g/plant)	-	-	207.0^a	568.3^b

Numbers sufficed with different letters in same rows by crop species are significantly different. N/A means "not available".

Figure 4. Delayed maturity of bush beans on ground bed as compared to maturity of beans on top growth bed.

observations for all the parameters are presented in Table 3. All the mean values were very significantly ($P \leq 0.001$) higher on the top growth bed than on the ground bed for the two plant species and for all monitored parameters, except the plant growth. Bush bean biomass and grain yields were 27.4 and 43.6% lower on ground bed than on top bed, respectively, while a drastic 73.6% tomato fruit yield reduction was noted on the ground bed as compared to the yield on the top bed.

Otherwise, bush bean plants grew longer on the ground bed than on the top bed. This was also true for tomatoes. Moreover, not only the numbers of bush bean flowers and pods were higher on top bed, but also the maturity of pods and ripening of tomatoes were faster. A visual example is given for beans in Figure 4.

Effects of treatments on total yields and yield components of bush beans and tomatoes

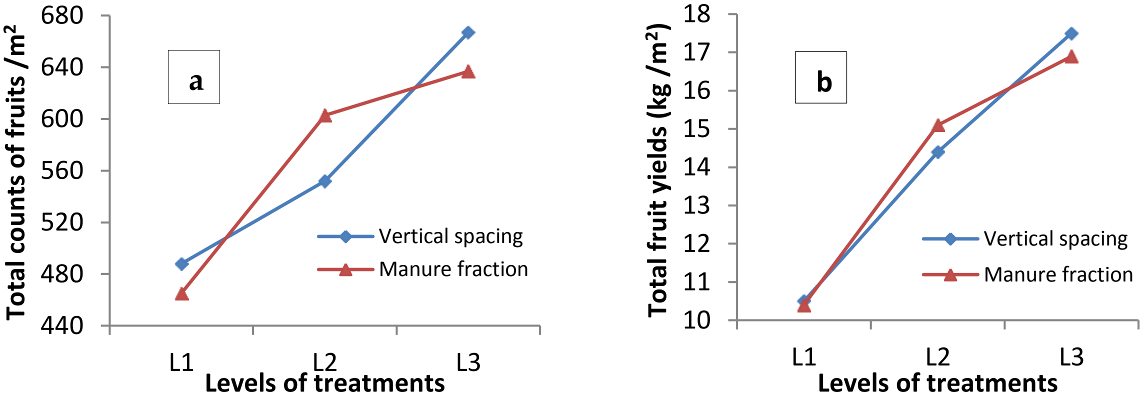
The interest of multi-level growth beds lie in their multiplicative potential of yields on the same land area unit. In this respect, in Table 4 are presented the results on sums of flower counts, pod counts, biomass yields, and grain yields of bush beans. The vertical spacing factor only significantly impacted on total bush bean grain yields ($P \leq 0.05$) with VS120 and VS160 equally yielding, and yielding higher than VS80.

For the growth medium factor, significant differences only noticed for the bush bean flower counts ($P \leq 0.01$) and grain yields ($P \leq 0.05$) indicated that GM40 yielded higher than any other growth medium level.

Table 4. Multiplicative effects of a two-level growth beds on yields and yield components of bush beans as influenced by the treatments

Factorial treatments	**Vertical spacing (cm)**			**Growth medium manure content (%)**		
Measured parameter	**80**	**120**	**160**	**0**	**40**	**60**
Flower counts /m^2	534^a	451^a	484^a	472^a	525^b	484^a
Pod counts/m^2	342^a	318^a	331^a	328^a	342^a	320^a
Biomass (kg/m^2)	1.35^a	1.49^a	1.49^a	1.30^a	1.60^a	1.43^a
Grain yields (kg/m^2)	0.74^a	0.95^b	0.88^b	0.81^a	0.96^b	0.80^a

Numbers sufficed with different letters in same row are significantly different.



Figures 5. Total fruit counts (a) and fruit yields (b) of tomatoes as influenced by the two factors (L_1, L_2, and L_3 respectively stand for 80 cm, 120 cm, and 160 cm for vertical spacing or 0, 40, and 60% for manure content in the growth medium).

Regarding tomatoes, the vertical spacing factor did not significantly affect the sums of counts and yields of tomato fruits. However, the actual values of total fruit counts linearly stretched out from 488 counts m^{-2} for VS80, through 552 counts m^{-2} for VS120 to 667 fruit counts for VS160 per land square meter (Figure 5a). In the same order, the actual values of total fruit yields varied from 10.5 kg m^{-2}, through 14.4 kg m^{-2} out to 17.5 kg m^{-2} of land area (Figure 5b) for VS80, VS120, and VS160, respectively.

On the contrary, very significant differences were found between the three added-manure levels of the growth medium for each of total fruit counts and fruit yields. These differences were reflected in significantly linear effects visualizable in Figure 5a for fruit counts ($P \leq 0.01$) and Figure 5b for fruit yields ($P \leq 0.001$). GM40 and GM60 produced equal numbers of fruits, which were 29.5 and 36.9% higher than GM00, respectively. With regard to fruit yields, all the three levels of the growth medium were different from one another, yielding 10.4^2, 14.4, and 17.5 kg m^{-2} for GM00, GM40, and GM60, respectively.

The regression equations developed from statistically significant key results as related to bush beans and tomato fruit yields revealed that maximum values of each factor generating maximum yields were computed and the results presented.

For bush bean grain yield (Table 4):

$$Y_{VS}= -9e\text{-}^{5}X^{2} + 0.0228X – 0.52$$

with R^2= 1; Y_{VS} variable is the grain yield while X variable is the vertical spacing distance of growth beds; a calculated distance of 127 cm provides for the maximum grain yield.

$$Y_{GM}= -0.0002X^{2} + 0.0116X + 0.81$$

with R^2 = 1; Y_{GM} variable is the grain yield while X variable is the added manure fraction in the growth medium; a calculated 29% rate of manure in the growth medium provides for the maximum grain yield.

For tomato fuit yields (Figure 5b).

$$Y_{VS}= 0.087X + 3.6333$$

with R^2 = 0.9957; Y_{VS} is the tomato fruit yield while X is the vertical spacing factor. No maximum yield could be achieved within the studied interval of the vertical spacing factor.

$$Y_{GM}= 0.1096X + 10.479$$

with $R^2 = 0.9962$, no maximum yield can be achieved within the studied interval of the growth medium factor. However, a maximum number of fruit counts could be projected for a growth medium containing 80% added manure.

DISCUSSION

Vertical spacing factor

Under natural conditions of vertical farming, the vertical spacing effect is directly linked to how much of sunlight energy reaches the lower growth beds. In fact, the plant growth and productivity is highly determined by the radiation absorption, the photosynthetic efficiency and the respiration efficiency (Bugbee, 1995). In this study, any significant effect of the vertical spacing factor must have impacted on these three components for each crop growing on the lower bed. The impact of sunlight efficiency was effective with regard to bush beans grain yields as well as tomatoes fruit counts and yields (Table 1). Bush bean grains and tomato fruit counts and yields were lowest under VS80 while VS160 yielded highest tomato fruits counts and yields. The impact of sunlight efficiency was much more obvious when the two growth beds were compared with regard to the yields and yield components (Table 3). Significantly highest values were systematically monitored on top beds for all of the measured parameters except the plant growth rate. Such differences resulted from the difference in daily light integral received by plants growing on the two superimposed beds (Morgan, 2013; Dorais, 2003). The higher growth of plants on the ground beds implies the insufficiency of sunlight and their elongation is an attempt to expose to as much energy as possible. Overall, the yield reductions of 43.6% for bean grains and 73.6% of tomato fruits were tangible evidence of reduced radiations on ground beds in comparison to top beds. Also, when yields from the two beds were summed up for each parameter and total values were analyzed with regard to the vertical spacing factor (Table 4), VS120 and VS160 significantly yielded 28.4 and 18.9% bean grains higher than VS080, respectively. The bush bean maximum grain yield could be achieved with a projected vertical spacing of 127 cm (≅ 130 cm) distance between the two beds.

For tomatoes, the maximum levels of fruit counts and fruit yields could not be achieved within set boundaries of vertical spacing (Figure 5). The optimum spacing distance for optimum fruit counts and yields fall beyond the interval of this study. This implies that a higher spacing distance was needed to achieve maximum production potential of tomatoes in such a vertical farming system. In fact, previous findings indicated that tomato was a more light-demanding plant than bush beans (Morgan, 2013; Papadopoulos, 1991). However, both bush beans and tomatoes exerted an exceptionally high productivity with yield averages of 0.86 kg/m^2 of dry bean grains and 14.1 kg/m^2 of tomato fruits for the potential of these crops in the region. Of course, this high productivity allows for increased food availability (Drost and Wytsalucy, 2014) while fostering the protection of the environment by reducing the need for more agricultural land (Edgerton, 2009). In addition, this vertical farming technology allowed for a sequenced period of crop maturity with plants on the top bed maturing earlier than plants on the ground bed (Figure 4), thus making fresh food available for a longer period of time. The increase of both of plant productivity and food availability is what the major interest of vertical farming technology is all about.

Growth medium factor

The manure fraction in the growth medium plays a physical role through improved water holding capacity, aggregate stability, and drainage potential (Nalivata et al., 2017). It also plays a chemical role as a plant nutrient source (Sankaranarayanan and Karemangingo, 2012). In this study, the impact of the growth medium was dependent on monitored parameters and the plant species (Tables 2 and 4). In general, the impact was almost none on bush beans for most of the parameters although GM40 statistically or actually produced the highest values for flower counts, maturity pods, aerial biomass, and grain yields. Moreover, total yields of bush beans were statistically higher under GM40 than under any other level (Table 4). The GM40 treatment might have offered the best growing conditions to bean plants in terms of drainage conditions and adequate nutrient supply through mineralization. Bush beans thrive better in well drained soils (Worku, 2015) such as sandy loams and sandy clay loams (Liebenberg, 2002). The maximum yields of bean grains could be achieved with a projected growth medium containing 30% manure (the calculated rate was 29%).

As regards tomatoes, GM60 resulted in the highest impact, statistically or in actual values for all the parameters (Table 2). The same is true when the factor’s levels were compared with regard to total counts of tomato fruits (Figure 5a) and total fruit yields (Figure 5b) from the two beds. Tomato plants prefer growth media containing high rates of organic materials as supported by several studies (Atif et al., 2016; Lin et al., 2015; Nabi et al., 2002). The actual GM60 did not allow for achieving the maximum productivity of tomatoes. It is however close to the 3:1 ratio of organic materials and soil mix recommended by Lin et al. (2015) and the 2:1 best growth medium mixes reported by Atif et al. (2016) and

Gama et al. (2015) for growing tomatoes in containers. In the present study, a projected growth medium containing 80% manure could have achieved maximum tomato fruit counts only.

Interaction effects of vertical spacing and growth medium

On bush beans**,** the maximum grain yield is achieved when GM40 is combined with any level of the vertical spacing factor. In particular, GM40 and VS120 interacted to significantly produce highest yields than any other factorial combinations (Figure 2). On tomatoes, the fruit yield from GM40 constantly increased under increasing VS factor and no maximum yield could be obtained within the range of the study for the VS factor (Figure 3). Therefore, based on the above discussion on the main effects of the two factors, vertically farming bush beans and tomatoes in built structures shall require specific growth media and specific vertical spacing distances of the growth beds.

Conclusion

This study aimed at determining the productivity of bush beans and tomatoes in a constructed two-level vertical farming structure. Two factors comprising vertical spacing distance of growth beds and growth medium were tested in a split plot design and three replicates. Three levels of vertical distance, namely VS80, VS120, and VS160 were tested in the main plots while three levels of growth medium, namely GM00, GM40, and GM60 were tested in subplots. Monitored parameters included plant growth rate, stem diameter, flower counts, bean pods and tomato fruit counts, bean above-ground biomass, bean grain yields, and tomato fruit yields.

Key findings indicated that both bush beans and tomato yields were reduced on ground beds when compared to top bed yields. When the data from the two beds were considered as same sets of observations, the productivity response of the two crops were variable: Bush beans significantly yielded highest grains (480 g/m^2) under VS120 while tomatoes significantly yielded highest fruits (468 g/plant) under VS160. Also, bush beans grew better in GM40 while tomatoes did not achieve their potential even in GM60. When the sums of data collected from the two beds were considered, VS120 and GM40 generated the highest bean grain yields in the amounts 0.95 and 0.96 g/m^2, respectively. For tomatoes, the higher the level of each factor, the higher the fruit counts and fruit yields in actual values or statistically. The actual values of total fruit yields varied from 10.5 kg m^{-2}, through 14.4 kg m^{-2} out to 17.5 kg m^{-2} of land area unit. The high productivity of the vertical farming technology is clearly demonstrated by the above yields of the two crops (as weight/land area unit).

Yield projections based on the regression equations of yields on each factor indicated that bush bean maximum total yield would be achieved in a 30%-added manure growth medium while tomato plants might require up to 80%-added manure growth medium. For vertical spacing, projections for maximum bean yields could be achieved with 130 cm distance between the two superimposed growth beds. Tomatoes would possibly achieve their maximum production potential for a vertical spacing distance higher than the upper boundary of the present study for the factor.

Overall, vertical farming technology represents an opportunity for urban cities to significantly contribute to national food security. However, how the high productivity of the technology would compensate for the investment effort required to get it established is still lacking for the region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Influence of GA_3 on seed multiplication of CMS lines used for hybrid rice development

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In Pakistan, the F_1 seed production ranks very low in the three-line system. One of the reasons is poor panicle exertion and less out crossing rate in the female lines. Gibbrillic Acid (GA_3) increase the seed and improve panicle exertion as well as outcrossing. This study was designed to identify the suitable doses of GA_3 application in the seed multiplication block of different CMS lines. The material comprising both the parental lines (cytoplasmic male sterility and maintainer) used in the development of hybrid rice. GA_3 powder used in the experiment is 90% concentration in three level concentration that is 0, 100, 200, ppm. The outcomes indicated that GA_3 positively increased plant height, stigma exertion, panicle exertion, duration of floret opening, angle of floret opening and panicle length. Increased seed production is 0.3 to 1 t/ha compared to the control (0 ppm GA_3). Application of GA_3 concentration at the rate of 200ppm gave the best results as compared to the other treatments like control and 100 ppm in all the tested CMS lines increased productivity. So, 200 ppm concentration is recommended in seed multiplication of parental lines used in hybrid rice development.

Key words: Hybrid rice, CMS lines, GA_3, seed multiplication.

INTRODUCTION

Rice is a significant crop for food security everywhere in the world. Rice demands are very high since maximum of the people consumes rice. There are two ways to meet the rice needs: intensifying the rice planting area and increased production, or together. But in the upcoming time, expansion in area will be tougher and non-economical, significant advancement can be done over the implementation of hybrid rice (Nguyen, 2010). Amongst the several possible methods, hybrid rice cultivation is the most reasonable and practical one in estimation of its 10-15% yield benefit over the high yielding conventional varieties. The accomplishment in growing rice production through hybrid rice has been proven in China. The rise in production is 15-20% greater than the best commercial inbred rice, with a planting area grasped more than 50% of the total rice cultivated area (You et al., 2006). Hybrid rice has added meaningfully to food safety in China in the last 25 years. Rice is autogami plant so that the level of cross breeding is naturally low (Sheeba et al., 2006), so the hybrid rice is developed by male sterility system. The genetic systems known for developing rice hybrids are two type systems. First,

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cytoplasmic-genetic male sterility (CMS) for the development of three-line hybrid rice. Second, photo/ thermo-sensitive genetic male sterility (PGMS or TGMS) for developing two-line hybrid rice (Yuan et al., 2003).

In Pakistan, hybrid rice is developed by the three line systems. The drawback of F_1 system level for seed production is very low in this system. Poor panicle exertion, due to which about 25 to 30% panicle remained inside sheath of flag leaf and low outcrossing rate are also the main reasons of low seed production. Gibberellic Acid application is an effective means to increase seed production rate by coping the problems; it also enhances cell elongation. In hybrid rice seed production, GA_3 plays a vital role to obtain high seed yield. In China, application of Gibberellic acid (GA_3) at fairly high concentration (150-225 g/ha) plays a significant role in solving the problem of poor panicle exertion, better stigma exertion and stigma receptivity; besides enhancing wider glume opening, thereby increasing the outcrossing rate (Duan and Ma, 1992).

It can upsurge the rate of stigma exertion, increase the duration of floret opening, increase the rate of panicle exertion from the flag leaf sheath, modify plant height, and make the later taller and productive (Virmani and Sharma, 1993; Yuan et al., 2003; Viraktamath and Ilyas, 2005; Gavino et al., 2008). The behavior of GA_3 on development and growth of plants is determined by environment and varieties. In a new growing environment

of hybrid rice adoption in Sindh province of Pakistan, it is essential to recognize the suitable concentration of GA_3 for some hybrid rice varieties. Thus, it is required to conduct a research to study the impact/effect of GA_3 different concentration on some hybrid rice varieties. This research was expected to provide some information regarding appropriate GA_3 concentration levels to support the process of pollination between male sterile lines with maintainer, in Pakistan

MATERIALS AND METHODS

The experiment was conducted at Rice Research Institute, Kala Shah Kaku, during kharif seasons, 2016 and 2017, in randomized complete block design with 3 replications. The material comprise both the CMS lines and their maintainer lines. The GA_3 powder was used in the experiment with 90% concentration in three levels, that is 0, 100, 200 ppm. The age of seedling for transplanting was 25 days for ten CMS (A), named KSK and maintainer (B) lines. B lines or maintainer lines are morphologically similar to the A line expect fertility, due to which it produce the pollens to pollinate the A line. Both A lines and B-lines were sown on 3 days intervals. Then, the equal proportion of seedlings of B lines transplanted on two different dates were mixed equally before transplanting. Maintainer lines were transplanted in the pared rows with two/three seedlings per hill keeping 30x15 cm spacing and A line was 15x15 cm and space was 20 cm between A and B line, in a plot of 4x5 m. The row ratio of B:A was 2:6 used in the experiments. Each CMS lines along with maintainer were individually positioned out in the field. The polyethylene plastic was used in testing the area as barrier to isolate different CMS lines and other experimental material at 2.5 m height ,during the flowering stage confirm the F_1 seed purity. Every plot of CMS line was sprayed with GA_3 in two spraying, using knapsack sprayer. Initially sprayed, A lines were at 5-10% heading stage. Second time spraying was done three days after first time spraying. One, which remained untreated, was used as the control. The data were recorded on 5 randomly selected plants from each replication for quantitative characters. The characters studied were plant height (cm), productive tillers per plant, panicle length (cm), panicle exertions of male sterile lines (%), stigma exertions of male sterile line (%), seed set (%), angle of floret opening (°) and seed yield (kg/ha). The over-all reference for data assortment was by standard evaluation system for rice (SES) (IRRI, 2002). The data assembled was statistically investigated, using the analysis of variance (ANOVA) in Split Plot Design (parental lines were in main plot and sub plot was dosages of GA_3) to the test the significance for each trait. On the significance of results, the treatments were compared using 5% level of significance of Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1993).The list of plant material, including CMS lines along with their maintainer, is as shown in Table 1.

RESULTS AND DISCUSSION

The GA_3 concentrations significantly affect all studied parameters except the number of productive tillers per plant and 50% flowering age in the analysis of variance. The interface between CMS lines and GA_3 concentration revealed in panicle exerts plant height panicle length, stigma exertion, and seed set and seed yield (Biradarpatil and Shekhargouda, 2006; Tiwari et al., 2011; Susilawati et al., 2014). The panicle exertion is also affected by the application of GA_3 (Gavino, 2008; Rumanti, 2012; Susilawati et al., 2014). Plant height of the CMS lines increases with the increase in dose from 0-200 ppm. It increases on application of 100 ppm GA3, with range of 8-10 cm. GA_3 concentration of 200 ppm causes the increase in plant height from 7to 18 cm (Table 1). The rise in plant height was due owing to improved action of cells division, elongation and enlargement. Regulation of several processes of plant growth and development is censored by the Gibberline, which is a hormone important for cell elongation also (Hedden and Phillips, 2000; Sakamoto et al., 2004; Sun, 2004; Tiwari et al., 2011). Values with different letter(s) within a column differ significantly at 5% level probability (LSD).

Panicle length and panicle exertion influenced by the interaction between varieties with GA_3 spraying at 100, 200 ppm significantly improved the panicle length linked to controls on all CMS lines (Table 1). Impact on panicle elongation due to GA_3 spraying is from 12cm-18cm within application of GA_3, which rises panicle length due to elongation and cell division (Yuan et al., 2003; Tiwari et al., 2011). The point from where the panicle comes out from the flag leaf sheath to total panicle length in the panicle exertion is measured. All the parental lines tested in this experiment of GA_3 application showed significant increase in panicle exertion rate. On both treatments of 100 and 200 ppm, the values showed the GA_3 at 100 and 200 ppm, which shows the highest effect (87 to 89%) compared to controls treatment (74 to 79%). Yin et al.,

Table 1. List of Cytoplasmic CMS lines and their maintainer lines used in seed multiplication.

S/N	Name of A line and B lines	Code name
1	KSK1310A	A1
2	KSK1310B	B1
3	KSK1315A	A2
4	KSK1315B	B2
5	KSK1313A	A3
6	KSK1313B	B3
7	KSK1401A	A4
8	KSK1401B	B4
9	KSK1402A	A5
10	KSK1402B	B5
11	KSK1317A	A6
12	KSK1317B	B6
13	KSK1301A	A7
14	KSK1301B	B7
15	KSK1319A	A8
16	KSK1319B	B8
17	KSK1302A	A9
18	KSK1302B	B9
19	KSK1318A	A10
20	KSK1318B	B10

(2007) study has presented the influence of GA3 on panicle base elongation of CMS lines and the panicle inside flag leaf sheath was filled out, so the chances of grain filling increases in the rise of panicle exertion. In this research finding, the panicle exertion doses not reach 100% while the concentration of GA_3 application of 200 ppm on all spikelets emerged fully. For study one, CMS lines A8, A9, and A10 showed the maximum panicle exertion on 200 ppm, although the panicle is not fully exerted but all the spikelets were out of flag leaf sheath. However, in stiff panicles the percentage of panicle exertion got to 100%, but also lowered the yield of plant. The percentage of panicles exertion, 100%, can result in brittle panicle by wind and rain; thus, it will lower the yield, particularly in the rainy weather (Gavino et al., 2008). Improved panicle exertion positively increase seed set. Chances of outcross breeding also increased if the panicle exertion is increased by a CMS line/variety, ultimately seed set improved. Application of GA3 increased the panicle exertion by 20 to 30%, and rise in yield as much as 35 to 60% (Jagadeeswari et al., 1998). In this study, increase in seed set due to GA3 spraying ranged from 5 to 40% (Table 2).

The angle of floret opening is significantly affected by spraying of GA_3 application as linked to the zero application (control) of GA_3 in spraying. The proper angel of floret opening is also helpful in more seed setting due to cross pollination, ultimately the seed setting will be high. Proceeding exhibited that high degree of cross-pollination in hybrid rice is affected by the angle of floret opening, large stigma surface and long duration of floret opening (Singh and Shirisha, 2003; Biradarpatil and Shekhargouda, 2006; Gavino et al., 2008; Susilawati et al., 2014), save for the lengthier pollination activities.

Values with dissimilar letter(s) within a row vary significantly at 5% level of probability (LSD). Seed yield indicated that all CMS tested for seed multiplication by application of GA_3 resulted in higher productivity as compared to control treatments values (Table 3).The GA_3 application with the doses of 100 and 200 ppm gave the increased yield. In previous studies, it is shown that the treatment of 200 ppm gave the highest seed yield as compared to 300ppm, because by application of 300 ppm the plant height of cms lines increased panicle stalk broke in rainy season and faced yield loss due to wind (Yuan 1985; Prasad et al., 1988; Gavino et al., 2008; Susilawati et al., 2014). Consequently, application of GA_3 must be improved with responsiveness of cm line, seasons and agro-ecological conditions and.

Conclusion

Treatment using two dissimilar GA3 concentration positively increase plant height, panicle length, panicle exertion, angle of floret opening and enhanced some growth and flowering traits which are involved the rise in seed set/seed production of CMS lines by 0.3 to 1.2 t/ha.

Table 2. Agronomic traits of ten male sterile lines in several different applications of GA3 Concentration (Pooled data of two rice crops/seasons).

Parameter	Concentration of GA3 (ppm)			
	Plant Height			
	0	100	200	Average
A Lines				
A1	97	110	127	111
A2	92	105	121	106
A3	93	108	115	104
A4	105	115	132	117
A5	102	111	123	112
A6	75	87	105	89
A7	88	98	117	101
A8	100	104	125	110
A9	101	112	125	113
A10	95	105	115	
Average	**95^c**	**105^b**	**110^a**	
Number of productive tillers/plant				
A1	9	10	11	10
A2	13	12	10	12
A3	13	15	17	15
A4	17	16	16	16
A5	9	8	12	10
A6	15	13	12	13
A7	12	14	16	14
A8	16	14	17	16
A9	18	17	15	17
A10	13	11	17	14
Average	**13**	**13**	**14**	
Panicle Length (cm)				
A1	23	24	25	24
A2	28	29	30	29
A3	30	32	33	32
A4	24	26	27	26
A5	22	24	25	24
A6	19	23	25	22
A7	22	24	25	24
A8	26	28	29	28
A9	26	27	28	27
A10	22	23	24	23
Average	**24^c**	**26^b**	**27.1^a**	
Flowering age 50%				
A1	79	79	79	79
A2	75	75	75	75
A3	78	78	78	78
A4	75	75	75	75
A5	76	76	76	76
A6	71	71	71	71
A7	72	72	72	72

Table 2. Contd.

A8	76	76	76	76
A9	75	75	75	75
A10	73	73	73	73
Average	**75**	**75**	**75**	

Table 3. Panicle exertion, angle of floret opening, seed set, and seed yield of four male sterile lines in several different applications of GA3 concentration (Pooled data of two rice crops/seasons).

Parameter	**Concentration of GA3 (ppm)**			
	Panicle exertion			
	0 ppm	**100 ppm**	**200 ppm**	**Average**
CMS line				
A1	75	82	84	80
A2	74	82	85	80
A3	79	84	86	83
A4	76	85	85	83
A5	71	83	84	80
A6	75	87	83	82
A7	76	85	86	82
A8	79	86	88	85
A9	77	85	87	83
A10	75	84	89	83
Average	75.7	84.3	86.1	
Angle of floret opening				
A1	22.5	26.4	28.4	26
A2	24.7	28.2	30.1	28
A3	23.3	27.1	29.2	27
A4	22.4	26.2	27.8	25
A5	23.1	27.4	29.5	27
A6	24.3	28.5	30.7	28
A7	25.2	29.2	31.1	29
A8	23.5	26.7	28.7	26
A9	24.4	27.7	29.4	27
A10	22.5	26.5	28.7	26
Average	23.59	27.39	29.36	
Seed set %				
A1	17	25	26	23
A2	13	23	25	20
A3	16	32	34	27
A4	20	30	34	28
A5	19	21	23	21
A6	22	28	30	27
A7	24	31	35	30
A8	21	31	34	29
A9	23	31	33	29
A10	23	28	32	28
Average	20	28	31	

Table 3. Contd.

Seed Yield kg/ha				
A1	750	1120	1433	1434
A2	325	520	820	588
A3	640	841	1140	1040
A4	870	1015	1431	1472
A5	810	912	1550	1291
A6	590	740	914	650
A7	690	830	1017	1320
A8	810	1012	1387	1379
A9	780	920	1360	1110
A10	810	1017	1623	1217
Average	777.5^{c}	962.7^{b}	1710.4^{a}	

Application of GA_3 concentration at the rate of 200 ppm gives the best results as compared to other treatments like control and 100 ppm, in all the tested CMS lines productivity increased. Thus, concentration of 200 ppm is recommended in seed multiplication of parental lines for hybrid rice development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Anatomical adaptations in species of Poaceae growing in Al-Ha'ir region of Riyadh, Saudi Arabia

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The aim of this work was to determine the anatomical adaptations of leaves and stems of four species of *Poaceae* (*Cynodon dactylon* L. Pers., *Chloris barbata* SW. (Chloridoideae), *Setaria verticillata* L. P. Beauv., and *Panicum coloratum* L. (Panicoideae)) growing naturally at Al-Ha'ir region near Riyadh, Saudi Arabia. Cross-sections of the leaves revealed that the epidermis cells were spherical to oval and formed one layer with a thick cuticle as well as numerous bulliform cells in a fan shape and prickles. Ground tissue (mesophyll) consists mostly of chlorenchyma cells. A large vascular bundle surrounded by two bundle sheaths, outer sheath parenchyma and inner sheath sclerenchyma were observed in *C. barbata* and *C. dactylon,* while one bundle sheath of sclerenchyma surrounds the vascular bundle in *S. verticillata* and *P. coloratum*, while one bundle sheath with chlorenchymatous cells surrounded the small vascular bundles. Our results showed that all species contained a Kranz anatomy indicative of a C4 photosynthetic pathway despite belonging to two different subfamilies, *Panicoideae* and *Chloridoideae*. Oil droplets appeared in the mesophyll tissue of *P. coloratum* and *S. verticillata*. Cross-sections of stems revealed an epidermis which consists of one layer of cells with spherical to oval shape and had a thick cuticle. Ground tissue contains strands of chlorenchyma cells followed by sclerenchyma tissue surrounding vascular bundles, thereby making a continuous cylinder. The vascular bundles were scattered in the ground tissue, with each vascular bundle surrounded by a single sclerenchymatous bundle sheath. Our results indicate that these plants were characterized by anatomical adaptations that enhance drought-tolerance capabilities, facilitating survival in arid and semi-arid regions such as Al-Ha'ir and thus these plants can be used to increase vegetation cover and pasture area in dry environments.

Key words: Anatomy, adaptations, Poaceae, *Cynodon dactylon*, *Chloris barbata*, *Setaria verticillata*, *Panicum coloratum*.

INTRODUCTION

The *Poaceae* family comprises a large number of economically important crops such as Triticum and Hordeum (Al-War'a et al., 1997), and several saline-tolerant species, including *Cynodon dactylon* (Poljakoff-

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Mayber, 1988) In addition, several plants in the *Poaceae* have important medicinal and aromatic benefits; for example, dried *C. dactylon* leaves are often used in anabolic and antiseptic substances (Gangwar et al., 2011), and the cereals *S. italica* (L.) P. Beauv is used to treat chicken pox in some areas of Pakistan (Ahmad et al., 2011). Moreover, *C. barbata* has been shown to have antibacterial activity (Natrajan et al., 2012). Several terrestrial plant species of family Poaceae were studied from the anatomical point of view (Kellogg, 2015; Panizzo et al., 2017). The plant species in our study were *C. dactylon* L. Pers. and *Chloris barbata* SW that belong to the *Chloridoideae* subfamily, while *P. coloratum* L and *S. verticillata* P. Beauv were part of the *Panicoideae* subfamily of the *Poaceae* family. These plants were considered permanent herbs, with the exception of *S. verticillata*, grow wild in Al-Ha'ir, an area south of Riyadh city in Saudi Arabia. However, the morphological characteristics of these species can vary significantly. Peng et al. (2017) studied the anatomical feature of swamp plant species that reflects the adaptation of plants to different environmental variables paralleled with morphological, eco-physiological and growth responses. Many studies have focused on the anatomical aspects of the *Poaceae*, such as Shaheen et al. (2012), who observed differences in leaf epidermal characteristics among six *Panicum* spp. native to Pakistan; these included variations in the shape of the phytoliths, prickles, large and small trichomes, and stomata, factors that were often used as important taxonomic indicators at the genus level. Ahmad et al. (2011) in a study of leaf epidermal characteristics of five plant species in Pakistan, including *C. barbata* and *C. dactylon*, found that differences in stomatal apparatus, subsidiary cells shape, the long epidermal cells, and silica cells were useful for distinguishing among species. Hameed et al. (2010) examined the anatomical adaptations of *C. dactylon* collected from saline and non-saline environments in Pakistan, reported that adaptations in plants growing in saline environments included saline excretions on the plant surface, accumulation of ions in the parenchyma tissue, reductions in stomata size on the leaf upper epidermis, increased bundle sheathing, and increased leaf bulliform cells size. Leaves epidermal characteristics of 13 species within 10 genera of Poaceae were studied by Nazir et al. (2013). They characterized the plants that grow in coastal zones by the presence of rows of silica bodies, which differed in shape and size among the plant species, silica bodies were cross- or dumb-bell-shaped or intermediate between the two and accompanied by cork cells.

Vanesa and Cambi (2010) distinguished the occurrence of parenchyma tissue in the midrib region and prickles in leaves of *Trichloris crinita*, and further noted a set of sub-epidermal fibers alternating with chlorenchyma tissue, whereas in *Pappophorum phillippianum* and *T. crinita*, sclerenchyma cells were ring-shaped around vascular bundles. Eltahir and Abuereish (2010) reported the occurrence of small oil droplets in epidermal cells of leaf and mesophyll of *Cymbopogon schoenanthus* and *Cymbopogon citratus*. Carvalho Santos et al. (2013) evaluated variations in anatomical characteristics of 38 different genotypes of *Brachiaria ruziziensis* belong to *Poaceae* to identify genotypes that could be useful in plant breeding. Photosynthesis in C_4 plants was more active under environments that stimulate carbon loss through photorespiration, for example high light intensity and temperatures and reduced water obtainability in the case of stomatal closing (Carmo-Silva et al., 2009). The results of previous studies have shown that plants and grasses of the *Poaceae* were globally ubiquitous and often have great economic, medical and pastoral importance. Research has revealed numerous anatomical modifications among plants, which has greatly improved our understanding of how plants were able to overcome unsuitable environmental conditions and increase their growth and productivity. Our objective here was to determine the anatomical modifications of several of the plant species growing in Al-Ha'ir that contribute to their tolerance to the arid conditions of this part of Saudi Arabia. Improving our understanding of these adaptations may assist in enhancing vegetation cover in desert areas.

MATERIALS AND METHODS

The four plant species we focused on were members of the Poaceae, which grow naturally in Saudi Arabiathese species, consists of *C. dactylon* (L). Pers., *C. barbata* SW. (Chloridoideae), *S. verticillata* (L). P. Beauv. and *P. coloratum* L. (Panicoideae). Plant samples were collected at the flowering stage in the Al-Ha'ir region near Riyadh in the spring of 2017 and identified by botanists in the Herbarium, Department of Botany and Microbiology, King Saud University (KSUH). All samples were kept in 70% ethyl alcohol. Permanent sections were prepared in paraffin wax following the procedures described by Al-Khazraji and Aziz (1989) and Doaigey et al. (1997) for studying the internal anatomical characteristics of leaves and stems of the samples. Sections were examined and photographed with a TK-C1381EG video camera Japan, attached to an Olympus BX4LTF light microscope, Japan.

RESULTS

Leaf anatomical characteristics

Cross-section of the blades of *C. dactylon*, *C. berbata*, *S. verticillate* and *P. coloratum* leaves showed that the leaves were V-shaped, and the vascular bundles appeared in a single row parallel to the lower and upper surfaces of the leaf.

Coastal regions

Upper epidermis

In leaves of all studied species, the upper epidermis

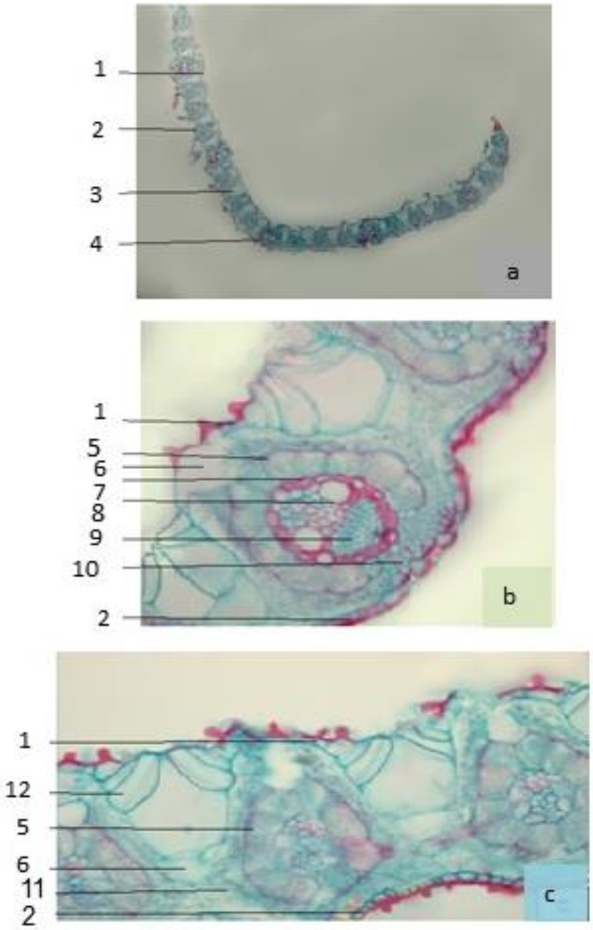

Figure 1. Cross sections of the *C. dactylon* (L.) Pers. (leaf). a-Blade shape 50×, b-Coastal region 400×, c-Intercostal region 400×, 1-Upper epidermis, 2-Lower epidermis, 3-Intercoastal region, 4-Coastal region, 5-Parenchyma shealth, 6-Aerenchyma, 7-Sclerenchyma shealth, 8-Xylem, 9-Phloem, 10-Sclerenchyma cells, 11-Chlorenchyma tissue and 12-Bulliform cells.

consists of one layer of cells spherical to oval shaped, with thick outer walls, thin internal walls and protected with a thick cuticle. The epidermis cells in cross sections of *C. dactylon* appeared to be papillary shaped (Figure 1b), and leaves of *C*. *barbata* contain many prickles (Figures 3a and 3b). In *S. verticillata* leaves, a thick cuticle covered the epidermis that contains bulliform cells (Figure 5a), whereas epidermis cells in leaves of *P. coloratum* were spherical to palisade-shaped, contained numerous large bulliform cells, and covered by a thick cuticle (Figures 7b and 7c).

Ground tissue

In *C*. *dactylon* ground tissue consists of an aerenchyma followed by 2 to 3 rows of chlorenchyma cells, filled with chloroplasts and surrounded by thin cellulose walls (Figure 1b). The ground tissue in *C*. *barbata*, consists of 4 to 7 irregular- and large-sized parenchyma cells with thin cellulose walls free of chloroplasts, followed by 2 to 3 layers of chlorenchyma cells Containing chloroplasts (Figure 3a). Ground tissue in *S. verticillata* consists of 2 to 10 layers of irregularly-shaped parenchyma cells with thin cellulose walls free of chloroplasts followed by 2 to 3 layers of chlorenchyma cells filled with chloroplasts (Figure 5a). Finally, in *P. coloratum*, the ground tissue consists of 4 to 6 layers of irregularly shaped parenchyma cells, free of chloroplasts, with thin cellulose walls followed by 2 to 3 layers of chlorenchyma cells filled with chloroplasts (Figures 7a and 7b).

Vascular tissue

Vascular tissue in the leaves of these species consists of oval-shaped, closed collateral bundles in oval shape. The bundle consists of xylem that is oriented towards the upper epidermis in an approximately V-shape and contains large cavities and thick lignin walls in vessels, with an inverted metaxylem occupying the V-shape head. The phloem, containing companion cells and sieve tubes, was located near the lower epidermis. Large vascular bundles were surrounded by two layers of cells that define the perimeters of the bundle sheaths; its outer layer contained large parenchymal cells, containing some chloroplasts, and surrounded by cellulose thin walls. However, the inner layer was composed of small lignified thick-walled sclerenchyma cells. In the leaves of all the four plants a bundle sheath of parenchyma cells containing chloroplasts were surrounded the small vascular bundles (Figures 1b, 3b, 5a and 7b). Large vascular bundles were connected to the lower epidermis via a group of sclerenchyma cells with lignified walls; sclerenchyma cells in 3 to 5 layers were presented in *C. dactylon* and *P. coloratum* (Figure 7a), 2 to 4 layers occur in *S. verticillata* (Figure 3b) and 5 to 7 layers were found in *C. barbata* (Figure 5a).

Lower epidermis

It was similar in appearance to the upper epidermis in leaves of all four plants, except that prickles were absent in the leaf epidermis in C*. barbata* (Figure 3b).

Intercostal region

Ground tissue

Ground tissues in all plants had 3 to 4 layers of chlorenchyma cells, filled with chloroplasts, and surrounded by thin cellulose walls. The results showed that C. *dactylon* (Figure 1c) was different compared to *C.*

barbata (Figure 3c) in the presence of aerenchyma, whereas *S. verticillata* (Figure 5d) and *P. coloratum* (Figure 7d) were dissimilar in the occurrence of oil droplets in some cells.

Lower epidermis

It was similar to the upper epidermis of the four plants under study in its Characteristics, except the absence of bulliform cells and a thick cuticle in *C. dactylon* (Figure 1c), *C. barbata* (Figure 3c), and *S. verticillata* (Figure 5c). The lower epidermis in ground tissue of *P. coloratum* consisted of a single layer of spherical-to palisade-shaped cells, with thick outer walls covered by a thin cuticle and thin internal walls (Figure 7c).

Stem anatomical features

Cross-sectioned stems were circular in both *C. dactylon* (Figure 2a) and *C. barbata* (Figure 4a), but heart-shaped in *S. verticillata* (Figure 6a) and *P. coloratum* (Figure 8a).

Epidermis

Stem epidermis was composed of a single layer of spherically- to elliptically-shaped cells with thick outer and thin internal walls. Outer walls were covered by a thick cuticle in *C. dactylon* (Figure 2a) and *C. barbata* (Figures 4a and b), but the cuticle was thin in *S. verticillata* (Figure 6a) and *P. coloratum* (Figure 8a).

Ground tissue

For *C. dactylon*, the results showed that ground tissue (chlorenchyma tissue) consists of 2 to 3 layers of thin-walled parenchyma cells containing chloroplasts followed by 3 to 5 layers of sclerenchyma cells in a cylinder shape, which was connected to the external vascular bundle sheath. The remaining ground tissue consists of parenchyma cells free of chloroplasts and with thin cellulose walls, with cell size increasing towards the center of the pith and containing large intercellular spaces (Figures 2a and 2b). Ground tissue in *C. barbata* was composed of 3 to 4 layers of chlorenchyma cells with thin cellulose walls, and containing chloroplasts. Clusters of 3 to 4 layers of sclerenchyma cells with lignin walls were connected to the epidermis, and were followed by 3 to 4 layers of sclerenchyma cells that form a cylinder shape. The sclerenchyma cylinder connects to the sheath of the vascular bundle. The remaining ground tissue consists of thin-walled parenchyma cells free of chloroplasts, with cell size increasing towards the center of the pith, and with clear intercellular spaces (Figures 4a and 4b). In *S. verticillata*, ground tissue consisted of 4 to 5 layers of small parenchyma cells with thin cellulosic walls, which represent the initial ground-tissue layers, along with 3 to 4 layers of sclerenchyma cells with lignified walls, which were connected to the vascular bundles. The ground tissue towards the pith center (Figure 6a and 6b) was similar to that in *C. barbata*; however, crystalline sand was also found in the parenchyma cells (Figure 6c). Finally, in *P. coloratum*, the ground tissue consisted of 4 to 5 layers of chlorenchyma cells with thin cellulose walls containing chloroplasts, along with 4 to 5 layers of sclerenchyma cells that form a complete cylinder, which connect to the sheaths around the vascular bundles. The remaining ground tissue in this species consists of parenchyma cells free of chloroplasts with thin cellulose walls, with cell size increasing towards the center of the pith and containing small intercellular spaces (Figure 8b).

Vascular tissue

Vascular bundles were scattered throughout the ground tissue in all four species, and the stem center was free of vascular bundles, except in *P. coloratum*. The vascular bundles form closed collateral and were oval shaped. Vascular bundles consist of xylem and phloem, with the primary xylem including metaxylem vessels, the latter was located at the head of the V-shape, and vessels with thick lignin walls and large cavities, and protoxylem vessels were replaced by air spaces or protoxylem cavities. The phloem was located toward the outer side and contained companion cells and sieve tubes. The vascular bundles were distributed throughout the primary tissue in all four plant species and were surrounded by sclerenchyma cells in one layer (that is, a bundle sheath; Figures 2a and b; Figures 4a and b; Figures 6a and b and Figures 8a and b, respectively).

DISCUSSION

Our examination revealed several of the internal anatomical adaptations of the leaves and stems associated with higher drought tolerance in *C. dactylon* (L). Pers., *C. barbata* SW, *S. verticillata* (L). P. Beauv. and *P. coloratum* L (Figures 1 to 8). As shown in Figures 1, 3, 5, and 7, the leaf blade was narrow and V-shaped in *C. dactylon* and *C. barbata* (Figure 1a and Figure 3a), but V-shaped and divergent in *S. verticillata* and*, P. coloratum* (Figure 5a and Figure 7a). Our results accord with those of several previous studies in which it was suggested that the U-shaped (Metcalfe, 1960) or V-shaped (Doğan and Tosunoğlu, 1992; Mavİ et al., 2011) leaf blades of members of the *Poaceae* were an adaption to reduce the leaf-surface exposure to light, thus reducing water loss; moreover, the flat blade may facilitate absorption of vital minerals at times when water was plentiful.

Our analyses of leaf cross-sections (Figures 1, 3, 5,

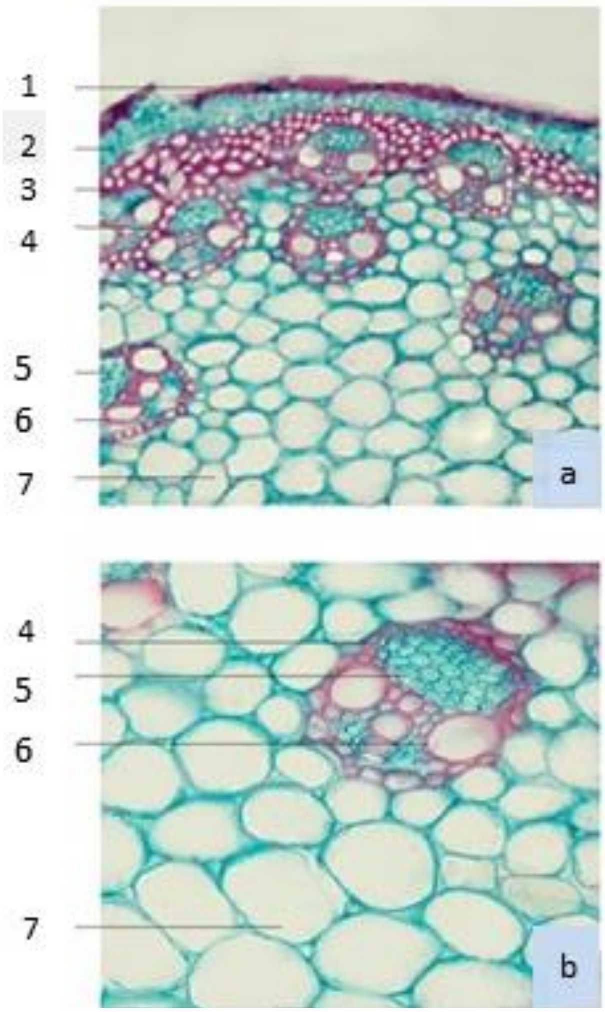

Figure 2. Cross sections of *C. dactylon* (L.) Pers. (Stem). a-200×, b-400×. 1-Epidermis, 2-Chlorenchyma tissue, 3-Sclerenchyma cells, 4-Schlerenchyma shealth, 5-Phloem, 6-Xylem and 7-Parenchyma cells

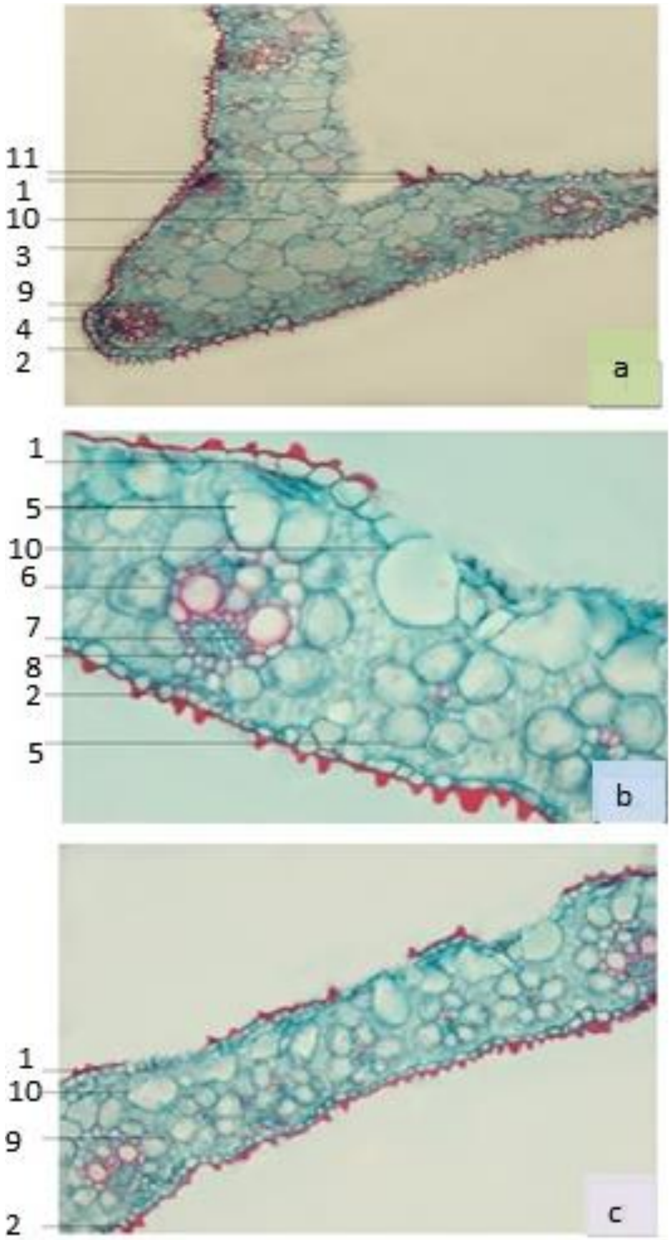

Figure 3. Cross sections of *C. barbata* SW. (leaf). a-Blade shape in leaf and coastal regions 200×, b-Vascular bundles in coastal regions 400×, c-Intercoastal region 200×, 1-Upper epidermis, 2-Lower epidermis, 3-Intercoastal region, 4-Coastal region, 5-Parenchyma shealth, 6-Schlerenchyma shealth, 7-Xylem, 8-Phloem, 9-Chlorenchyma tissue, 10-Bulliform cells and 11-Prickles.

and 7) indicate that the epidermis was covered with a thick cutin cuticle, which aids in water retention. The presence of stomata on both epidermal leaf surfaces, as well as the large intercellular spaces in leaf mesophyll tissue, facilitates gas exchange. Thin-walled bulliform cells with large vacuoles were plentiful in the upper epidermis above the prominent midrib region, and play a role in the folding or shrinking of the leaves when plants were under water stress, which reduces the amount of exposed leaf surface area and lowers rates of transpiration and light absorption. These results were consistent with the reports of Carmo-Silva et al. (2009), Gangwar et al. (2011) and Grigore and Toma (2017). In addition, the presence of different forms of silica cells, cork cells with suberized walls, and sclerenchyma cells in bundles that link with the epidermis via the large vascular bundles, were all traits that increase the strength and rigidity of the plants and provide protection against grazing herbivores.

The internal anatomical characteristics of the main vein leaves were very similar in *C. dactylon* (Figure 1a) and *C. barbata* (Figure 3a) and in both species ground tissues were composed of parenchyma and chlorenchyma cells. Vascular tissues consist of closed collateral vascular

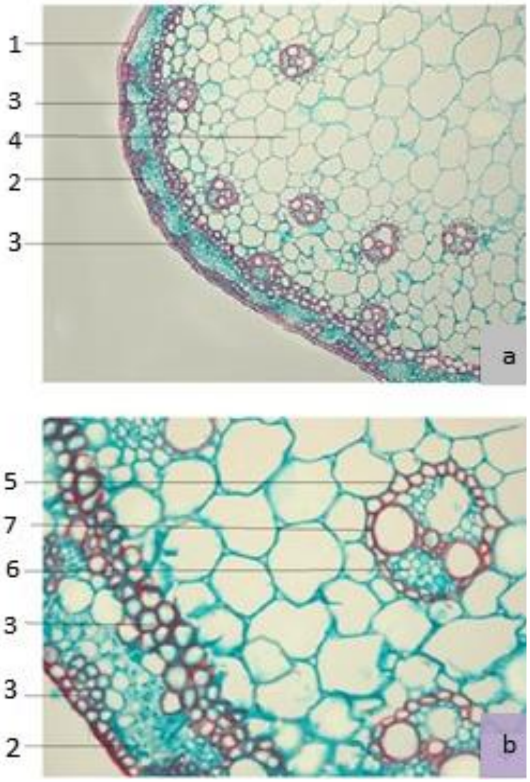

Figure 4. Cross sections in *C. barbata* SW. (Stem). a-100×, b-400×. 1-Epidermis, 2-Chlorenchyma tissue, 3-Sclerenchyma cells, 4-Parenchyma cells, 5-Sclerenchyma shealth, 6-Phloem, 7-Xylem.

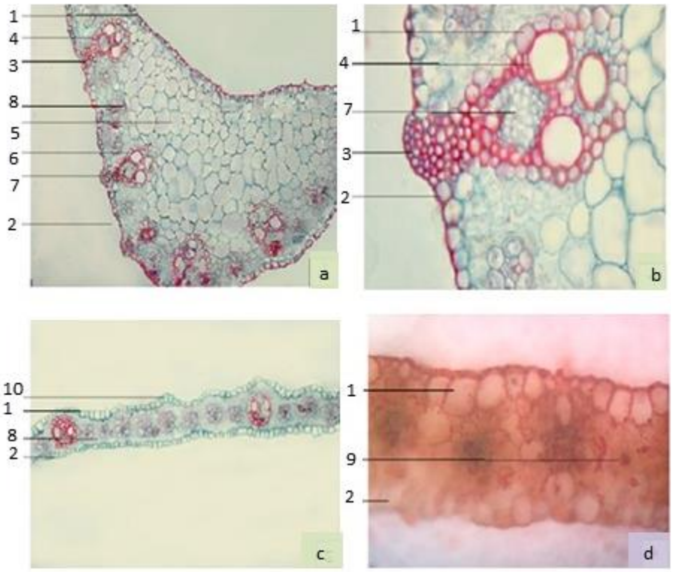

Figure 5. Cross sections in *S. verticillata* (L.) P. Beauv. (leaf). a-Blade shape in leaf and coastal regions 100×, b-Vascular bundles in coastal regions 400×, c-Intercoastal region 100×, d-Oil droplets in Intercoastal region 400×. 1-Upper epidermis, 2-Lower epidermis, 3-Sclerenchyma cells, 4-Sclerenchyma shealth, 5-Parenchyma cells, 6-Xylem, 7-Phloem, 8-Chlorenchyma tissue, 9-Oil drops, 10-Bulliform cells

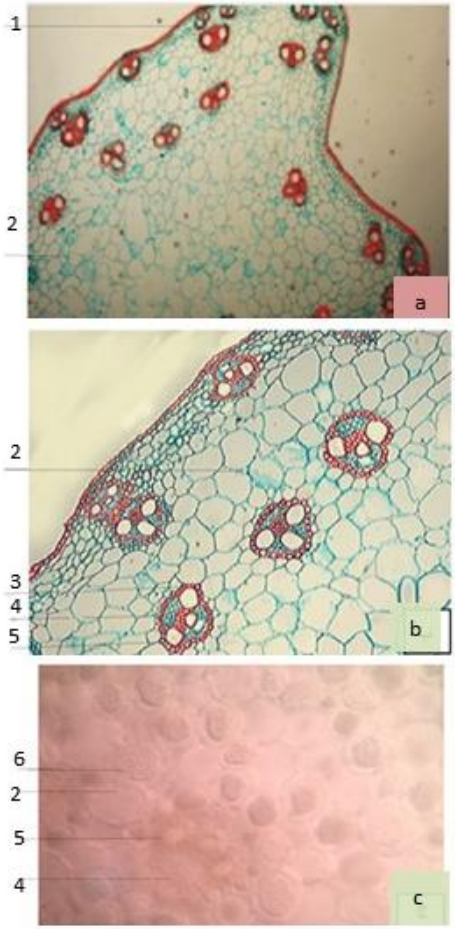

Figure 6. Cross sections of *S. verticullata* (L.) P. Beauv. (Stem). a, b-Parts of cross sections in stem, (a-50×, b-100×), c-Crystal stand in stem parenchyma tissue 200×. 1-Epidermis, 2-Parenchyma cells, 3-Sclerenchyma shealth, 4-Phloem, 5-Xylem and 6-Crystal sand.

bundles in an oval shape, with each bundle surrounded by two bundle sheaths composed of radially-shaped cells (chlorenchyma outside and sclerenchyma inside), whereas in *S. verticillata* (Figures 5a and b) and *P. coloratum* (Figures 7a and b), each vascular bundle was surrounded by a single bundle sheath composed of sclerenchyma cells and partially surrounded by chlorenchyma cells. In all four species, lateral vascular bundles were surrounded by a single bundle sheath of parenchyma cells, even though the four species belong to different genera and sub-families (*Chloridoideae* and *Panicoideae*).

Leaf blades contain a large number of vascular bundles, reflecting a greater ability to absorb water (Carmo-Silva et al., 2009), moreover, we observed oil droplets in the ground tissues of *P. coloratum* (Figure 5d) and *S. verticillata* (Figure 7d) leaves. Such oil droplets, which reduce water loss and can serve as an energy source

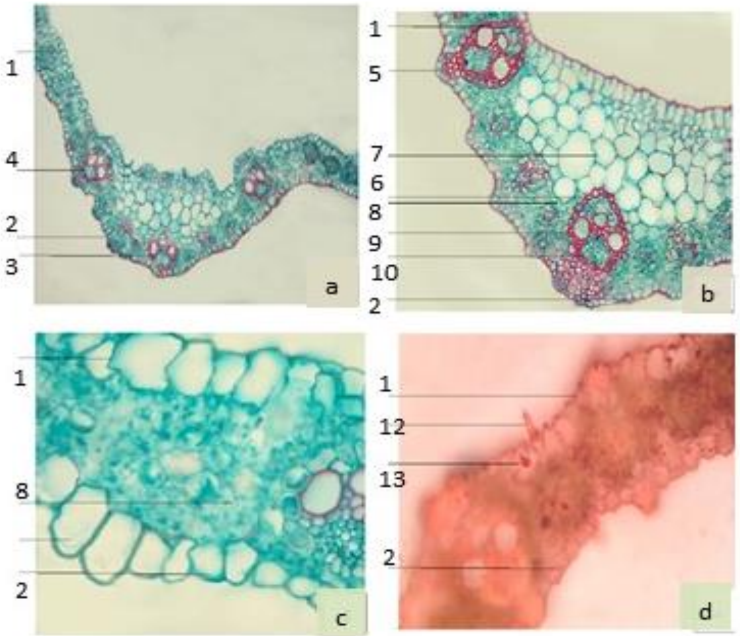

Figure 7. Cross sections in *P. coloratum* L. (leaf). a-Blade shape of leaf and coastal regions 100×, b-Coastal regions 200×, c-Intercoastal region 400×, d-Oil drops in an intercostal region 400×, 1-Upper epidermis, 2-Lower epidermis, 3-Coastal regions, 4-Intercostal region, 5-Sclerenchyma cells, 6-Sclerenchyma shealth, 7-Parenchyma cells, 8-Chlorenchyma tissue, 9-Xylem, 10-Phloem, 11-Bulliform cells, 12-Prickles and 13-Oil droplets.

(Eltahir and Abuereish, 2010), have been found in other epidermal cells of Poaceae species. Analysis of the stem cross-sections indicated (Figures 2, 4, 6, and 8) the presence of thick epidermal cellular walls due to an abundance of sclerenchyma tissue, which assists in the regulation of water loss, along with the presence of parenchyma cells that make up the ground tissue, which increases water storage capacities. We observed an abundance of vascular bundles in the ground tissues and metaxylem elements, which serve to enhance transport efficiency and provide maximal amounts of water and minerals to the vegetative parts of the plant for the proper functioning of photosynthesis and other biological processes. Moreover, the presence of many sieve tube elements in the phloem also improves nutrient transport (Hameed et al., 2010) and storage.

The results of our analysis indicate that the internal structures of the leaves and stems of these four plants exhibit a variety of adaptations that enhance drought tolerate capacity. These anatomical adaptations allow these species to thrive in the arid and semi-arid regions of Saudi Arabia, and can be used to increase vegetation cover and increase pasture area.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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