



## Morphological Characterization of Four *Pandanus* Species of Different Genetic Background

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### Abstract

The present study was carried out in the lab. of plant tissue culture at King Abdulaziz University to investigate morphological characterization of four *Pandanus* species of different genetic background. The used *Pandanus* species were *Pandanus odoratissimus* naturally grown in different regions of Saudi Arabia and *Pandanus dubius*, *Pandanus tectorius* var 'Variegata' and *Pandanus tectorius* var 'Utilis' collected from the agriculture market of Jeddah, Saudi Arabia. About 15 seedling of each species were organized in Randomized Complete Block (CRD) using 5 replicates. Morphological characterization was carried out using leaves of different ages of *Pandanus* species. The results showed that *Pandanus tectorius* var 'Variegata' revealed highest leaf breadth, leaf thickness and leaf contents of P (%). *Pandanus odoratissimus* illustrated highest *Ch a* and *Ch b* contents (mg/100g). *Pandanus dubius* recorded highest leaf length (cm). leaves of medium ages of *Pandanus* (leaves found in the middle layers of plant crown) revealed highest values of leaf length (cm), leaf breadth (cm), leaf thickness (mm), leaf fresh and dry weight (g), and *Ch a* and *Ch b* (mg/100g). The new young leaves (found in the first internal layers of plant crown) revealed highest contents of N (%) and P (%). Length, breadth and thickness of the middle leaves were successfully utilized for morphological identification of *Pandanus odoratissimus*, *Pandanus dubius*, *Pandanus tectorius* var 'Variegata' and *Pandanus tectorius* var 'Utilis'.

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## Introduction

Kewda (*Pandanus* spp.) is native to south peninsular and south-east Asia. It is widely distributed both in east and west coasts of India that include states of Orissa, Andhra Pradesh, Tamil Nadu, Kerala and Gujarat. In Saudi Arabia, one genus of 'kewda' (*Pandanus odoratissimus*) is reported to be found and broadly distributed in the southern regions particularly Jazan governorate. However other species of the genus *Pandanus* (*P. odoratissimus*, *P. tectorius*, *P. tectorius* 'Variegata' and *P. dubius*) were observed (Stone, 1983; Lim, 2012; Al-Ghamdi *et al.*, 2013). Botanically the plants are dioecious and the unisexual male and female flowers take place on separate individuals. The plants have simple or branched stems which frequently supported by adventitious roots. The plant Leaves are narrow, sheathing at the base. The female flowers take the pineapple shape and having no fragrance. Male plants form about 30 branches and flower annually. The male flowers are highly fragrant, small, white, and arranged in clusters with large white bracts. Male flowers are with stamens that are usually many, hypogynous, spicate or umbellate on the axis; anthers are 2-celled with longitudinal dehiscence (Thomson *et al.*, 2006). The male flowers are used to isolate perfumed oil which containing about 60% -80% of 2-phenylethyl methyl ether ( $\beta$ -phenylethyl methyl ether). Also, the oil is containing minor components as free alcohol, 2-phenylethanol ( $\beta$ -phenylethyl alcohol), acetic acid ester, terpinene-4-ol (up to 15%),  $\alpha$ -terpineol and  $\gamma$ -terpinene (Yahya *et al.*, 2010; Yahya *et al.*, 2011).

The pandanus male plants have great economical importance due to its male inflorescence (spadix) that used in the perfume industry and natural food additive for flavoring. Medicinally *Pandanus* extracts from anthers, flowers, fruits and roots used individually or in combination with other ingredients to treat a wide range of human diseases (Burri *et al.*, 1989; Thomson *et al.*, 2006; Peng *et al.*, 2010; Tan *et al.* 2010). The *pandanus* genera and species are morphological characterized and identified by its pistillate flowers and 8 subgenera and 86 sections were recognized (Huynh, 1982; Laivao *et al.*, 2006; Buerki *et al.*, 2012; Callmander *et al.*, 2012).

However, the taxonomy of *Pandanus* still presents many challenges regarding relationships among and within the genera are not well understood. Additionally, species identification is often problematic, even for experienced botanists. The time has now come to incorporate evidence from molecular inferences and biogeography in an effort to refine generic and infra-generic delimitations, especially within *Pandanus*. The present study aims to morphological characterizations of four species of the genus *Pandanus* of the family Pandanaceae that can be found in Saudi Arabia.

## Materials and methods

### Plant materials

This study was conducted at the lab of plant tissue culture, Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdul Aziz University. The presented research was designed to study morphological characterizations between four different Kewda (*Pandanus* spp.) populations. Seedlings of four Kewda species were used. The local specie '*Pandanus odoratissimus*' was considered to be naturally grown in different regions of Saudi Arabia. Three species, *Pandanus tectorius* 'Variegata', *Pandanus tectorius* var 'Utilis' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia. A total of 15 seedlings from each species were used for morphological characterizations.

### Morphological characterization of Kewda (*Pandanus* spp)

Morphological characteristics between Kewda (*Pandanus* spp) species were investigated. Seedlings of the local Kewda species (*Pandanus odoratissimus*) were collected from three different valleys of South-Western regions of Saudi Arabia. Seedling of *Pandanus tectorius* 'Variegata', *Pandanus tectorius* var 'Utilis' and *Pandanus dubius* were obtained from agriculture market of Jeddah province, Saudi Arabia. Fifteen seedlings from each *Pandanus* species were used.

### Statistical design

The experiment was carried out in the plant of plant propagation, Department of Arid land Agriculture, King Abdulaziz University. The experiment was laid out in Completely Randomized Block (CRD) using 5 replicates.

### Assessed parameters

The following parameters were used to indicate morphological differences between the collected *Pandanus* species:

### Leaves parameters

Average leaf length (cm), average leaf breadth (mm), average leaf thickness (mm), average stem diameter (cm), leaves fresh weight (g) and leaves dry weight (g)

### Leaves contents of N %, P % and K%.

Total nitrogen in leaves and fruit flesh was determined according to the Kjeldahl method (Jackson, M. L. 1973) using Kjeltex auto 1030 analyzer. Total P was measured calorimetrically after digestion with perchloric- nitric method (Shelton, W. R., and H. J. Harper. 1941) Total K was measured using flame emission spectrophotometer (Lachica *et al.*, 1973).

### Leaves contents of Chlorophyll a and b

The quantity of *Chlorophylls a* and *b* were measured using 80% acetone solution and the extract samples were measured at two different wavelength 644nm and 663nm using UV- spectrophotometer (Metzner *et al.* (1965).

The following formula was applied on the recorded data to calculate the Chlorophyll a and b:

$$Chl a = \frac{(10.3 \times E663) - (0.918 \times E644)}{1000 \times W} \times V$$

$$Chl b = \frac{(19.7 \times E644) - (3.87 \times E663)}{1000 \times W} \times V$$

Where: E663: the Chlorophyll amount at wavelength of 663

E664: the Chlorophyll amount at wavelength of 663

10.3, 0.918, 19.7 and 3.87 are fix values

W: weight of the leaf sample (0.1g)

V: the volume of the solvent (Acetone).

### Data analysis

All experiments were laid out in Completely Randomized Design (CRD) using 5 replicates. Analysis of variance related to CRD experiments was conducted using the Statistical Analysis System (SAS) program *verg* (ver. 9.00, SAS Institute, Cary, NC, USA). The treatment means were compared by F-test and the Least Significant Differences test (LSD) at 5% probability level (Gomez and Gomez, 1984).

## Results and discussion

### Average leaf length (cm)

Plant leaf length (cm) was significantly differed due to genotypic effects of *Pandanus* spp. and leaf types while the interaction was not significant (Table 1).

**Table 1.** Mean squares for the genotypic effects and leaf types (LT) on average leaf length (cm), average leaf breadth (cm), average leaf thickness (mm) and average stem diameter (cm) of four *Pandanus* spp. The species *Pandanus tectorius* var 'Utilis', *Pandanus tectorius* var 'Variegata' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia), and *Pandanus odoratissimus* naturally grown from three different regions of Saudi Arabia.

Source	df	Average leaf length (cm)	Average leaf breadth (cm)	Average leaf thickness (mm)
Rep	4	663.75 <sup>ns</sup>	151.83 <sup>ns</sup>	0.042 <sup>ns</sup>
<i>Pandanus</i> spp (PS)	3	7223.96 <sup>**</sup>	3728.12 <sup>**</sup>	0.668 <sup>**</sup>
Leaf Types (LT)	2	11335.11 <sup>**</sup>	58.18 <sup>ns</sup>	0.554 <sup>**</sup>
LT* PS	6	536.14 <sup>ns</sup>	25.58 <sup>ns</sup>	0.058 <sup>*</sup>
Error	32	241.64	20.57	0.017

ns= non significant, \*\* significant at  $p < 0.01$ .

Plants of the species *Pandanus tectorius* var 'Utilis' produced the tallest leaves (106.08 cm), followed by *Pandanus dubius* with 86.24 (cm) and the local species (*Pandanus odoratissimus*) with 81.06 cm. Shortest leaves were found on plants of *Pandanus tectorius* var. 'Variegata' (52.90 cm) (Fig. 1A).

Regarding leaf types the results showed that leaves of medium age (found in the middle layers of plant crown) revealed the highest length (103.37 cm), followed by old age leaves (found in the outer bottom layers of the plant crown) (85.18 cm).

**Table 2.** Effects of interaction between *pandanus* spp. and leaf types (new, medium and old ages leaves) on average leaf thickness (mm) as indicator for Morphological characterization of four *Pandanus* spp.

Leaf Types	<i>Pandanus</i> spp			
	<i>P. odoratissimus</i>	<i>P. dubius</i>	<i>P. tectorius</i> var Variegata	<i>P. tectorius</i> var Utilis
Old leaves	5.9	8.84	10.72	6.92
Medium leaves	5.94	7.48	12.88	9.41
New leaves	3.91	5.59	7.45	5.83
LSD <sub>0.05</sub>	0.167			

The new young leaves (found in the first internal layers of plant crown) registered an average length of 56.16 cm (Fig. 1B).

There were reported considerable variations in leaves length among *Pandanus* species. The maximum length of fully expanded leaves of *Pandanus* species ranged from 1-3m (David, 1984; Brink and Janson, 2003). Watke *et al.* (2010) reported that the length of fully expanded leaves of *Pandanus amaryllifolius* Roxb ranged from 0.90m to 1.0 m. Rahayu *et al.* (2011) studied some morphological features of the

vegetative parts, peduncle, fruiting cephalium, drupes and style in *Pandanus bantamensis*, *Pandanus pseudolais*, *Pandanus scabrifolius*, *Pandanus tectorius* var. littoralis and *Pandanus odoratissimus*. They found that leaves length ranged from 216–441cm for *Pandanus bantamensis*, 299.4–574.5 cm for *Pandanus pseudolais* and 204–372cm for *Pandanus scabrifolius*. Additionally, they illustrated that Leaf dimension did not have any consistent differences between *Pandanus tectorius* var. littoralis (ranged from 112–199cm) and *Pandanus odoratissimus* (ranged from 98–126cm).

**Table 3.** Mean squares for genotypic effects and leaf types (LT) on leaf fresh weight (g), leaf dry weight (g), chlorophyll a (mg/100g) and chlorophyll b (mg/100g) of four *Pandanus* spp. The species *Pandanus tectorius* var 'Utilis', *Pandanus tectorius* var 'Variegata' and *Pandanus dubius* obtained from the local market in Jeddah, Saudi Arabia, and *Pandanus odoratissimus* collected from three different regions of Saudi Arabia.

Source	df	Leaf fresh weight(g)	Leaf dry weight (g)	Ch a (mg/100g)	Ch b (mg/100g)
Rep	4	110.01 <sup>ns</sup>	6.28 <sup>ns</sup>	0.31 <sup>ns</sup>	0.169 <sup>ns</sup>
<i>Pandanus</i> spp (PS)	3	107.65 <sup>ns</sup>	3.86 <sup>ns</sup>	11.54 <sup>**</sup>	1.312 <sup>**</sup>
Leaf Type (LT)	2	905.67 <sup>**</sup>	48.12 <sup>**</sup>	7.84 <sup>**</sup>	2.153 <sup>**</sup>
LT* PS	6	37.27 <sup>ns</sup>	1.35 <sup>ns</sup>	2.27 <sup>ns</sup>	0.611 <sup>**</sup>
Error	24	18.09	0.911	1.713	0.142

ns= non significant, \*\* significant at p < 0.01.

#### Average leaf breadth (cm)

Average leaf breadth (cm) was significantly affected by genotypic of the tested *Pandanus* species and types of leaf, while the interaction was not significant (Table 1). The results demonstrated that the imported

*pandanus* species (*Pandanus tectorius* var 'Variegata') produced leaves with the greatest breadth (6.86 cm). Narrow breadth were observed for leaves of the species *Pandanus dubius* (3.50 cm) followed by *Pandanus tectorius* var 'Utilis' (3.87 cm) and the

naturally grown local species *Pandanus odoratissimus* (3.81 cm) (Fig. 2A). As presented in Table (1) leaves breadth was significantly affected by leaf types. The widest leaves were observed in the middle layers of plant crown (4.64 cm), new young leaves (leaves found in the first internal layers of plant crown) (4.57 cm). The least width was observed for the old age leaves (found in the outer bottom

layers of the plant crown) (4.32 cm) (Fig. 2B). Watke *et al.* (2010) found that the breadth of newly internal leaves of *Pandanus amaryllifolius* Roxb. ranged from 2-5cm and the fully expanded leaves from 7-9cm. Brink and Janson, (2003) illustrated that breadth of fully expanded leaves of *Pandanus tectorius* leaves ranged from 11-16cm.

**Table 4.** Effects of interaction between *pandanus* spp. and leaf types (new, medium and old ages leaves) on leaf contents of *Ch a* (mg/100g) as indicator for Morphological characterization of four *Pandanus* spp.

Leaf Types	<i>Pandanus</i> spp			
	<i>P. odoratissimus</i>	<i>P. dubius</i>	<i>P. tectorius</i> var Variegata	<i>P. tectorius</i> var Utilis
Old leaves	1.278	0.768	0.732	0.690
Medium leaves	1.958	0.664	1.832	1.468
New leaves	0.964	0.564	0.890	1.83
LSD <sub>0.05</sub>	0.167			

**Table 5.** Mean squares for the genotypic effects and leaf types (LT) on leaf contents of N (%), P (%) and K (%) of four *Pandanus* spp. The species *Pandanus tectorius* var 'Utilis', *Pandanus tectorius* var 'Variegata' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia), and *Pandanus odoratissimus* naturally grown from three different regions of Saudi Arabia.

Source	df	N %	P %	K %
Rep	4	0.038 <sup>ns</sup>	0.0002 <sup>ns</sup>	0.069 <sup>ns</sup>
<i>Pandanus</i> spp (PS)	3	0.098 <sup>ns</sup>	0.0004 <sup>**</sup>	0.348 <sup>ns</sup>
Leaf Type (LT)	2	3.325 <sup>**</sup>	0.0002 <sup>ns</sup>	1.616 <sup>**</sup>
LT* PS	6	0.059 <sup>ns</sup>	0.0001 <sup>ns</sup>	0.057 <sup>ns</sup>
Error	24	0.063	0.0001	0.05

ns= non significant, \*\* significant at  $p < 0.01$ .

#### Average leaf thickness (mm)

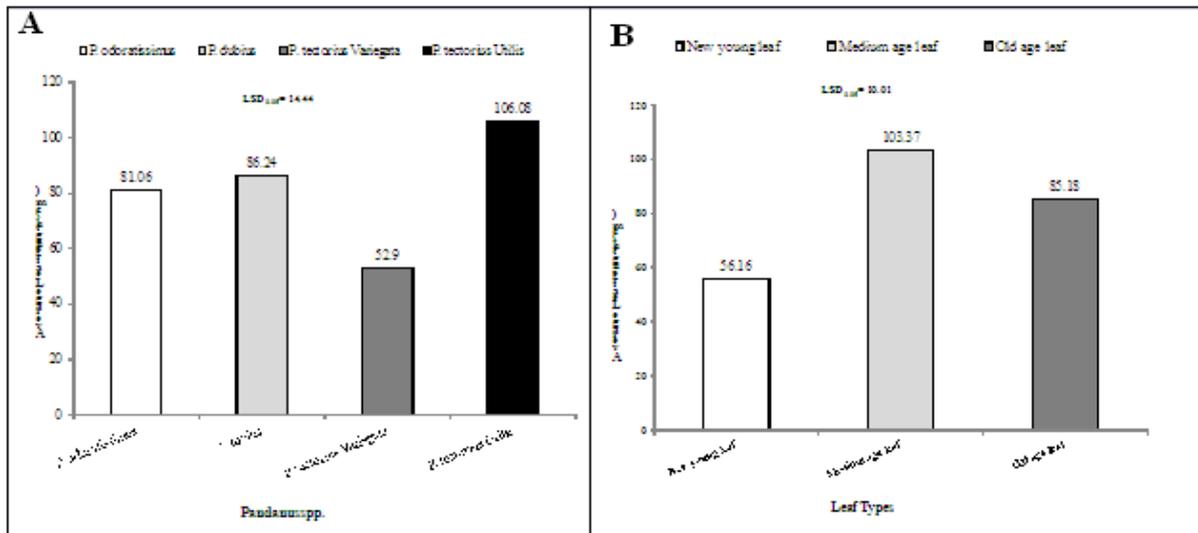
There were significant differences due to genotypic effects of *Pandanus* species, leaf types and their interaction on average leaf thickness (mm) (Tables 1).

Leaves of medium age (leaves found in the middle layers of plant crown) of *Pandanus tectorius* var 'Variegata' illustrated the greatest thickness (12.88 mm). Additionally, *Pandanus tectorius* var 'Variegata' produced old age leaves with greatest thickness (10.72 mm) as compared to other *pandanus* species. The new young leaves (leaves found in the first internal layers of plant crown) *Pandanus tectorius* var 'Variegata' were thicker than the new young leaves of other tested *Pandanus* spp (Table 2). Contrary, *Pandanus odoratissimus* (naturally grown local

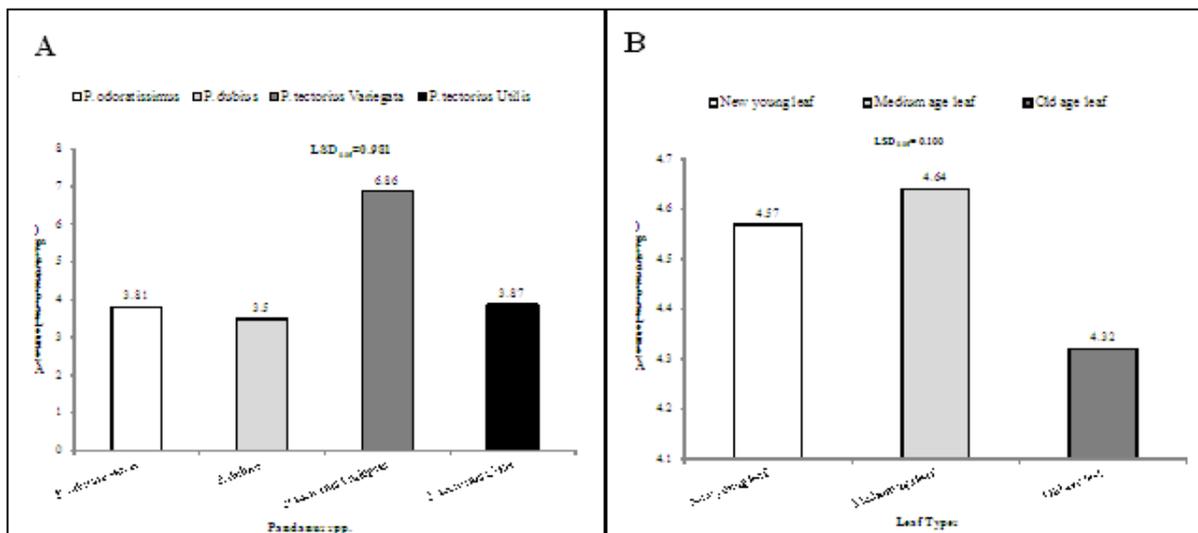
*pandanus* in Saudi Arabia) produced leaves with the least thickness (3.91mm) (new young leaves) as compared to *Pandanus tectorius* var 'Variegata' (7.54 mm), *Pandanus dubius* (5.59 mm) and *Pandanus tectorius* var 'Utilis' (5.41 mm). Also, *Pandanus odoratissimus* produced medium and old ages leaves with lower thickness as compared to other tested *Pandanus* spp (Table 2). Leaf dimension, leaf shape, cephalium size and shape, and the number of phalanges per cephalium were reported to not have any consistent differences between *Pandanus* species (Rayham *et al.*, 2011). However, they observed that *Pandanus tectorius* var. littoralis had leaf apex prickles only on one surface (*Pandanus odoratissimus* has prickles on both surfaces of the leaf apex); and carpel apices that were essentially

fused, with our any deep grooves between carpels (*P. odoratissimus* had carpel apices that were free, leaving deep grooves between carpels). Stone (1972) found that the species recognized within the

*Pandanus furcatus* complex shared some morphological similarities, but could be distinguished by leaf base colour, peduncle shape, fruit shape, style shape, and stomatal characteristics.



**Fig. 1.** Average leaf length (cm) as indicator for Morphological characterization of four *Pandanus* spp. Three species *Pandanus tectorius* var *Utilis*, *Pandanus tectorius* var '*Variegata*' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia and *Pandanus odoratissimus* was considered to be naturally grown in three different regions of Saudi Arabia. A) genotypic effects and B) leaf types effects.



**Fig. 2.** Average leaf breadth (cm) as indicator for Morphological characterization of four *Pandanus* spp. Three species *Pandanus tectorius* var *Utilis*, *Pandanus tectorius* '*Variegata*' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia and *Pandanus odoratissimus* was considered to be naturally grown in three different regions of Saudi Arabia. A) genotypic effects and B) leaf types effects.

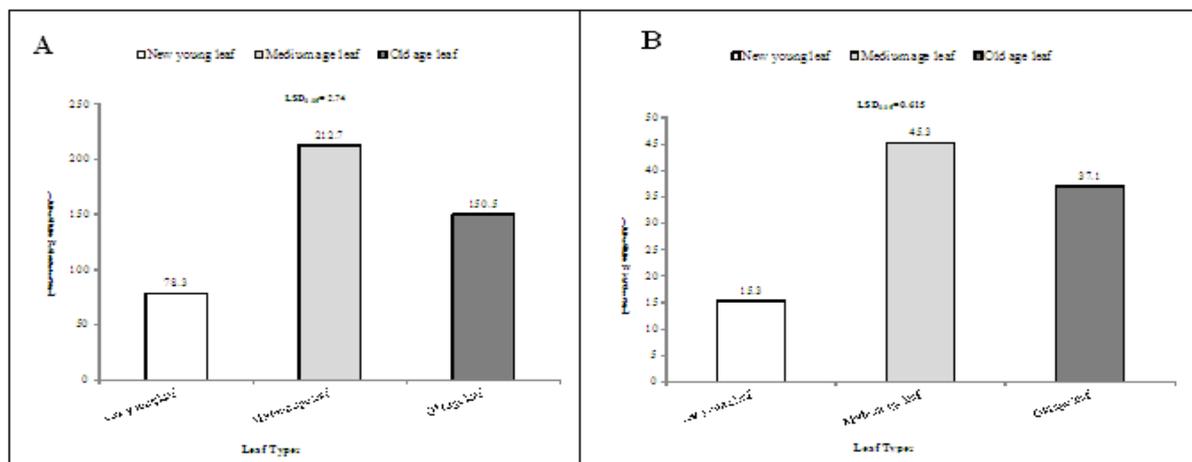
*Leaf fresh and dry weights (g)*

Leaf fresh and dry weights were significantly affected by leaf types, while genotypic effects and the

interaction between treatments were not significant (Table 3). Leaves of medium age (leaves found in the middle layers of plant crown) revealed highest fresh

(212.70g) and dry (45.30 g) weights flowed by old age leaves (leaves found in the outer bottom layers of plant crown) with 150.50 g and 36.20 g for fresh and dry weights, respectively (Fig 3 A & B). New young leaves (leaves found in the first internal layers of plant crown) registered the least fresh and dry weights with 78.30g and 15.30g, respectively.

Maximum leaves fresh and dry weights were observed for plants of *Pandanus tectorius* var 'Variegata' with 176.70 g and 37.60 g followed by *Pandanus tectorius* var 'Utilis' with 156.9g and 36.20g, *Pandanus dubius* with 142.0 g and 2.86 g and *Pandanus odoratissimus* with 113.10 g and 27.80 g.



**Fig. 3.** Effects of leaf types on morphological characterization of four *Pandanus* spp. Three species *Pandanus tectorius* var Utilis, *Pandanus tectorius* 'Variegata' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia and *Pandanus odoratissimus* was considered to be naturally grown in three different regions of Saudi Arabia. A) leaf fresh weight (g) and B) leaf dry weight (g).

#### *Chlorophyll a* (mg/100g)

The results revealed significant differences between tested *Pandanus* species and leaf types with regard *Ch a* (mg/100g), while the interaction was not significant (Table 3).

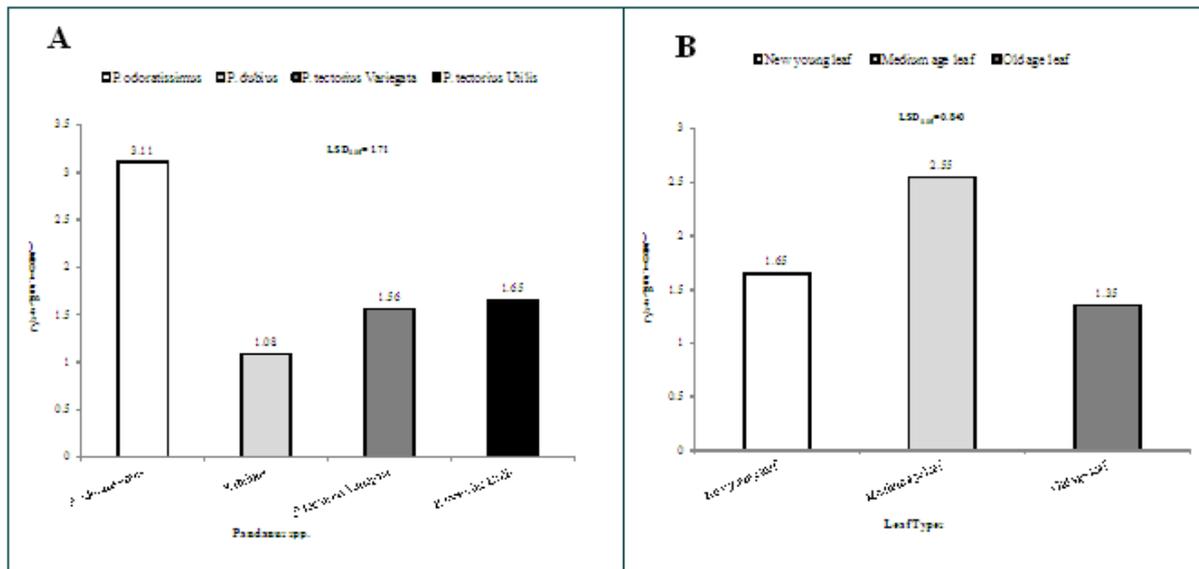
The naturally grown local species in Saudi Arabia *Pandanus odoratissimus* illustrated highest *Ch a* contents with 3.11 mg/100g. Leaves of imported *Pandanus* species *Pandanus dubius* contained least *Ch a* contents (1.08 mg/100g) while the results were not significantly different from *Pandanus tectorius* var 'Variegata' (1.56 mg/100g) and *Pandanus tectorius* var 'Utilis' (1.65 mg/100g) (Fig 4A).

Regarding impact of leaf types on *Ch a* contents the results were presented in Figure (4B). Highest *Ch a* contents were registered for *Pandanus* leaves of medium age (leaves found in the middle layers of plant crown) (2.55 mg/100g) followed by new young age leaves (leaves found in the first internal layers of plant crown) with 1.65 mg/100.

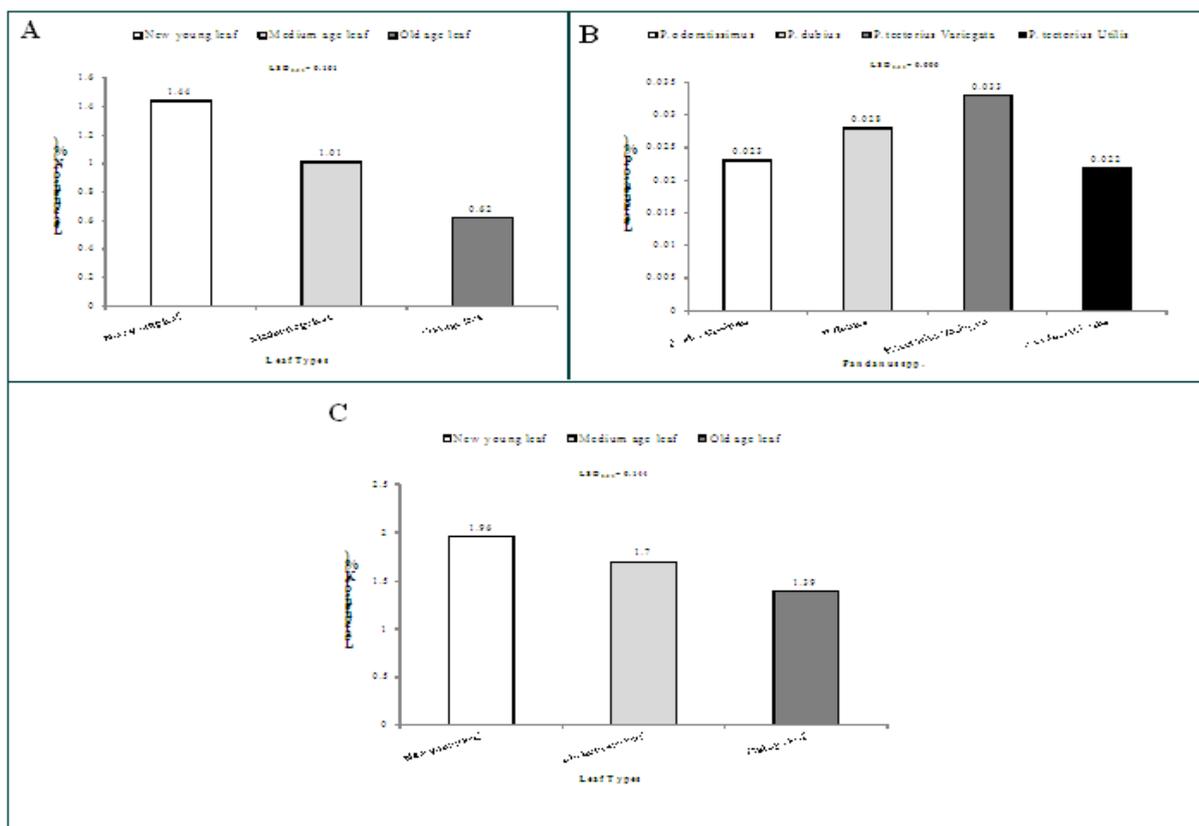
The old age *pandanus* leaves (leaves found in the outer bottom layers of plant crown) produced the least *Ch a* contents with 1.35 mg/100g (Fig 4 B).

#### *Chlorophyll b* (mg/100g)

There were observed significant differences due to interaction between *Pandanus* genotypes and leaf types on *Ch b* contents (mg/100g) (Table 3). The results revealed that the medium age leaves of the naturally grown local *Pandanus* species in Saudi Arabia *Pandanus odoratissimus* illustrated attained highest *Ch b* contents (1.958 mg/100g) with no significant differences from medium age leaves of *Pandanus tectorius* var 'Variegata' (1.832 mg/100g). Among the new young leaves *Pandanus tectorius* var 'Utilis' produced highest *Ch b* contents by 1.380 mg/100g (Table 4). Additionally, among leaves of old age *Pandanus odoratissimus* produced high *Ch b* contents (1.278 mg/100g). Medium, new young and old ages leaf of *Pandanus dubius* produced least *Ch b* contents where it contained 0.664, 0.654 and 0.768 mg/100, respectively (Table 4).



**Fig. 4.** Morphological characterization of four *Pandanus* spp. Three species *Pandanus tectorius* var Utilis, *Pandanus tectorius* 'Variegata' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia and *Pandanus odoratissimus* was considered to be naturally grown in three different regions of Saudi Arabia. A) Effects of *Pandanus* genotypes on Ch contents and B) effects of leaf types on Ch a contents.



**Fig. 5.** Morphological characterization of four *Pandanus* spp. Three species *Pandanus tectorius* var Utilis, *Pandanus tectorius* 'Variegata' and *Pandanus dubius* were obtained from the local market in Jeddah, Saudi Arabia and *Pandanus odoratissimus* was considered to be naturally grown in three different regions of Saudi Arabia. A) effects of leaf types on N (%) contents and B) Effects of *Pandanus* genotypes on P (%) and C) effects of leaf types on K (%) contents.

#### Leaf content of N (%), P (%) and K (%)

Leaf types were significantly affected leaf N contents (%), while the effects of *pandanus* species and interaction between leaf types and genotypes were not significant (Table 5). Medium age leaves were attained highest percentage of N (1.01 %). Old age leaves and new you leaves of *Pandanus* contained low level of N without significant differences (Fig. 5 A). There were significant genotypic effects on leaf content of P(%) of *Pandanus* species, but effects of leaf types and its interaction with *Pandanus* species were not significant (Table 5).

Leaves of *Pandanus tectorius* var 'Variegata' contained the highest percentage of P (0.033%) followed by *Pandanus dubius* (0.028%) and *Pandanus odoratissimus* (0.023%). The least percentage of P was observed for leaves of *Pandanus tectorius* var 'Utilis' (0.022%) (Fig. 5 B). Percentages of K in *Pandanus* leaves were significantly affected by leaf types, but Leaf types were significantly affected leaf N contents (%), while *Pandanus* species and its interaction with leaf types had no significant effects on leaf content of K (%) (Table 5). The new young leaves (leaves found in the first internal layers of plant crown) attained highest percentage of K by 1.96 %, followed by leaves of medium age (leaves found in the meddle layers of plant crown) by 1.70 %. Least percentage of K found for leaves of old age (leaves found in the outer bottom layers of plant crown) (1.39%) (Fig. 5C).

#### Conclusion

*Pandanus tectorius* var 'Variegata' can be morphologically identified by leaf breadth (mm), leaf thickness (mm) and leaf content of P (%). Leaves chlorophyll contents were effectively recognized *Pandanus odoratissimus*. Leaves length (cm) were

considered as morphological markers to identify *Pandanus dubius*. Leaves of medium ages (leaves found in the meddle layers of plant crown) can be effectively used for further studies for the morphological differentiation between *Pandanus* species.

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