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Anatomical Studies on Six Cultivars of *Allium sativum* L. Cultivated at Mandalay Region, Lower Sagaing Region and Shan State

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Abstract

The present studies deal with the anatomical characters of six cultivars, *Allium sativum* L. (garlic) known as kyetthun phyu. These cultivars were included cultivar Au tlaone khayan, Au talone phyu, cultivar Shan hmwar phyu, cultivar Shan hmwar khayan, cultivar Kyun au and cultivar Shwe pan gone. All cultivars were collected from Yat Sauk from Southern Shan State, Tagaung Sub- township located in Mandalay Region and Wetlet Township of Sagaing Region, in this year 2012-2013.The anatomical characteristic of the leaf blade were studied, described, compared and discussed. The maximum thickness of leaf blade was found in cultivar Au talone phyu. The distinct character included, multiple layers of spongy was 6-10 layers found in cultivar Shan hmwar khayan and 1-8 layers of phloem was found in Shan hmwar phyu. In this research work also observed that the anatomical characteristic of the six cultivars were found to be similar with one another in term of type of stomata and vascular bundle.

Keywords: *Allium sativum* L., six cultivars, anatomical characters

Introduction

Genus *Allium* belong to family Alliaceae. *Allium sativum* L.(garlic) is the second most important vegetable among the spice and condiment. It is closely related to the onion, shallot, leek, chive and rakkyo (Gafar *et al*. 2012).Most of the garlic had been consumed today, come from China, South Korea, India, Spain and the United States. It was used as food, spice and medicinal crops (Wyk & Wink 2004).

The family Alliaceae was composed of 13 genera and 600 species. Generally distinct characters were bulbous herbs, with basal, usually narrow

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leaves, an umbellate inflorescence, and a usually superior ovary (Simpson 2006).Garlic is a sterile species and reproduces only by vegetative propagation. Although is an asexually propagated crop; it displays great morphological diversity in bulb, leaf size, color, shape, height, flower color, fertility and bulbil (topset) development (Abdoli *et al*. 2009).

Garlic was grown in globally, but China is the largest production from 13,674,400 t (FAO 2002). Garlic are divided into several informal cultivar group: the *Longicuspis* group of Central Asia; the *sativum* group from Mediterranean; the *Ophioscorodon* group from central and eastern Europe; the Pekiense group of China; the subtropical group from Indian, Vietnam and Myanmar, which have small bulbs and are selected for eating as fresh levels (Fritsch & Friesen 2002 as cited in Brewster 2008).

Single bulb *Allium sativum* L., locally known as Au talone kyetthun phyu is renowned for its versatile use of medicinal property. Shan garlic was observed to be the most popular cultivars for culinary purpose it is also commercially cultivated at high altitude of Southern Shan State. Whereas, Myanmar garlic (or) lowland commercial cultivars are mostly cultivated at Wetlet and Tagaung Sub-township. Among then, Tagaung cultivar, locally known as Kyun au has reputation for it's pungency of flavoring quality for culinary purpose and it was also highly demanded ones.

According to FAO data 2012, there are altogether 97 garlic producing countries in the world. Among then Myanmar has reputation for being included in the list of Top Ten garlic producing country. Myanmar stands as in the position of 6^{th} largest producing countries and it's production was about 185,900 t.

Basic two main types of garlic are hardneck and softneck. Hardneck garlic, woody central stalk at extend down to the balsl plate at the bottom of the bulb. They send up a flower stalk and umbel covered by pointed spathe. Softneck garlic have a non-woody pseudostem formed from overlapping leaf sheath and rarely send up flower stalk, unless stressed by climatic conditions (Meyer 2006)

Beside morphological identification and anatomical observation is also important for Myanmar cultivars of *Allium sativum* L. Edwin *et al*. (2006) mentioned that leaves were very important vegetative organs because they were mainly concerned with photosynthesis and transpiration. Pandey & Chadha (2000) stated that the anatomical features of vegetative as well as reproductive organs have been studies to help in studies on systematic, particularly for resolving controversial taxonomic problems. In fact, such studies have been

successfully employed for the solution of taxonomic problems and to the elucidation of phylogenetic relationships. In several cases, anatomical studies have helped in identifying the fragmentary material which lacked flowers and fruits in the herbarium specimens.

In *Allium sativum* L. two group of non-bolting (softneck) and bolting (hardneck) can be categorized. Therefore it very difficult for using formal taxonomic identification. In that condition, designation the identifying character for certain cultivars has been essentially needed. Htwe Htwe Tin Maung (1948) had been described a comparative morphology and anatomy of the cultivation *Allium* species of Burma. In 2006, Soe Soe Hlaing conducted the plant genetic resources of garlic *Allium sativum* L. conducted the plant genetic resources of garlic *Allium sativum* L. grow in Upper Myanmar. In recent studies can provide the anatomical information , that can be used as tool for artificial key and varieties identification.

Therefore, the present study focused on six cultivars of *Allium sativum* L. with the following aims and objectives to know the different cultivars of *Allium sativum* L. and to provide the similarities and differences of their anatomical characters in six cultivars of *A*. *sativum* L.

Materials and Methods

Six cultivars of *Allium sativum* L.(garlic) were collected from Yat Sauk from Southern Shan State, Tagaung Sub-township located in Mandaly Region and Wetlet Township of Sagaing Region, from 2012-2013. Both morphology and taxonomy characters were classified according to Hooker (1879), Backer & Brink (1968), Hill & Small (1933), Dassanayake (2000) and Kaul *et al*. (2006). In anatomical observations, the study was restricted only to vegetative parts of leaf blade. For anatomical study, after killing and fixation, the dehydration, infiltration, embedding, staining staining and mounting were made according to Johansen's (1940) method. The paraffin sections were cut by using a rotary microtome to a thickness of cut 25µ. Infiltration, embedding, microtoming, and staining were made at Department of Botany Mandalay University. Leaf blade were macerated by using equal volume of hydrogen peroxide and glacial acetic acid Jeffery (1917) method.

Results

1.Anatomical Studies

1.1 Internal structure of leaf blade *Allium sativum* L. from three different localities (Figure 1-6)

Leaf blade

In transverse section leaf blade were Au talone khayan 384-504 μm thick, Au talone phyu 470.4-6336.6 μm thick, Shan hmwar phyu 384-422.4 μm thick, Shan hmwar khayan 331.2-528 μm thick, Kyun au 297.6-600 μm thick and Shwe pan gone 364-470.4 μm thick.

Dermal tissue system: Composed of epidermal cells and guard cell of the stomata. In surface view, adaxial and abaxial epidermal cells are similar in shape, parenchymatous, cell wall thin. Stomata present on both surface, abundant, anomocytic type, oval, guard cells reniform. In transverse section both adaxial and abaxial epidermis 1 layered, both epidermal cells are anticlinal walls straight, slightly convex on adaxial and abaxial epidermis. Cuticle presence thin on both surface.

Ground tissue system: Mesophyll differentiated into outer palisade and inner spongy; palisade parenchyma on both surface, 1 layered; laticifera present on both surface; spongy parenchyma were Au talone khayan 8-9 layered, Au talone phyu 7-8 layered, Shan hmwar phyu 6-8 layered, Shan hmwar khayan 6-10 layered, Kyun au 5-8 layered and Shwe pan gone 7-9 layered, the cell rounded or oval shaped. Calcium oxalate crystals tetragonal, prismatic and rod shape.

Vascular Tissue System: Vascular embedded in the ground tissue, close collateral type, oval shaped, Au talone khayan 43.2-168 μm in length and 28.8-72 μm in width, Au talone phyu 33.6-120 μm in length and 19.2-72 μm in width, Shan hmwar phyu 28.8-126.6 μm in length and 28.8-72 μm in width, Shan hmwar khayan 33.6-168 μm in length and 24-72 μm in width, Kyun au 19.2-158.4 μm in length and 19.2-81.6 μm in width, Shwe pan gone 33.6-96 μm in length and 33.6-62.4 μm in width; bundle cup parenchymatous, 1 layered, phloem were Au talone khayan 1-4 layered, Au talone phyu 1-4 layered, Shan hmwar phyu 1-8 layered, Shan hmwar khayan 2-4 layered, Kyun au 1-3 layered and Shwe pan gone 1-4 layered, the layers were 24-48 μm thick in Au talone khayan, 9.6-19.2 μm thick in

Au talone phyu, 1-8 µm in Shan hmwar phyu, 19.2-48 µm thick in Shan hmwar khayan, 14.4-19.2 µm thick in Kyun au and 14.4-19.2 µm thick in Shwe pan gone, phloem composed of sieve tube, companion cells, phloem fibers and phloem parenchyma; xylem were 2-4 layered in Au talone khayan, 1-5 layered in Au talone phyu, 1-4 layered in Shan hmwar phyu, 2-5 layered in Shan hmwar khayan, 1-4 layered in Kyun au and 2-4 layered in Shwe pan gone, the layers were 24-48 µm thick in Au talone khayan, 9.6-38.4 µm thick in au talone phyu, 14.4-33.6 µm in Shan hmwar phyu, 24-48 µm thick in Shan hmwar khayan, 19.2-28.8 µm thick in Kyun au and 14.4-48 µm thick in Shwe pan gone, cells polygonal; xylem composed of vessel elements, tracheids, fibers and xylem parenchyma. Vessel elements thick-walled lateral walls straight, end walls oblique of transverse, thickening spiral, perforation plate simple, cells 105-115 µm in length and 30-90 µm width in Au talone khayan, 135-310 µm in length and 50-125 µm width in Au talone phyu, 350-425 µm in length and 150-195 µm width in Shan hmwar phyu,70-245 µm in length and 15-100 µm width in Shan hmwar khayan, 205-250 µm in length and 85-150 µm width in Kyun au, 70-115 µm in length and 15-100 µm width in Shwe pan gone; tracheids elongated, lateral walls straight, end wall straight, thickening spiral , cells Au talone khayan 275-600 µm in length and 10-15 µm in width, Au talone phyu145 - 2670 µm in length and 20-25 µm in width, Shan hmwar phyu 100-1885 µm in length and 10-15 µm in width, Shan hmwar khayan 210-1325 µm in length and 15-25 µm in width, Kyun U 235- 1100 µm in length and 10-15 µm in width, Shwe pan gone 150-450 µm in length and 5-10 µm in width; fibers long, lumen narrow, lateral walls straight, end walls acute, cells Au talone khayan 425-1100 µm in length and 15-30 µm in width, Am talone phyu 335-2000 µm in length and 10-30 µm in width, Shan hmwar phyu 340-1000 µm in length and 10-20 µm in width, Shan hmwar khayan 365-890 µm in length and 15-25 µm in width, Kyun au 300-875 µm in length and 10-15 µm in width, Shwe pan gone 2372-2925 µm in length and 15-25 µm in width.

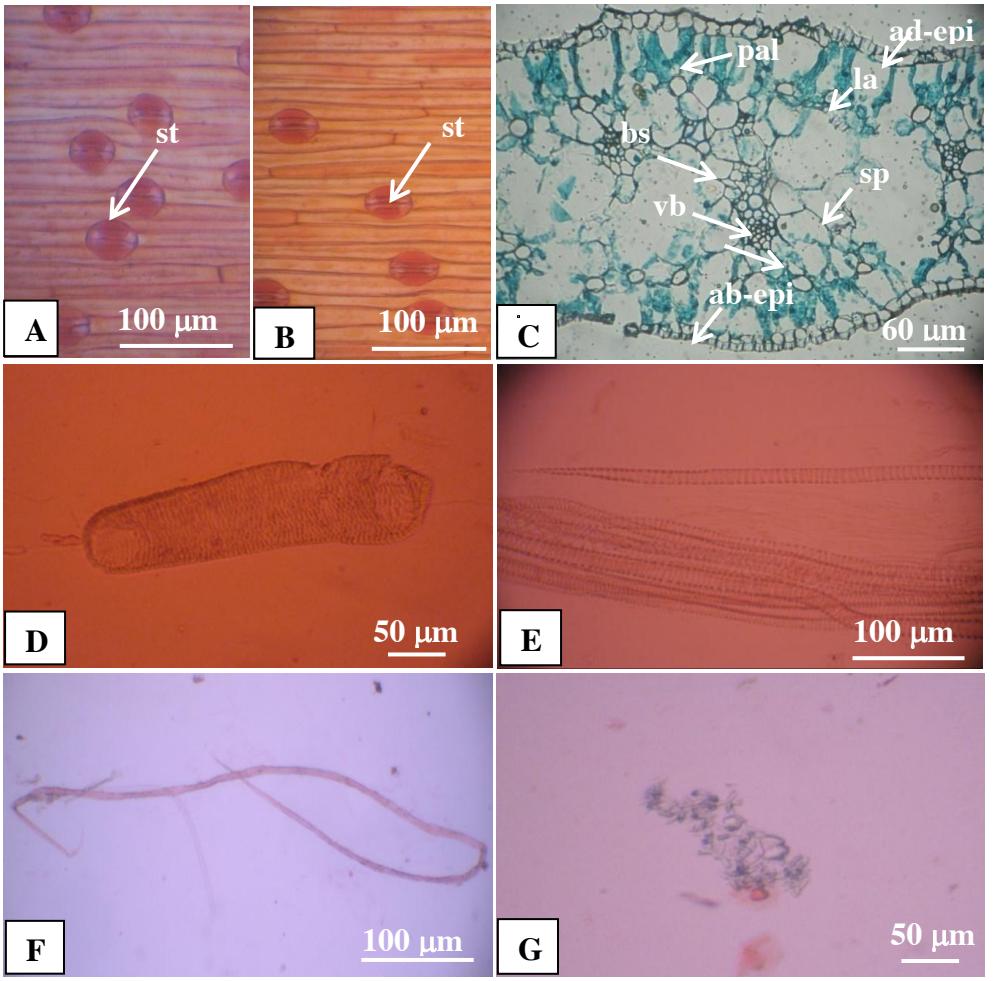

Figure 1. Internal structure and macerated elements of leaf blade *Allium sativum* L.
Au talone khayan
A. Adaxial surface view of leaf blade
B. Abaxial surface view of leaf blade
C. T.S of leaf blade D. Macerated vessel element
E. Tracheid with spiral thickening F. Fiber
G. Calcium oxalate crystals
(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

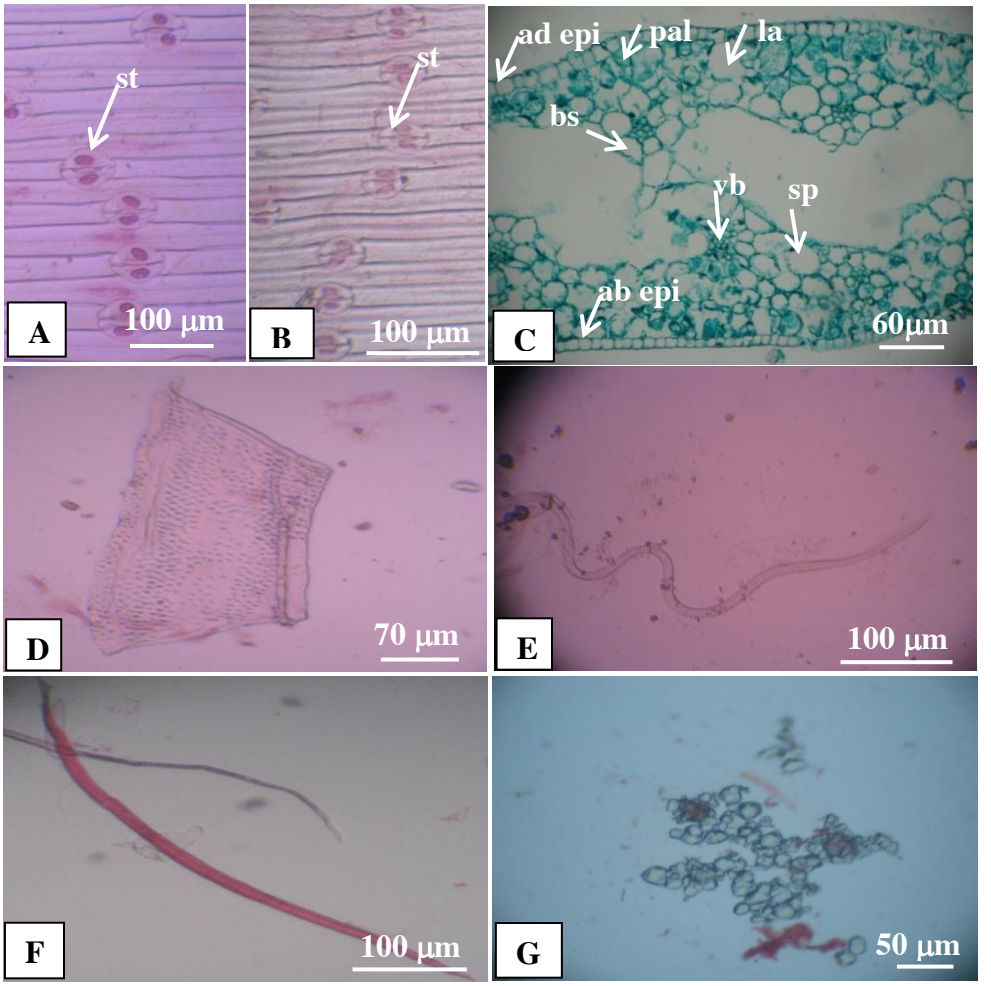

Figure 2. Internal structure and macerated elements of leaf blade *Allium sativum* L. Au talone phyu

A. Adaxial surface view of leaf blade
B. Abaxial surface view of leaf blade
C. T.S of leaf blade
D. Macerated vessel element
E. Tracheid with spiral thickening
F. Fiber
G. Calcium oxalate crystals

(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

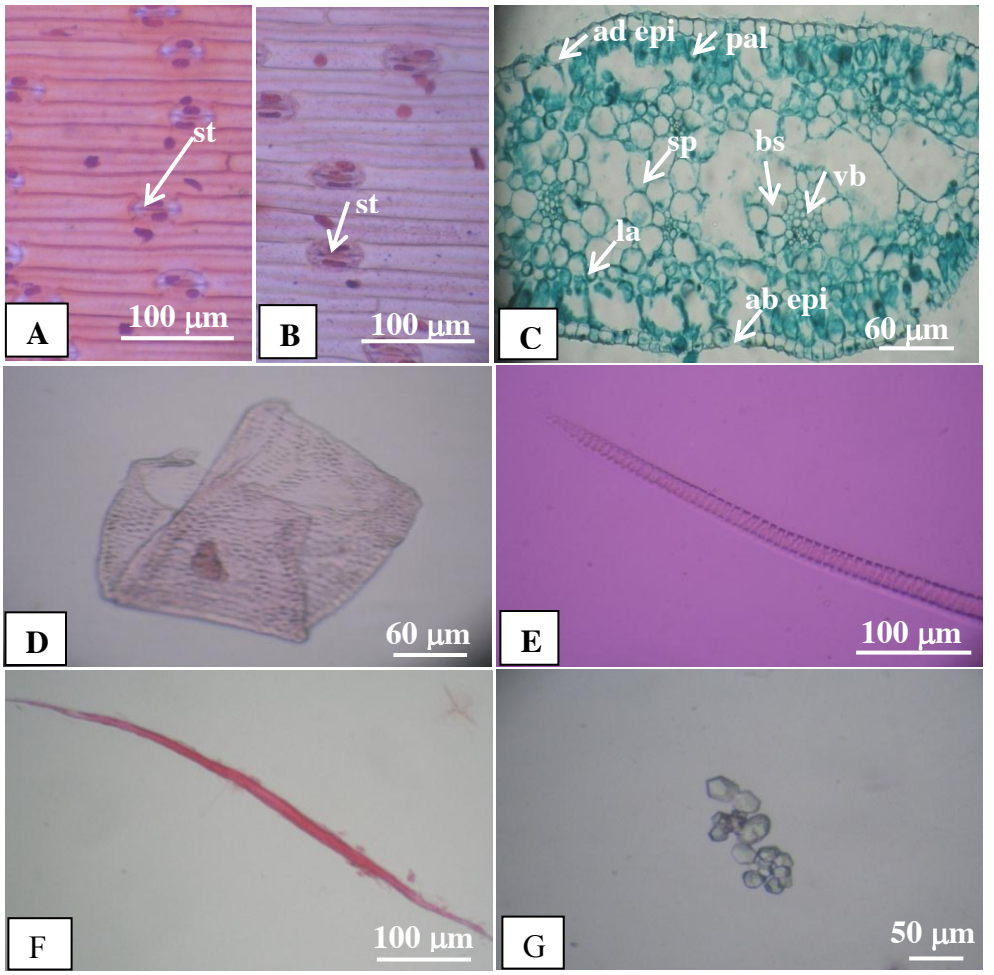

Figure 3. Internal structure and macerated elements of leaf blade *Allium sativum* L.
Shan hmwar phyu
A. Adaxial surface view of leaf blade
B. Abaxial surface view of leaf blade
C. T.S of leaf blade D. Macerated vessel element
E. Tracheid with spiral thickening F. Fiber
G. Calcium oxalate crystals
(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

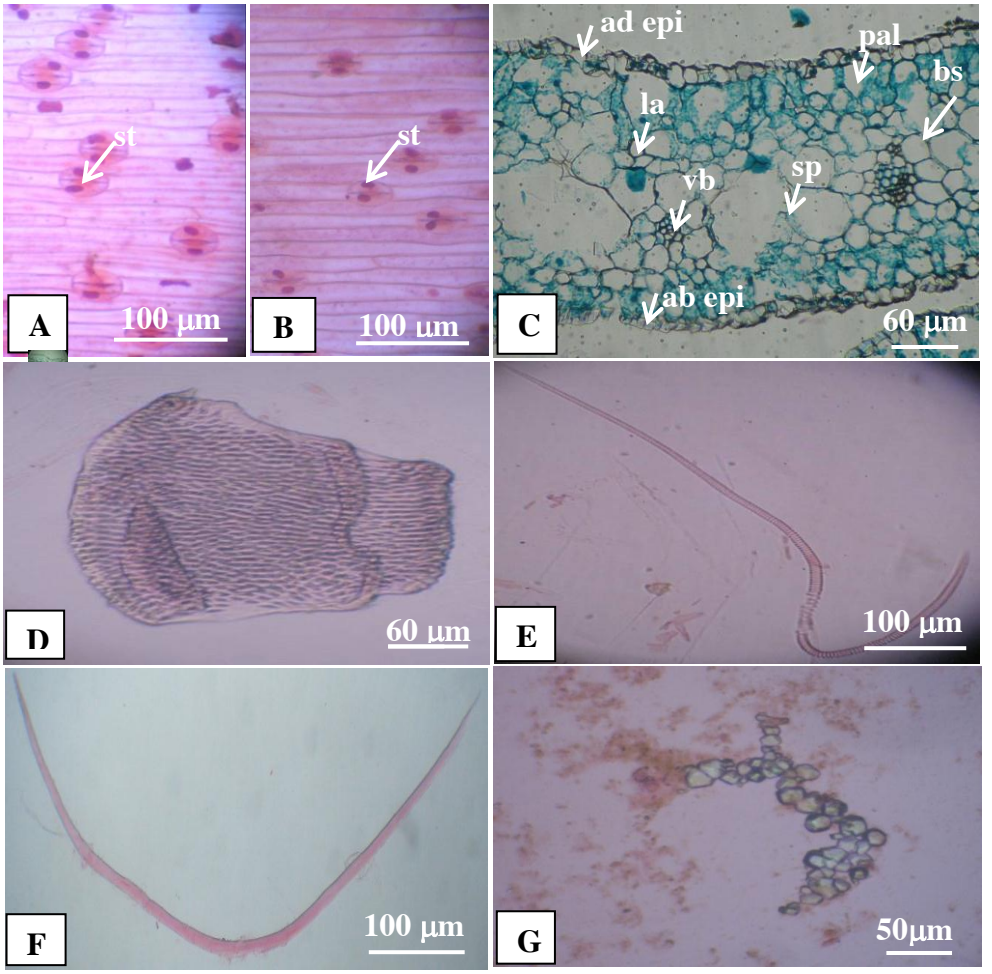

Figure 4. Internal structure and macerated elements of leaf blade *Allium sativum* L. Shan hmwar khayan

A. Adaxial surface view of leaf blade

B. Abaxial surface view of leaf blade

C. T.S of leaf blade

D. Macerated vessel element

E. Tracheid with spiral thickening

F. Fiber

G. Calcium oxalate crystals

(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

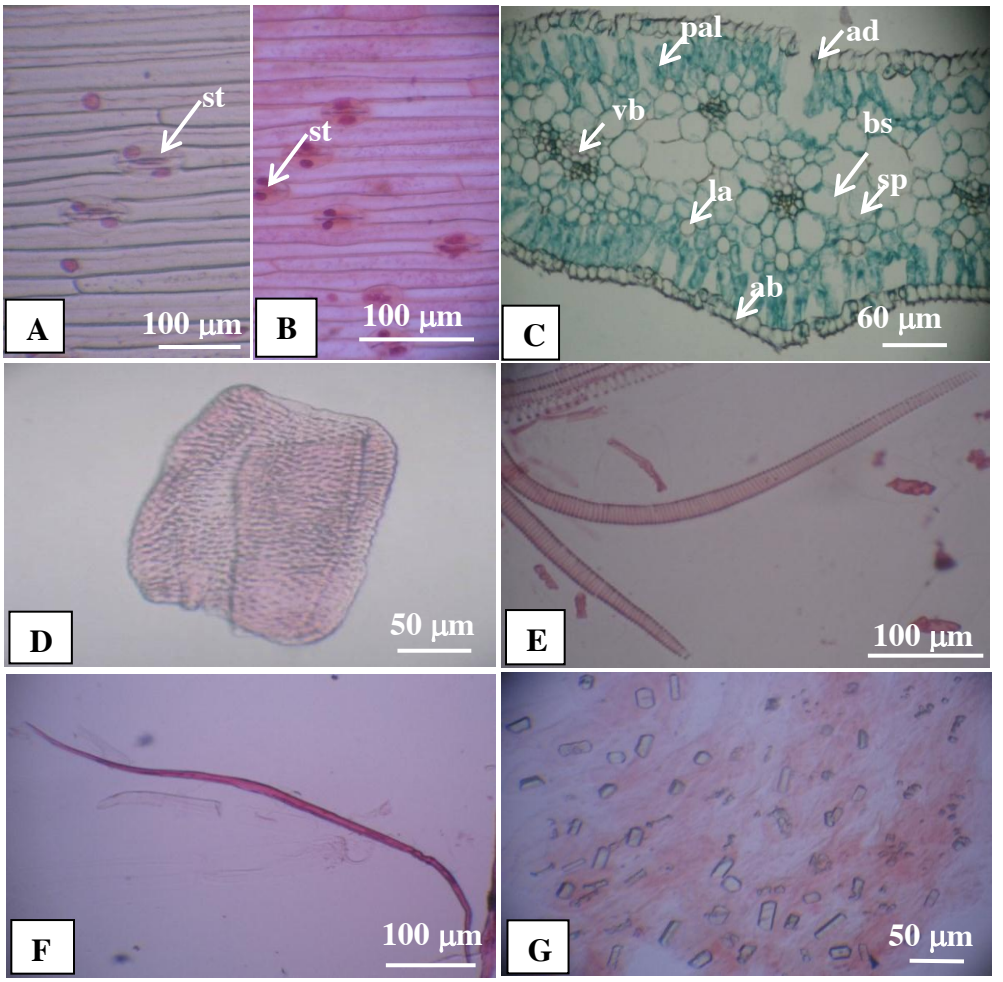

Figure 5. Internal structure and macerated elements of leaf blade *Allium sativum* L. Kyun au

A. Adaxial surface view of leaf blade
B. Abaxial surface view of leaf blade
C. T.S of leaf blade
D. Macerated vessel element
E. Tracheid with spiral thickening
F. Fiber
G. Calcium oxalate crystals

(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

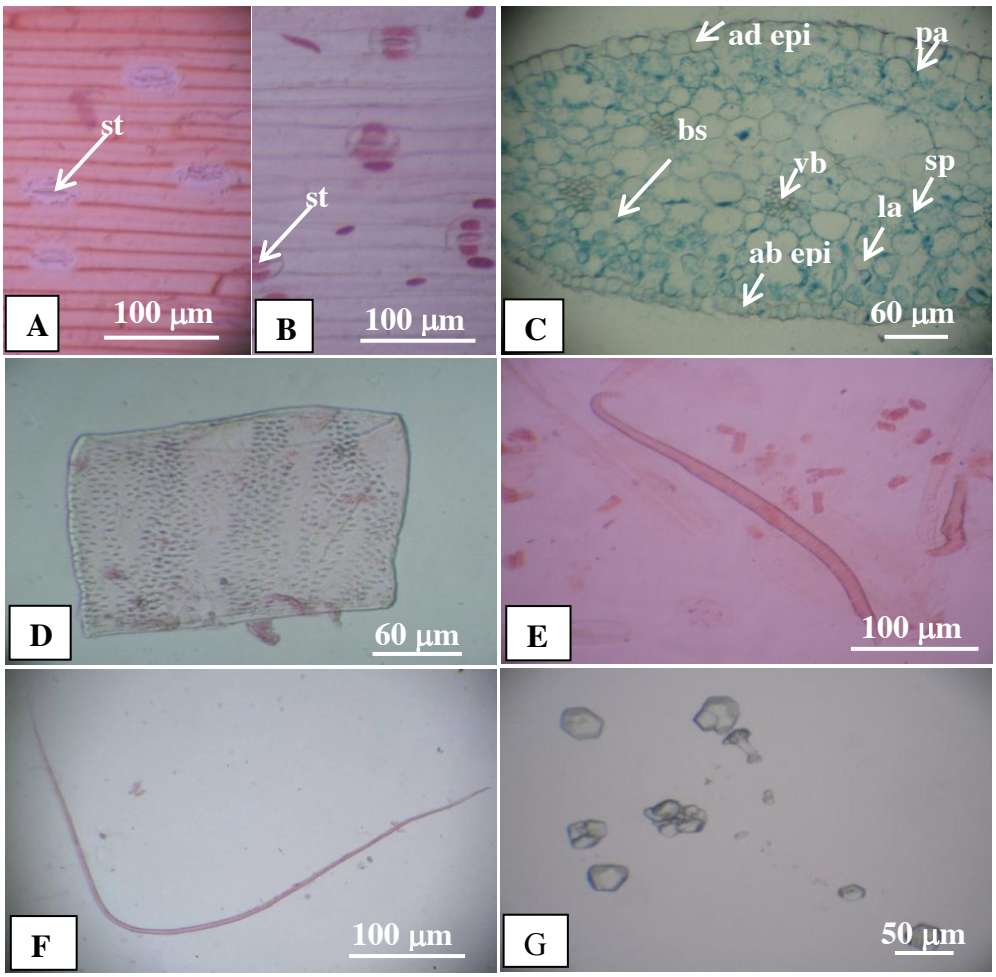

Figure 6. Internal structure and macerated elements of leaf blade *Allium sativum* L. Shwe pan gone

A. Adaxial surface view of leaf blade
B. Abaxial surface view of leaf blade
C. T.S of leaf blade
D. Macerated vessel element
E. Tracheid with spiral thickening
F. Fiber
G. Calcium oxalate crystals

(ad epi-adaxial epidermis, ab epi-abaxial epidermis, st-stomata, la-laticifera, pal-palisade parenchyma cell, sp-spongy parenchyma cell, vb-vascular bundle, bs-bundle sheath, xy-xylem, ph-phloem)

Table 1. Anatomical characters of the leaf blades of six cultivars *Allium sativum* L.

Cultivar names	Leaf blades thick	Epidermis		Stomata Type	Palisade parenchyma	Spongy parenchyma	Type of Vasular bundles	Cuticle thick
		Upper	Lower					
Allium sativum L. U talone khayan	384-504 µm	One layered	One layered	Anomocytic	One layered	8-9 layered	Collateral type, oval in shape	4.8 µm
A.sativum L. U talone phyu	470.4-633.6 µm	One layered	One layered	Anomocytic	One layered	7-8 layered	Collateral type, oval in shape	4.8 µm
A.sativum L. Shan hmwar phyu	384-422.4 µm	One layered	One layered	Anomocytic	One layered	6-8 layered	Collateral type, oval in shape	4.8 µm
A.sativum L. Shan hmwar khayan	331.2-528 µm	One layered	One layered	Anomocytic	One layered	6-10 layered	Collateral type, oval in shape	4.8 µm
A.sativum L. Kyun U	297.6-600 µm	One layered	One layered	Anomocytic	One layered	5-8 layered	Collateral type, oval in shape	4.8 µm
A.sativum L. Shwe pan gone	364-470.4 µm	One layered	One layered	Anomocytic	One layered	7-9 layered	Collateral type, oval in shape	4.8 µm

Table. 2 Macerated elements of the leaf blades of six cultivars *Allium sativum* L.

Cultivar names	Vessel elements		Tracheids		Fibers	
	Length	Width	Length	Width	Length	Width
Allium sativum L. Au talone khayan	105-125 µm	30-160 µm	275-600 µm	10-15 µm	425-1100 µm	15-30 µm
A. sativum L. UAutalone phyu	135-230 µm	50-125 µm	145-2670 µm	20-25 µm	335-2000 µm	10-30 µm
A. sativum L. Shan hmwar phyu	350-425 µm	150-195 µm	100-1885 µm	10-15 µm	340-1000 µm	10-20 µm
A. sativum L. Shan hmwar khayan	70-245 µm	15-100 µm	210-1325 µm	15-25 µm	365-890 µm	15-25 µm
A. sativum L. Kyun au	205-250 µm	85-150 µm	235-1100 µm	10-15 µm	300-875 µm	10-15 µm
A. sativum L. Shwe pan gone	70-115 µm	15-100 µm	150-450 µm	5-10 µm	2373-2925 µm	15-25 µm

Discussion and Conclusion

The present studies concentrated which the anatomy characters of six cultivars such as *Allium sativum* L., these are cultivar Au talone khayan, cultivar Au talone phyu, cultivar Shan hmwar phyu, cultivar Shan hmwar khayan, cultivar Kyun au and cultivar Shwe pan gone there are commercially important cultivated in Upper Myanmar. Cultivars Au talone khayan, Au talone phyu, Shan hmwar phyu and Shan hmwar khayan were cultivated in Shan State at the altitude 600-2500 M ASL. Cultivars Shwe pan gone was cultivated at Sagaing Region at the altitude of 80-100 M ASL. Cultivar Kyun au was cultivated at Mandalay Region at altitude 200-480 M ASL.

Shan cultivars were high altitude cultivars while Myanmar cultivars were low-altitude once. Cultivar Kyun au was cultivated at the alluvial flood plain of Ayeyawady river at the altitude of 200-480 M ASL. According to the market data cultivar Kyun au was found to be the most expensive cultivar owning to it's quality of having very thin sheet, spicy odor and pungent taste. It can be postulated that cultivar Kyun au was feted to the local ecological condition of Tagaung Sub-township and which can be regarded as ecological niche-based crop of Tagaung area.

The transverse sections of leaf blade was shown in three tissue systems of dermal tissue system, ground tissue system and vascular tissue system. The leaf blades of six cultivars were found to be isobilateral type, this character agreed with Metcalfe & Chalk 1960 & Pandey & Chadha 2000. Dermal tissues system, six cultivars were found to be a single layer. The cells were compact, radially elongated, and convered by a cuticle layer at outer surface on both side. Anomocytic type of stomata were found on both surface of the six cultivars. Stomata has none subsidiary cells agreed with Stebbins and Kush 1961 as cited in Esau 1965. Types of stomata agreed with Frolich & Barthloot 1988 as cited in Kubitzki 1998. All of the epidermal cells were anticlinal walls straight and inner walls slightly convex. Elongated epidermal cells were found on both side of the six cultivars leaf blades, these characters agreed with Esau 1965 and Htwe Htwe Tin Maung 1984. The maximum thickness of leaf blades was found in Au Talone phyu 470.4-633.6 μm.

The ground tissues composed of leaf blades were differentiated into palisade and spongy parenchyma, this characters agreed with Htwe Htwe Tin Maung 1984. Palisades were one layered on both surface under the epidermis and right angles of the leaf epidermis in all of the six cultivars *A*. *sativum* L., these character was agreed with Pandey & Chadha 2000. Laticiferas were present on both surface of under palisade layer. All cultivars owned laticifer present in leaf mesophyll. They were between the second and third layers of parenchyma, this character coinsided with Esau 1965; Cronquist 1981and Htwe Htwe Tin Maung 1984.

The spongy cells were variable shapes and were loosely arranged with abundant intercellular spaces. The intercellular spaces which maintain containuity with stomatal chamber facilitate the gaseous exchange. In six cultivar, spongy were 5-10 layered, cells were rounded or oval inshape.The multiple layer of spongy was found in cultivar Shan hmwar khayan 6-10 layered. The calcium oxalate were common as octahedral, cubical, prismatic and rod like crystals were found in six cultivars this character was also agreed with Metcalfe & Chalk 1960 & Htwe Htwe Tin Maung 1984.

Vascular tissue system was composed of bundle sheaths and collateral type vascular bundles, oval or rounded in shape. The bundles were collateral in all cultivars and the phloem and the xylem were in contact with each other directly. In transverse sections, vascular bundles were surrounded by bundle sheaths occur in six cultivars of *A*. *sativum* L. leaf blades, this character also agreed with Esau 1965 & Pandey & Chadha 2000. The collateral type of vascular bundles were found on six cultivars of *A*. *sativum* L., this character was agreed with Edwin *et*

al. 2006 & Pandey & Chadha 2000, who stated that, monocotyledonous leaves was also, the bundle is conjoint, collateral and closed.

The multiple layer phoem was found in Shan hmwar phyu 1-8 layered. The perforation plate of vessel elements was found to be simple perforation plates in all cultivars. The longest length of vessel elements was found in cultivar Shan hmwar phyu ranged in 350-425 µm and the shortest in Shwe pan gone cultivar ranged in 70-115 µm. The highest level of vessel elements width was found in cultivar Shan hmwar phyu ranged in 150-195 µm and the lowest in cultivar Shwe pan gone 15-100 µm.

The longest length of tracheids was observed in cultivar Au talone phyu 145-2670 µm and the shortest in cultivar Shwe pan gone 15.-450 µm. The range of tracheid width was 5-25 µm in observed in all cultivars.The longest length of fiber was found in cultivar Shwe pan gone ranged 2373-2925 µm and the shortest in cltivar Kyun au ranged 300-875 µm. The range of fibers width was 10-30 found in all cultivars of the six cultivars.

The anatomical of characters of cultivar Au talone khayan was similar to cultivar Shan hmwar khayan and cultivar Au talone phyu was similar to cultivar Shan hmwar phyu. Therefore they were not different cultivars.

In the present research work, anatomical characters of six cultivars were found to be basically similar but slightly different in size and shape of cell, thickness of tissue layers and number of xylem layers.

Since Myanmar plays important role in garlic exporting countries at the level of the 6^{th} position in the global scale, Myanmar garlic cultivars have become increasingly important for generating foreign currency. Therefore, specific identification, morphological characters and anatomical characters are also increasingly important in classification of garlic cultivation because selection of commercial cultivars essentially relies on morphological and anatomical identification.

These characters were shape of epidermis, type of stomata, palisade parenchyma, spongy parenchyma, presence of calcium oxalate crystals and types of vascular bundle. Thus, these distinct anatomical characters mentioned above can contribute the specificity and characterization of each cultivar and also provide the feature taxonomic analysis of grouping of *A*. *sativum* L.

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