

ANATOMICAL INVESTIGATION OF *CROTALARIA LINIFOLIA* L.

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Abstract:

Fabaceae is economically and culturally very important family of flowering plants. It is easily recognized by their fruit legume or pod. The plants have ability to fix atmospheric nitrogen reduced fertilizer costs for farmers and gardeners who grow legumes, means that legumes can be used as in crop rotation to replenish soil that has been depleted of nitrogen. The genus *Crotalaria* L. is very large important genus of family Fabaceae composed of 81 species in India, and it is economically and medicinally useful. It is commonly known as 'rattlepods'. The *Crotalaria linifolia* L. is commonly known as 'Narrow-leaved crotalaria'. Morphological and anatomical study of different parts of *Crotalaria linifolia* L. like root, stem and leaf were done.

Key words: Anatomy, *Crotalaria*, Fabaceae.

Introduction:

Crotalaria L. is one of the large genus of the family Fabaceae. The studies on *Crotalaria* species are very little. It is an economically and medicinally important genus. It has wide uses. It is high quality fiber yielding genus and it is used in making fishing nets, cordage and canvas and also used for making cigarette papers. Many species are used as green manure, cover crops, nematode control, as forage and also for the control of soil erosion. *Crotalaria linifolia* L. used in folk medicine and traditional Chinese medicine.(Yadav S. R. and Sardesai M.M. 2003). It also helpful in taxonomic studies.

Material and methods:

The plant material were collected from Melghat region of Amravati district. The collected plant material were identified with the help of standard floras (Naik 1998; Cooke 1958; Dhore 2002). Detailed morphological characters were studied with the help of fresh material. The epidermal study for trichome and stomata were done by peeling method, the peeled part were stained in safranin and mounted in glycerin on a slide. The anatomical study were done by the free hand cut transverse section of fresh and preserved material i.e root, stem and leaves and the sections were dehydrated with different alcohol grades and stained with safranin and light green by double permant staining method.(Esau, 1965) These permanent prepared slides were observed under the microscope, and photographs were shoot out by using Trinocular microscope of Carl Zess.

Morphological Observation:

The plant body is erect, subfruticose, herbaceous, annual, up to 25-85 cm in height. Stem is cylindrical, branched, solid, densely silky hairy. 2-3.5×0.1-1.1 cm in size, with obtuse apex. Upper leaf surface hairy. Leaves simple, alternate opposite, exstipulate, sessile, linear, elliptical pubescent and lower leaf surface silky white shiny, hairy, with unicostate reticulate venation. Flowers are in terminal branched racemes 2-8 flowered racemes, bracteate, hairy, pedicelate, peduncle is 2-7 cm long. complete, bisexual, zygomorphic. Calyx green in color, hairy, gamosepalous, 5 toothed, two upper are 9 mm long subulate and three lower are 10 mm long, with obtuse apex. Corolla yellow consist of 5 petals. The uppermost largest standard with

9 × 8 mm in size having obovate shape, two lateral wings are with 7 × 5 mm in size, and two united petals to form keel with 8 × 5 mm in size. calyx and corolla are equal in length, after drying corolla convert in to black colour. The stamens are 10 in two bundles with 5+5 anthers bi-celled, dorsifixed, with staminal sheath length 4 mm and filament length 6 mm. The gynoecium is single, free, ovary superior with 8 mm in length, style is bent 13 mm in length, hairy, stigma simple. Pods quadrate, sessile, 0.2-0.4 cm long, hairy, green in colour, after drying pods are become black in color. Seeds are 3-6 in number brown, reniform.

Dermatology:

Trichome:

Trichomes present on both the surfaces, they are unbranched, non-glandular, uniseriate, with having one basal cell and elongated conical tapering tip, their average length of upper leaf surface 332.8 μm and in range 256 – 409.6 μm , and average length of lower leaf surface is 512 μm and in range 384 – 640 μm .

Stomata:

Leaves amphistomatic, (i.e present on both the surfaces), stomatas anisocytic, anomocytic, sometimes stomata with one guard cell, Thick Lining of subsidiary cells. Guard cells surrounded by three unequal subsidiary cells, among which one is distinctly smaller in size than the other two. Subsidiary cells are rectangular to polygonal in shape, with straight wall. From upper leaf surface average of pore is 14.5 × 5.9 μm , range 10.2 – 17.9 × 5.1 – 7.6 μm in size; guard cells average 22.1 × 16.2 μm , range 17.9 – 25.6 × 10.2 – 20.4 μm and subsidiary cells average 34.9 × 50.3 μm , range 25.6 – 43.5 × 38.4 – 61.4 μm in size. and from lower leaf surface average of pore is 16.2 × 8.5 μm , range 12.8 – 20.4 × 7.6 – 10.2 μm in size, guard cells average 25.6 × 18.7 μm , range 23 – 28.1 × 15.3 – 23 μm , and subsidiary cells average 39.2 × 59.7 μm and range 25.6 – 51.2 × 46 – 81.9 μm in size

Anatomical observation:

T. s of root:

The transverse section showed somewhat circular outline. 1 to 2 layered cork tissues becomes dead and ruptured at some places which measured 11 × 29.8 μm in average and 10.2 – 12.8 × 25.6 – 51.2 μm in range. Below the cork periderm or cortex 1 to 2 layered which measured 18.7 × 24.7 μm in average and 12.8 – 25.6 × 17.9 – 30.7 μm in range. Below the cortex present prominent single layered endodermis with barrel shaped compactly arranged cells, which measured 13.6 × 28.1 μm in average and 12.8 – 15.3 × 25.6 – 30.7 μm in range. Below the endodermis present parenchymatous pericycle, which measured 21.3 × 31.5 μm in average and 20.4 – 25.6 × 25.6 – 38.4 μm in range. Below the pericycle primary phloem present and secondary phloem found in patches, phloem composed of rounded, squarish and oval compactly arranged cells with few fibers which measured 13.6 × 23 μm in average and 7.6 – 20.4 × 15.3 – 30.7 μm in range. Below the phloem present strips of 5 to 6 layered rectangular flatten cambium tissue which measured 7.6 × 21.3 μm in average and 5.1 – 10.2 × 17.9 – 25.6 μm in range. Below the cambium present a broad massive xylem cylinder, proto-xylem present towards periphery which measured 38.4 × 33.2 μm in average and 28.1 – 35.8 × 25.6 – 46 μm in range. and meta-xylem towards centre which measured 73.3 × 64 μm in average and 64 – 79.3 × 51.2 – 76.8 μm in range. Vessels dense, scattered, present singly or in group rounded, oval squarish and polygonal, thick walled primary xylem present towards centre Protoxylem measured 25.6 × 21.3 μm in average and 20.4 – 30.7 × 17.9 – 25.6 μm in range. And metaxylem measured 38.4 × 43.5 μm in average and 25.6 – 51.2 × 35.8 – 51.2 μm in range. Medullary rays biseriate to multiseriate, multicellular, composed of oval, rectangular or elongated in shape, thin walled, which measured 29 × 19.6 μm in average and 25.6 – 30.7 × 15.3 – 25.6 μm in range. Medullary rays run from primary xylem through the secondary xylem to phloem, in the phloem region medullary rays get dilated shows wedge shape structure.

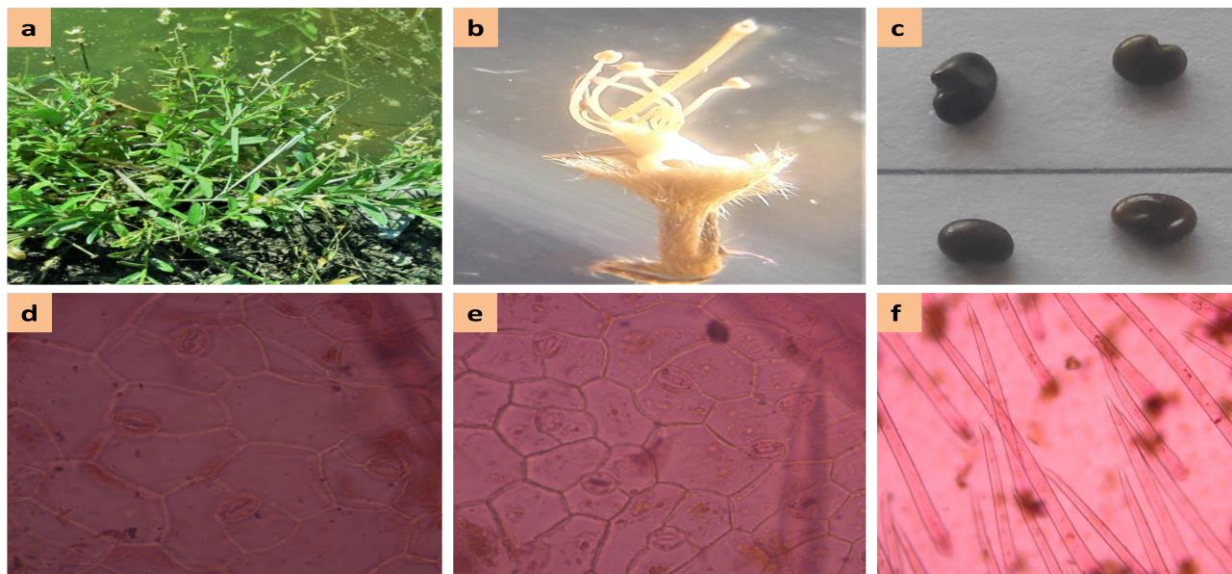
T. S of stem :

Transverse section of stem showed circular outline. Epidermis single layered covered externally with more thick cuticle, epidermal cells barrel shaped, rectangular compactly arranged which measured $20.4 \times 31.5 \mu\text{m}$ in average and $17.9 - 23 \times 25.6 - 38.4 \mu\text{m}$ in range. On the epidermis trichomes and stomatas observed. Stomatas present in small dipression like portion. Below the epidermis single layered hypodermis present cells, thick walled, collenchymatous which measured $11 \times 22.6 \mu\text{m}$ in average and $10.2 - 12.8 \times 20.4 - 24.3 \mu\text{m}$ in range. Below the hypodermis present parenchymatous cortex, 4 to 5 layered cells thin walled irregular in shape with some pigment granules, which measured $19.6 \times 12.8 \mu\text{m}$ in average and $12.8 - 28.1 \times 7.6 - 17.9 \mu\text{m}$ in range. Below the cortical region single layered prominent endodermis with barrel shaped, cells rectangular compactly arranged which measured $18.3 \times 35.8 \mu\text{m}$ in average and $17.9 - 19.2 \times 28.1 - 43.5 \mu\text{m}$ in range. Below the endodermis schlerenchymatous 4 to 5 layered patches of pericycle which measured $18.7 \times 19.6 \mu\text{m}$ average and $10.2 - 25.6 \times 12.8 - 30.7 \mu\text{m}$ in range. Phloem present next to pericycle 9 to 10 layered cells ovate, elliptical, squarish, and polygonal in shape which measured $10.2 \times 17 \mu\text{m}$ in average and $7.6 - 12.8 \times 12.8 - 20.4 \mu\text{m}$ in range. Below the phloem 2 to 3 layered flattened stripe like cambium present in between the phloem and xylem, which measured $6.4 \times 15.3 \mu\text{m}$ in average and $5.1 - 7.6 \times 12.8 - 17.9 \mu\text{m}$ in range. Broad continues xylem cylinder present meta-xylem present towards periphery and proto-xylem towards centre, vessels circular, squarish or polygonal arranged in rows protoxylem measured $33.2 \times 28.1 \mu\text{m}$ in average and $25.6 - 49.5 \times 23 - 35.8 \mu\text{m}$ in range. And metaxylem measured $53.7 \times 43. \mu\text{m}$ 5 in average and $46 - 64 \times 38.4 - 48.6 \mu\text{m}$ in range. Medullary rays parenchymatous uniseriate to biseriate, multicellular traversed through the xylem and phloem which measured $23.8 \times 19.6 \mu\text{m}$ in average and $17.9 - 28.1 \times 15.3 - 23 \mu\text{m}$ in range. Central large pith composed of rounded isodimetric, polygonal, thin walled parenchymatous loosely arranged with intercellular spaces, central cells of pith enlarge than peripheral one which measured $58 \times 64 \mu\text{m}$ in average and $30.7 - 102.4 \times 38.4 - 102.4 \mu\text{m}$ in range.

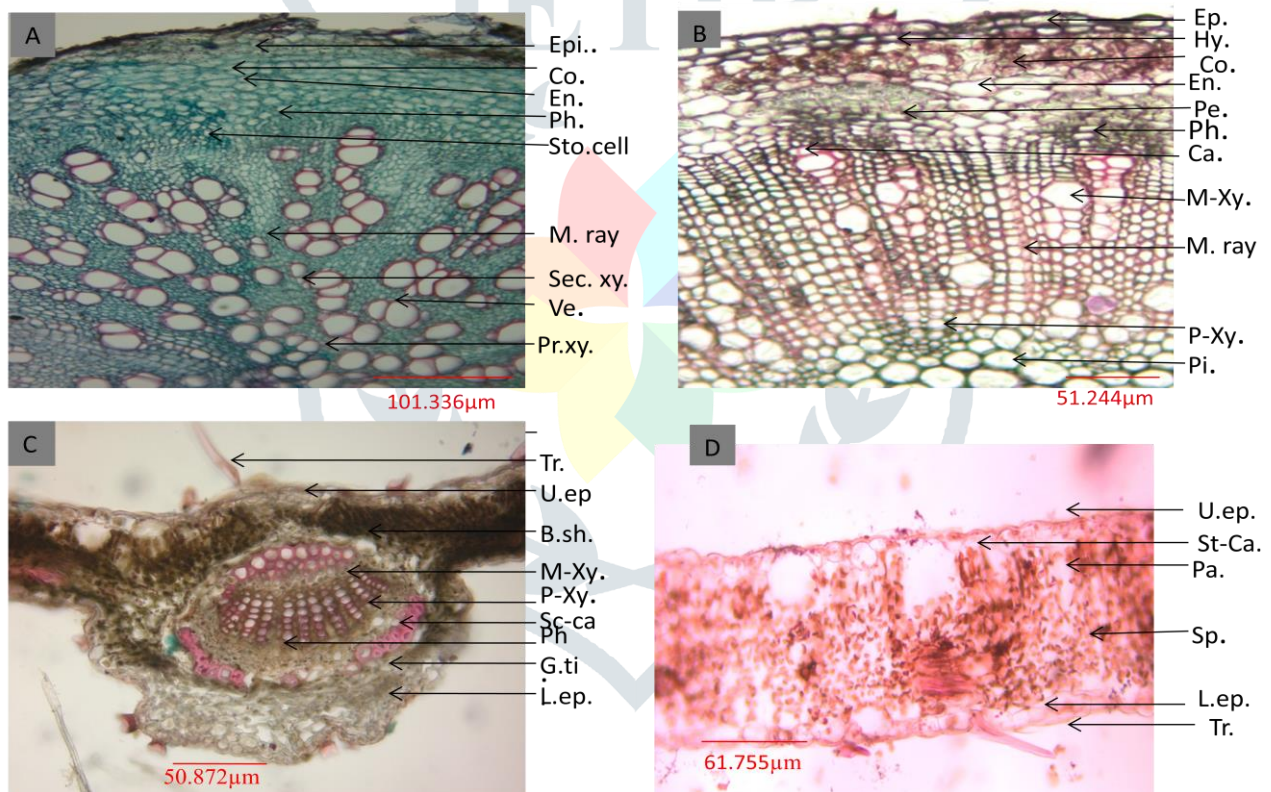
V. S. of leaf:

The vertical section of leaf showed typical bifacial / dorsiventral structure. The epidermis of both the surfaces such as upper and lower single layered covered with thick cuticle, leaf amphistomatic stomata's confined to upper and lower epidermis. Trichome's also confined to both the surfaces. The upper epidermis in midrib region composed of squarish, columnar, and rounded to rectangular compactly arranged cells which measured $26.4 \times 52 \mu\text{m}$ in average and $20.4 - 33.2 \times 40.9 - 64 \mu\text{m}$ in range. In the lamina region upper epidermal cells larger than midrib region. Cells rectangular, rounded, to flattened compactly arranged which measured $36.6 \times 43.5 \mu\text{m}$ in average and $25.6 - 48.6 \times 33.2 - 53.7 \mu\text{m}$ in range. Lower epidermal cells rounded, oval, squarish, rectangular, smaller than upper one which measured $17.9 \times 27.3 \mu\text{m}$ in average and $15.3 - 20.4 \times 25.6 - 30.7 \mu\text{m}$ in range. In arm region the cells bigger which measured $23.8 \times 29 \mu\text{m}$ average and $17.9 - 28.1 \times 25.6 - 33.2 \mu\text{m}$ in range. Mesophyll differentiated in to palisade and spongy parenchyma tissue, 1 to 2 layered columnar, elongated palisade parenchyma observed below the upper epidermis. Cells filled with full of chloroplasts, loosely arranged with intercellular spaces and air cavities which measured $20.4 \times 11 \mu\text{m}$ in average and $15.3 - 25.6 \times 7.6 - 12.8 \mu\text{m}$ in range. Following to lower epidermis present spongy tissue cells 4 to 5 layered oval, circular, polygonal to irregular thin walled loosely arranged with intercellular spaces, showed presence of chloroplast and some pigment granules, which measured $14.9 \times 20.4 \mu\text{m}$ in average and $12.8 - 16.6 \times 17.9 - 23 \mu\text{m}$ in range. In the mid rib region vascular tissue encircled by single layered bundle sheath composed of rounded, oval, flatten barrel shaped, compactly arranged thin walled parenchymatous cells which measured $23 \times 38.4 \mu\text{m}$ in average and $17.9 - 28.1 \times 23 - 58.8 \mu\text{m}$ in range. Below the bundle sheath on the upper and lower sides of the vascular tissue present schlerenchymatous caps. 3 to 4 layered. Cells rounded, hexagonal to polygonal thick walled compactly arranged which measured $19.6 \times 23 \mu\text{m}$ in average and $12.8 - 30.7 \times 15.3 - 30.7 \mu\text{m}$ in range. Following to lower epidermis present 5 to 6 layered parenchymatous ground tissue with rounded, polygonal irregular, cells with wavy cell wall, loosely arranged with intercellular spaces which measured $48.6 \times 50.3 \mu\text{m}$ in average and $46 - 51.2 \times 38.4 - 61.4 \mu\text{m}$ in range. Inside the lower schlerenchymatous cap phloem present in the form of griddle present abaxially (towards the lower surface) composed of squarish, rounded, irregular thin walled which measured $11.5 \times 10.2 \mu\text{m}$ in average and $8.9 - 15.3 \times 5.12 - 17.9 \mu\text{m}$ in range. Xylem present in adaxial position (towards the upper epidermis) meta-xylem observed towards periphery and proto-xylem towards centre, cells rounded to polygonal arranged in specific rows which measured $22.1 \times 16.2 \mu\text{m}$ average and $15.3 - 30.7 \times 12.8 - 17.9 \mu\text{m}$ in range. In the midrib region below the upper epidermis at the centre present 1 to 2 layered parenchymatous ground tissues.

Note: In range mentioned, length (i.e smaller to bigger) × width (smaller to bigger).



a) Habit , b) Androecium and gynoecium , c) Seeds , d) Upper leaf surface stomata , e) Lower leaf surface stomata , f) Trichomes



A- T. S. of root , B- T. S.of Stem , C- V. S. of Lea,f D- Leaf lamina, Epi- Epiblema, Ep- Epidermis , U-ep- Upper epidermis, L-ep- Lower epidermis, Pa- Pallisade, Sp- Spongy parenchyma, G-Ti Ground Tissue, Sc-Ca- Schlerenchymatous cap. Hy- Hypodermis, Pe- pericycle, Co.- Cortex, En- Endodermis, B.sh- Bundle sheath, Ph- phloem, Ca- Cambium, pr-Xy- Primary xylem, Sec.xy- Secondary xylem, M-xy- Metaxylem, P-xy- Protoxylem, M-ray- Medullary ray, Ve- Vessel, Pi- Pith, Tr- trichome. G. ti- Ground tissue , St-Ca.- stomatal cavity.

Table I : Morphological characters:

	Characters	Observation
Vegetative	Habit	Herb
	Plant Height	25-85
	Life form	Erect/subfuticose
	Surface	Silky-hary
	Type	Simple
	Shape	Linear/ Eliptical

Leaf	Dimensions (cm)	1.2-3.5×0,1-1
	Apex	Obtuse
	Upper leaf surface	Pubescent
	Lower leaf surface	White shiny
Inflorescence Bract	Position / type	Terminal raceme
	Peduncle (cm)	1.4 – 3
	No. of flowers	3-6
	Shape	Linear
Calyx	Pubescence	Pubescent
	Calyx tube (mm)	3
	Upper sepal (mm)	9
	Lower sepal (mm)	10
	Teeth shape	Subulate
	Apex	Obtuse
Corolla	Pubescence	Hairy
	Colour	Yellow
	Standard Size (mm)	9.3×8
	Standard shape	Obovate
	Wing size	7×5
Androecium	Keel Size	8.2×5
	Staminal sheath length	4
Gynoecium	Filment length	6
	Ovary length	8
	Style length	13
Pod	Style pubescence	Hairy
	Size (cm)	02-0.4
	Shape	Quadrate
Seeds	No. of seeds	3-6
	Shape	Reniform
	Colour	Brown

Table II : Root Anatomy

Cell type	Dimensions in <i>C. linifolia</i> L.	
	Average (µm)	Range (µm)
Epiblema	11 × 29.8	10.2 – 12.8 × 25.6 – 51.2
Cortex	18.7 × 24.7	12.8 – 25.6 × 17.9 – 30.7
Endodermis	13.6 × 28.1	12.8 – 15.3 × 25.6 – 30.7
Pericycle	21.3 × 31.5	20.4 – 25.6 × 25.6 – 38.4
Phloem	13.6 × 23	7.6 – 20.4 × 15.3 – 30.7
Cambium	7.6 × 21.3	5.1 – 10.2 × 17.9 – 25.6
Sec. Protoxylem	38.4 × 33.2	28.1 – 35.8 × 25.6 - 46
Sec. Metaxylem	73.3 × 64	64 – 79.3 × 51.2 – 76.8
Pri. Protoxylem	25.6 × 21.3	20.4 – 30.7 × 17.9 – 25.6
Pri. Metaxylem	38.4 × 43.5	25.6 – 51.2 × 35.8 – 51.2
Medullary Rays	29 × 19.6	25.6 – 30.7 × 15.3 – 25.6
Stone cells	12.8 × 11	10.2 – 15.3 × 7.6 – 12.8
Pith	-	-

Table III : Stem Anatomy

Cell type	Dimensions in <i>C. Linifolia</i> L.	
	Average (μm)	Range (μm)
Epidermis	20.4 × 31.5	17.9 – 23 × 25.6 – 38.4
Hypodermis	11 × 22.6	10.2 – 12.8 × 20.4 – 24.3
Cortex	19.6 × 12.8	12.8 – 28.1 × 7.6 – 17.9
Endodermis	18.3 × 35.8	17.9 – 19.2 × 28.1 – 43.5
Pericycle	18.7 × 19.6	10.2 – 25.6 × 12.8 – 30.7
Phloem	10.2 × 17	7.6 – 12.8 × 12.8 – 20.4
Cambium	6.4 × 15.3	5.1 – 7.6 × 12.8 – 17.9
Protoxylem	33.2 × 28.1	25.6 – 49.5 × 23 – 35.8
Metaxylem	53.7 × 43.5	46 – 64 × 38.4 – 48.6
Medullary rays	23.8 × 19.6	17.9 – 28.1 × 15.3 – 23
Pith cells	58 × 64	30.7 – 102.4 × 38.4 – 102.4

Table IV : Leaf Anatomy

Cell type	Dimensions in <i>C. linifolia</i> L.	
	Average (μm)	Range (μm)
Upper epidermis	26.4 × 52	20.4 – 33.2 × 40.9 – 64
Lower epidermis	17.9 × 27.3	15.3 – 20.4 × 25.6 – 30.7
Arm region upper epidermis	36.6 × 43.5	25.6 – 48.6 × 33.2 – 53.7
Arm region lower epidermis	23.8 × 29	17.9 – 28.1 × 25.6 – 33.2
Palisade Mesophyll	20.4 × 11	15.3 – 25.6 × 7.6 – 12.8
Spongy Parenchyma	14.9 × 20.4	12.8 – 16.6 × 17.9 – 23
Ground tissue	48.6 × 50.3	46 – 51.2 × 38.4 – 61.4
Schlerenchyma tissue	19.6 × 23	12.8 – 30.7 × 15.3 – 30.7
Endodermis	23 × 38.4	17.9 – 28.1 × 23 – 58.8
Phloem	11.5 × 10.2	8.9 – 15.3 × 5.12 – 17.9
Xylem	22.1 × 16.2	– 30.7 × 12.8 – 17.9

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