



Metamorphosis differences of caudiciform plants as an adaptation to arid conditions

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In the course of evolution, the formation of succulent variations in the underground and surface parts of plants in different taxa could occur simultaneously under the influence of similar abiotic factors, in particular as an adaptation to arid conditions, and similar structural features even in distant families are a manifestation of convergent evolution. Along with this, the development of such adaptive structures in ontogeny can occur in different ways in different taxa. Studying the anatomical and morphological features of the vegetative organs of *Fockea edulis* (Asclepiadoideae, Apocynaceae) at the early stages of ontogenesis and comparing them with those of other plants will improve the understanding of the features of growth and development of caudex-like plants. The research was conducted on seedlings, juvenile and immature plants of *F. edulis*. When comparing the features of the development of vegetative parts of three representatives of the Apocynaceae family, we found that the strategy of succulent traits development in *F. edulis* at the organ level is characterized by the active development of the hypocotyl already at the seedling stage, similar to *Adenium obesum*, and the subsequent formation of an expanded basal part of the stem due to the combined cortex-pith thickening and radish-like root due to the growth of xylem parenchyma. However, *Petopentia natalensis* is characterized by a mesophytic seedling. It was established that secondary thickening in the basal part of the stem occurs in two individual ways: in *Adenium obesum*, the cambium generates solid rings of phloem and parenchymatized xylem; in *P. natalensis* and *F. edulis*, the bundle type of conducting elements is preserved. The thickening of the tap root in *F. edulis* is similar to that in *A. obesum*.

Keywords: *Fockea edulis*; *Adenium obesum*; *Petopentia natalensis*; Apocynaceae; stem and root anatomy; drought adaptation; seedlings, juvenile plant; immature plant.

Introduction

The family Apocynaceae Juss. in its modern conception includes about 366 genera and more than 5000 species. Five subfamilies are distinguished within the Apocynaceae family: Apocynoideae, Asclepiadoideae, Periplocoideae, Rauvolfioideae and Secamonoideae (Endres et al., 2014). Most of the species are concentrated in the Holarctic, Palearctic and Neotropical regions, to a lesser extent in the Australian and Cape kingdoms. Many species of the family are common in arid or semiarid climates and are classified as succulents.

The group of perennial herbaceous succulent plants is concentrated in the subfamily Asclepiadoideae, mostly in the subtribe Stapeliinae, and is considered the largest in the family Apocynaceae. It includes more than 30 genera, the most famous of which are: *Stapelia*, *Huernia*, *Hoodia*, *Pseudolithos*, *Orbea*, *Quaqua*, etc. (Eggl, 2002; Albers & Meve, 2004). A characteristic feature of these plants is the formation of succulent single (*Pseudolithos*) or branched (*Stapelia*, *Huernia*) plagiotropically or orthotropically oriented stems. Representatives of the group are similar to individual succulent plants from the families Cactaceae Juss. and Euphorbiaceae Juss. in many anatomical and morphological features. This is confirmed by researchers who pointed out the features of convergent evolution between succulents originating from different rather distant families (Willert et al., 1992; Mauseth & Plemons-Rodriguez, 1998; Endres et al., 2014).

The group of tree or pachycaul succulents is represented by plants from the genus *Pachypodium*, which are part of the subfamily Apocynoideae (Rowley, 1999; Wickens et al., 2008; Hearn, 2009). Study of the anatomy and morphology of pachycaul representatives from the genera *Pachypodium*, as well as pachycaul succulents from other genera *Adansonia* L. (Malvaceae), *Cyphostemma* (Planch.) Alston (Vitaceae), *Dendrosicyos* Balf. f., *Dorstenia* L. (Cucurbitaceae), *Fouquieria* Kunth (Fouquieriaceae), *Moringa* Adans. (Moringaceae), found that in addition to cortex parenchyma growth, they are also characterized by the formation

of “juicy wood” formed due to the functioning of the cambium, which, in addition to tracheal elements, forms a large number of thin-walled xylem parenchyma cells. Water and nutrients accumulate in these parenchyma cells, like in parenchyma cells of the cortex (Olson & Carlquist, 2001; Olson, 2003; Chapotin et al., 2003, 2006; Wickens & Lowe, 2008). More detailed anatomical and morphological studies of pachycaul plants of the Apocynaceae family were conducted, only on the example of several taxa of the genus *Pachypodium* (*P. lanerei*, *P. horombense* H. Poisson) (Endress et al., 2014; El-Kashef et al., 2015).

Among the various forms of succulent plants, including the Apocynaceae family, a special group is represented by “caudex-like succulents”. For the first time, this term was used by G. Rowley in 1948 to unite succulents, the shoots of which have a modified highly extended, perennial basal part, which can be above-ground or partially submerged in the soil (Rowley, 1999).

Although the group of caudex-like succulents is not sufficiently studied, it includes a fairly large number of succulent shrubs and bushes from the family Asparagaceae Juss. (*Calibanus* Rose), as well as semi-woody forms (semi-shrubs) from various families: Dioscoreaceae R. Br. (*Dioscorea elephantipes* (L'Hér.) Engl., *D. sylvatica* Eckl.), Cucurbitaceae Juss. (*Kedrostis* Medik., *Gerrardanthus* Harv. ex Benth. & Hook. f., *Momordica* L., *Ibervillea* (S. Wats.) Greene, *Zehneria* Endl.), Passifloraceae Juss. ex Rousset (*Adenia* Forssk.), etc. According to F. Albers and U. Meve, mostly perennial semi-shrubs (hamephytes according to Raunkjer): *Bra-chystelma*, *Cibirhiza*, *Fockea*, *Microloma*, *Petopentia*, *Raphionacme*, *Schlechterella*, etc. are among the caudex-like succulents of the family Asclepiadaceae (Asclepiadoideae according to IPG III) (Albers & Meve, 2004). Caudex-like succulent shrubs include plants from the genus *Adenium* (Apocynoideae), which have gained high popularity as ornamental plants in recent years (Eggle, 2002; Gaidarzhy et al., 2015). Research conducted by D. Willert and colleagues for some time questioned the “succulence” of caudex-like plants, since, apart from the expanded basal

part of the shoots, their other vegetative organs do not undergo significant changes (Willert et al., 1990). This is due to the lack of appropriate critical analysis of the modified parts of the shoots of these taxa, which are based mostly on descriptive studies of model objects of different ages and origins in natural conditions. At the same time, insufficient information is known about the anatomical structure and features of the formation of these changes, which could determine the biomorphological status of these plants in more detail (Pate & Dixon, 1982; Eggle & Nyffeler, 2009).

The most in-depth anatomical and morphological studies conducted on adult plants of the genus *Adenia* (Passifloraceae) showed that many taxa of this genus are characterized by a significant expansion of the basal part of the stem and, in part, of the main root. Almost all studied taxa are characterized by noticeable primary thickening due to the growth of the pith and cortex parenchyma. Parenchymal thin-walled cells were noted for their large size, significant content of starch grains and various lipids. A developed specialized wood was found, which was characterized by significant parenchymatization and the formation of cavities of various sizes where water accumulates (Hearn, 2009; Hearn & Spicer, 2013). The researchers made an attempt to summarize the available information on the structure of stem, pachycaul and caudex-like succulents, and, based on existing data on anatomy and morphology, to identify possible ways of their evolution. As a result of the research, it was suggested that caudex-like plants may be the first evolutionary link in the acquisition of succulent features, while woody, stem succulents and pachycaul plants are more complex and specialized forms. The authors suggest that the formation of expanded basal parts of shoots in caudex-like plants can occur due to the development of the hypocotyl or the tap root (Hearn 2013; Hearn & Spicer, 2013). These assumptions partially coincide with the data of J. Mauseth et al., that during evolution the formation of succulent variations in underground and surface parts of Cactaceae plants is more likely than in others (Mauseth & Sajeve, 1992; Mauseth, 2000; Mauseth & Stone-Palmquist, 2001). The conclusions of D. Willert and colleagues additionally indicate that the acquisition of succulent properties in different taxa could occur simultaneously under the influence of similar abiotic factors and similar structural features even in distant families are a manifestation of convergent evolution (Willert et al., 1990, 1992).

Therefore, the establishment of evolutionary and family relationships between various succulent representatives, as well as the detection of the phenomenon of convergence, requires not only phylogenetic, but also anatomical and morphological research. U. Eggle pointed out that for a more complete understanding of the biological features of caudex-like plants and succulents in general, great importance should be attached to observing their development under the conditions of introduction (Eggle & Nyffeler, 2009). Previously, we conducted an anatomical and morphological study of the seeds and embryos of three representatives of the family: *Adenium*, *Petopentia*, and *Fockea*, which showed that the embryo of both *Adenium* and *Fockea* already has a thickened hypocotyl, where necessary nutrients are stored (Aviekin et al., 2016). The further goal of our study was to clarify the anatomical and morphological features of the vegetative organs of *Fockea edulis* (Asclepiadoideae, Apocynaceae) at the early stages of ontogenesis for a more complete understanding of the features of growth and development of caudex-like plants.

Material and methods

Fockea edulis was described by C. Thunberg in 1794 as *Pergularia edulis* Thunb., and in 1893 K. Schuman assigned this taxon the generic name *Fockea* nom. cons. According to the modern classification, *F. edulis* is one of the six species of the genus *Fockea*, which is distributed in the form of separate localities in the arid regions of the Western and Eastern Cape provinces of South Africa, as well as in the southern regions of the Kingdom of Lesotho (Albers & Meve, 2004). *Fockea edulis* is a succulent perennial semi-shrub, which has a massive root tuber of a rounded-elongated shape at first, and with age a rounded shape, partially submerged in the soil, up to 0.5 m in height and 1 m in diameter. The surface of the tuber is grayish-green, the bark is cracked with various wrinkles and outgrowths (Albers & Meve, 2004). Thin liana-like shoots of gray-green color are formed on the tops of the tuber, which partially die under unfavorable conditions. The leaves are green or light green, short-petioled up to 4 cm

long, elliptical or egg-shaped with a wavy edge, placed opposite. Five-sided greenish flowers, up to 3 cm in diameter, are collected in small tassell-like inflorescences formed from axillary buds. Fruits are spindle-shaped, dark green and with dorsal seams. According to Vasiliev et al. (1978), the life cycle of plants consists of the following periods: latent, pregenerative, generative and senile. In turn, the pregenerative period is divided into stages: seedling, juvenile and immature plants.

The research was conducted on seedlings, juvenile and immature plants of *F. edulis*, grown from the solution obtained from the collection plants of the Botanical Garden named after Academician O. V. Fomin of Taras Shevchenko Kyiv National University. For study of the anatomical structure, we took the middle part of the hypocotyl and the tap root of seedlings, as well as the middle part of the upper part of the stem, the basal parts of the stem and the tap root of plants of 6–7 weeks of vegetation, which are in the juvenile stage of development and of plants of 11–12 months of vegetation (immature stage of development). We fixed samples by FAA (formalin, acetic acid, alcohol), embedded in gelatin by the standard method (Romeis, 1948) and with the help of a freezing microtome we made cross-sections of the stem and root with a thickness of 10–15 μm . Sections were stained with safranin. Microscopic measurements were performed using an XSP-146TR microscope and Image J program (Wayne Rasband, NIH). Morphometric measurements of leaves, stems and roots were also made.

The data were analyzed in Prism Graphpad 6 (GraphPad Company, San Diego, USA, 2014). The values for different groups (significant difference of the parameter relative to the previous stage of development) were compared by ANOVA followed by a Bonferroni test. Photographs were taken with a Canon Power Shot A630 digital camera.

Results

Seedling. Germination of *F. edulis* seeds occurs on the 8–9th day after sowing, and the seedlings are released from the seed coats on the 11–13th day. Cotyledons are rounded, fan-shaped, dark green in color, hypostomatic, without expressive succulent features (Fig. 1A, Table 1). The hypocotyl is thickened, cylindrical in shape, clearly stands out compared to other parts of the seedling. Its surface is smooth, the same color as the cotyledons (Fig. 1A, Table 1). A cross-section of the middle part shows a single-layered epidermis, represented by cylindrical cells with slightly thickened walls. The cortex parenchyma is under the epidermis, which consists of 13–17 layers of thin-walled isodiametric cells with starch grains. Chloroplasts are present in the parenchymal cells bordering the epidermis.

In the thickness of the cortex parenchyma, next to the vascular bundles, there are non-articulated latex ducts. Seedlings of *F. edulis* have 17 to 20 vascular bundles of the collateral type. The phloem zones are less developed and located directly above the primary xylem. The pith is represented by vacuolated, thin-walled, isodiametric cells, in the thickness of which there are single non-articulated latex ducts (Fig. 2A).

The tap root of *F. edulis* is less developed compared to the hypocotyl (Fig. 1A). The conducting system is represented by 6–7 tangentially placed vascular bundles, which are located close to each other. Xylem tracheary elements are grouped by 4–6 pieces, above which less expressed phloem zones are observed (Fig. 2B).

Juvenile plants. After six to seven weeks of growth, seedlings of *F. edulis* form signs of juvenile plants, a shoot is formed, represented by 4–6 nodes with the opposite arrangement of leaves. Hypostomatic leaves are without expressed succulent features, 11.34 ± 2.60 mm long. The stem is morphologically heterogeneous, it can be conventionally divided into the upper (apical) non-succulent part and the thickened hypocotyl (basal part) of the stem (Fig. 1B, Table 1).

The upper part of the stem is orthotropic, rounded in cross section, grassy. The average length of the internode is 12.09 ± 5.48 mm. The surface of the light green stem is slightly rough (Fig. 1B). The epidermal layer is represented by cubic cells with thickened walls, some of the epidermal cells bear simple, unicellular covering trichomes 19.53 ± 3.64 μm long. The periderm is observed under the epidermis, consisting of one layer of cubic phellem cells with lignified cell walls, a single-layer phellogen, the thin-walled cells of which are tangentially flattened, and a layer of cylindrical thin-walled phelloderm cells with chloroplasts (Fig. 3A).



Fig. 1. Different age stages of the *Fockea edulis* plants: *a* – seedling, *b* – juvenile plant, *c* – immature plant: 1 – cotyledon, 2 – hypocotyl, 3 – basal part of the stem, 4 – upper part of the stem, 5 – tap root, 6 – lateral roots, 7 – juvenile leaves, 8 – leaves of immature plant

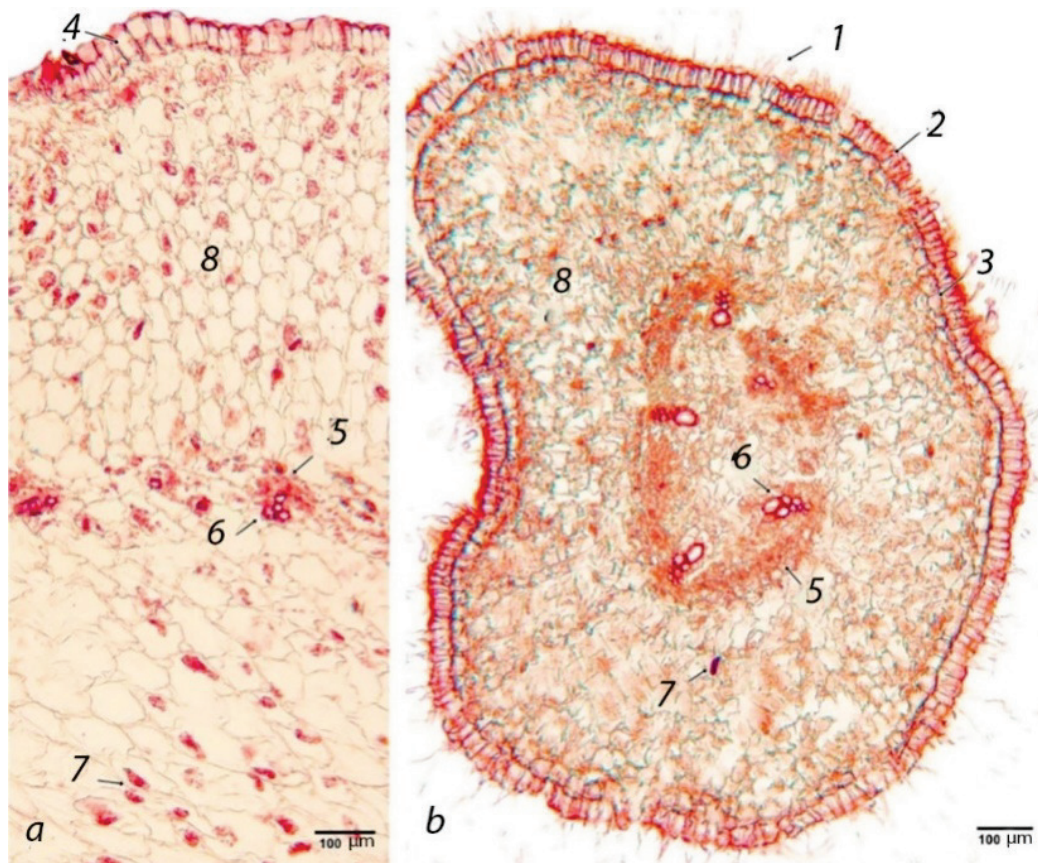


Fig. 2. Anatomical structure of *Fockea edulis* seedlings: *a* – hypocotyl, *b* – tap root: 1 – root hairs, 2 – epiblema, 3 – exodermis, 4 – epidermis, 5 – phloem, 6 – xylem, 7 – latex ducts, 8 – cortex parenchyma

The cortex parenchyma consists of 5–6 layers of thin-walled isodiametric cells, in the thickness of which are scattered single non-articulate latex cells. Chloroplasts are observed in the cortex parenchymal cells bordering the phelloderm, while the latter are almost absent in the cells of the deeper layers. Lignified xylem vessels are placed in radial bundles of 3–5 pieces, uniting into an almost continuous ring. Bundles of bast fibers with thickened lignified cellular walls are present. Intraxylary phloem is represented by separate tangentially placed zones of conductive elements.

Table 1

Morphometric indicators of *Fockea edulis* plants of different age states of the pregenerative period of ontogenesis ($\bar{x} \pm m$, $n = 6$, mm)

Organ	Parameter	Seedlings	Juvenile plants	Immature plants
Cotyledons/ leaves	l	5.42 ± 0.92	11.34 ± 2.57***	38.27 ± 4.34***
	h	5.13 ± 0.73	6.71 ± 0.89***	24.86 ± 2.52***
	b	0.24 ± 0.21	0.27 ± 0.13	0.45 ± 0.23*
Upper part of the stem	l	–	52.42 ± 13.53	546.73 ± 181.41***
	d	–	1.52 ± 0.27	2.33 ± 0.82*
Hypocotyl/basal part of the stem	l	9.84 ± 1.8	16.51 ± 3.11***	29.18 ± 6.89***
	d	3.72 ± 0.5	7.87 ± 1.42***	30.26 ± 5.32***
Tap root	l	7.35 ± 1.2	34.12 ± 11.71***	52.32 ± 19.45***
	d	1.13 ± 0.2	1.37 ± 0.45*	11.94 ± 6.34***

Notes: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ significant difference of the parameter relative to the previous stage of development was compared by ANOVA followed by a Bonferroni test; l – length, h – width, b – thickness, d – diameter.

The pith is weakly developed and is represented by small rounded, thin-walled cells, among which there are a large number of non-segmented latex ducts. The thickened basal part of the stem of juvenile *F. edulis* plants, which was formed due to the growth of the hypocotyl, has an ovoid shape. Its surface is green, slightly rough. The length and diameter of the basal part of the stem, compared to the length of the hypocotyl of the seedlings, increases almost twice (Fig. 3B, Table 1).

The cross-section revealed the formation of secondary integuments and almost complete peeling of the epidermis. The periderm is represented by three layers of cylindrical phellem cells with thin and weakly lignified walls and 2–3 layers of flattened thin-walled phelloderm cells. Phellogen is weakly determined and looks like a thin layer of tangentially flattened thin-walled cells. Chloroplasts are present in the protoplasts of phelloderm cells, similar to the upper part of the stem. The periderm thickness of the basal part of the stem is three times greater compared to its upper part, which is due not only to a greater number of cell layers, but also to an increase in the size of the cells (Fig. 3B). The cortex parenchyma is more developed and consists of 10–12 layers of large, isodiametric thin-walled cells. In the basal part of the stem, the conducting system is represented by the same number of vascular bundles as in the seedling hypocotyl, but due to the increase in the number of parenchymal cells, they are located at a greater distance (Fig. 2A, 3B, Table 2). One should note the increase in the area of vascular bundles, due to the increase in the number of xylem tracheal elements, which are larger in size compared to seedlings. Zones of intraxylary phloem were found in the vascular bundles, the area of which is much larger compared to the upper part of the stem. The pith, compared to other parts, develops significantly: not only the number of cells increases, but also their size (Fig. 3B, Table 2).

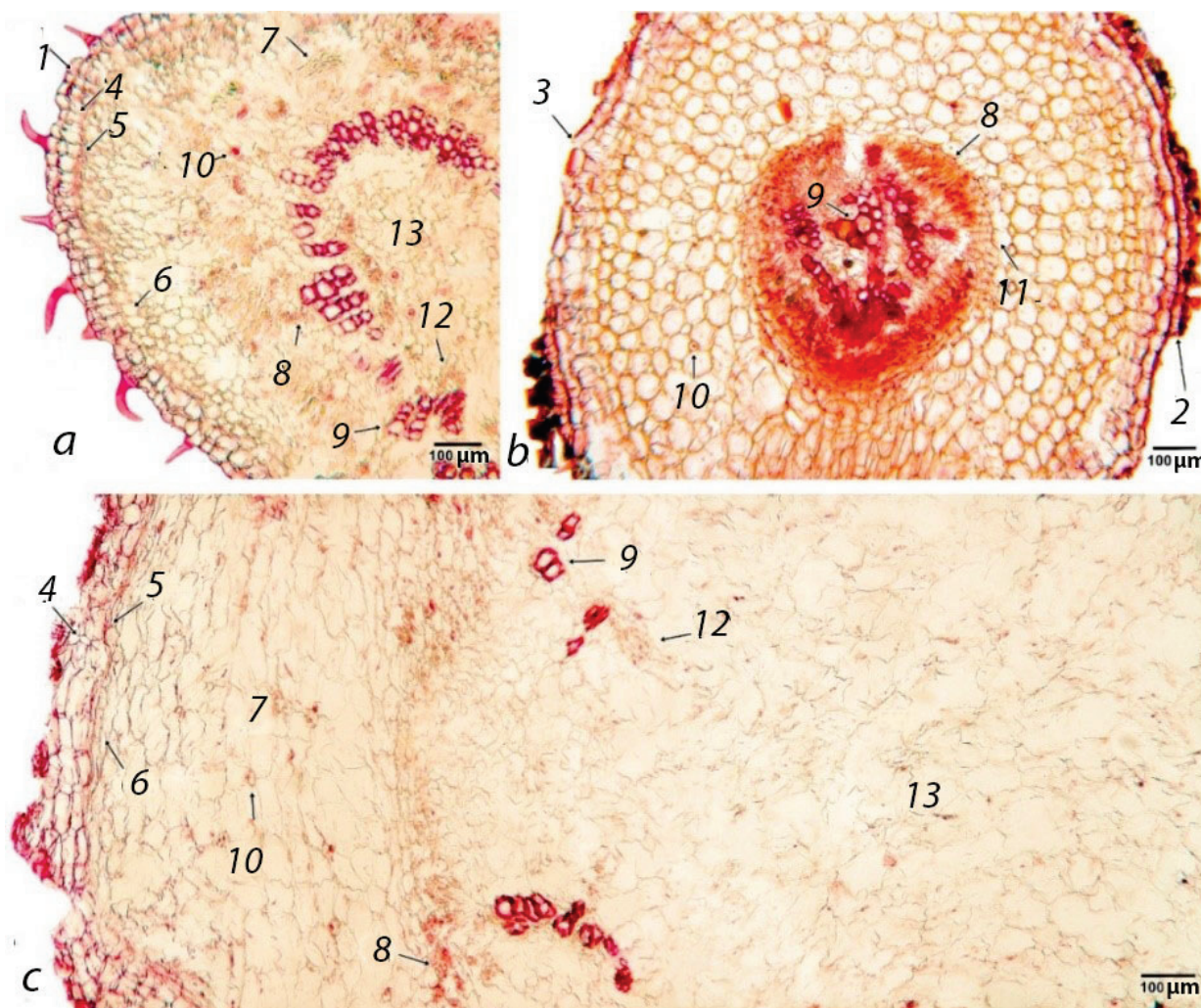


Fig. 3. Anatomical structure of juvenile *Fockea edulis* plants: a – upper part of the stem, b – tap root, c – basal part of the stem: 1 – epidermis, 2 – epiblema, 3 – exodermis, 4 – phellem, 5 – phellogen, 6 – phelloderm, 7 – cortex parenchyma, 8 – phloem, 9 – xylem, 10 – latex cells, 11 – endodermis, 12 – intraxylary phloem; 13 – pith

Compared to the hypocotyl of seedlings, the diameter of the pith of the basal part of the stem of juvenile plants approximately doubles. As in the parenchyma of the cortex, so in the pith, single latex ducts are observed. The root system of juvenile plants of *F. edulis*, compared to seedlings, is noticeably developing and branching. With a slight increase in the diameter of the tap root, its length increases more than five times (Fig. 1B). Also, 3 to 5 lateral roots develop additionally.

Minor changes are observed at the anatomical level (Fig. 3B, Table 2). The epiblema, the cells of which carry individual root hairs, loses its functional suitability and begins to exfoliate. The thickness of the root cortex parenchyma increases approximately twice, compared to seedlings,

and is represented by 7–9 layers of tangentially oblate thin-walled cells. Numerous latex ducts are among the cortex parenchymal cells. Compared to seedlings, the number of tracheal elements of xylem increases, while the thickness of phloem and xylem almost doubles (Table 2). There is a clearly visible endoderm layer with thickened anticlinal walls.

Immature plants. At the age of 11–12 months of vegetation, representatives of *F. edulis* acquire features of adult vegetative plants and reach immature age. Compared to juvenile plants, all vegetative organs are noticeably developed in immature plants, leaves 38.3 ± 4.3 mm long and 24.9 ± 2.5 mm wide are formed, which morphologically do not differ from the leaves of adult plants (Fig. 1B, Table 1).

Table 2

Morphometric indicators of plant tissue of different age states of *Fockea edulis* ($\bar{x} \pm m$, $n = 30$, μm)

Part	Tissue	Para-meters	Seedlings	Juvenile plants	Immature plants	
Upper part of the stem	epidermis	h	–	16.14 ± 2.83	18.86 ± 3.54	
	per-derm	phellem	h	–	39.35 ± 7.62	146.17 ± 39.82***
		phellogen	h	–	15.08 ± 4.26	18.59 ± 5.13*
		phellogen	h	–	28.54 ± 4.32	112.02 ± 4.43***
	cortex parenchyma	h	–	135.12 ± 20.23	153.47 ± 32.81	
	phloem sclerenchyma	h	–	59.28 ± 13.33	88.04 ± 17.24***	
	phloem	h	–	46.11 ± 8.14	117.66 ± 40.48***	
	xylem	h	–	168.01 ± 10.74	482.16 ± 13.21***	
pith	r	–	127.19 ± 24.52	164.05 ± 31.33***		
Hypocotyl / Basal part of the stem	epidermis	h	48.08 ± 6.21	49.12 ± 5.31	–	
	per-derm	phellem	h	–	167.43 ± 28.62	241.25 ± 58.43***
		phellogen	h	–	19.45 ± 5.71	22.13 ± 7.52
		phellogen	h	–	86.85 ± 10.12	114.37 ± 35.74*
	cortex parenchyma	h	492.2 ± 64.4	657.6 ± 99.4	2395.3 ± 295.4***	
	phloem	h	49.43 ± 7.91	159.45 ± 20.72***	232.45 ± 29.89***	
	xylem	h	54.53 ± 6.82	409.43 ± 71.36***	1320.5 ± 210.69***	
	pith	r	524.4 ± 84.2	902.4 ± 128.1***	9624.2 ± 597.4***	
Tap root	epiblema	h	18.45 ± 1.14	–	–	
	exodermis	h	36.87 ± 7.53	37.25 ± 6.23	–	
	per-derm	phellem	h	–	46.37 ± 4.03	242.48 ± 56.36***
		phellogen	h	–	14.06 ± 1.62	21.40 ± 3.15**
		phellogen	h	–	19.13 ± 2.39	12.38 ± 35.13***
	cortex parenchyma	h	232.4 ± 41.2	410.1 ± 74.8***	648.8 ± 102.2***	
	phloem	h	47.57 ± 8.23	77.82 ± 19.51*	257.47 ± 29.28***	
	xylem	h	94.56 ± 9.73	154.48 ± 42.49**	3815.2 ± 314.6***	

Notes: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ significant difference of the parameter relative to the previous stage of development was compared by ANOVA followed by a Bonferroni test; h – thickness, r – radius.

The length of the upper part of the stem increases more than tenfold, the diameter – only twofold compared to juvenile plants. The surface of the stem is green. Its older orthotropic part, represented by the first 4–6 internodes, is characterized by partial lignification and increased elasticity. The following internodes are less thickened, but have a longer length (58.33 ± 18.44 mm), forming a grassy, twisted part of the stem, which actively stretches uphill due to circular nutation. At the end of the vegetation cycle, the lignified part of the plant increases by one internode, and the other herbaceous part dies. This indicates that in immature plants of *F. edulis*, the upper part of the stem is represented by a gradually growing, perennial woody part and an annual herbaceous part, which regenerates at the beginning of each vegetation cycle. The lignified upper part of the stem of immature *F. edulis* plants begins to branch, forming 1–2 additional shoots from the second or third node. A gradual peeling of the epidermal layer is observed on the cross-section of the lignified upper part of the stem. The thickness of the periderm increases almost five times compared to the herbaceous part of the plant (Fig. 4A, Table 2). To a greater extent, this occurs due to phellem, which consists of 6–7 layers of larger cylindrical cells with thickened lignified walls. Phellogen is multi-layered and consists of very flattened cylindrical thin-walled cells, contains chloroplasts. The cortex parenchyma almost does not change. As in juvenile representatives, cortex parenchyma cells of the adjacent to the phellogen have chloroplasts, and calcium oxalate druses are present in some cells adjacent to the conducting tissues. It is possible to note an increase in the number and diameter of non-articulate latex ducts that penetrate the entire thickness of the cortex parenchyma. The thickness of phloem and xylem increases almost threefold compared to juvenile plants. The diameter of the vessels also doubles, they are placed in long radial strips, in which there are single, much more massive, thick-walled

metaxylem vessels (Fig. 4A, Table 2). Separate zones of the intraxylary phloem are also united into a solid ring, near which the formation of calcium oxalate druses is observed.

The basal part of the stem in immature plants of *F. edulis* develops noticeably, the length increases by about two times, and the diameter more than four times, compared to juvenile plants. At the same time, the shape of the basal part of the stem becomes inverted-ovoid. Its green-gray surface is rough, which is due to thickening and cracking of the periderm (Fig. 1B). The latter has the same structure as in the rest of the stem, with the exception of the phellem, the thickness of which is almost twice as large due to the larger size of the cylindrical cells and the number of their layers, which increases to 11–13 (Fig. 4B, Table 2). The phellogen is weakly determined, but isolated clusters of calcium oxalate druses are observed between the phellem and phellogen. Compared to juvenile plants, the thickness of the cortex parenchyma increases by more than four times, which is due to an increase in the number and size of thin-walled isodiametric cells. Chloroplasts and rounded calcium oxalate druses are present in the cortex parenchyma cells adjacent to the phellogen. Conductive tissues of the basal part of the stem have a similar structure to that of juvenile plants. The difference is in the increase in the number of xylem vessels in each of the bundles to 10–15, as well as a noticeable displacement deep into the pith of its individual conducting elements together with intraxylary phloem zones, due to very intensive formation of xylem parenchyma cells (Fig. 4B, Table 2). Therefore, the xylem zone thickens three-fold. The phloem zone thickens almost twice compared to juvenile plants. Single sclerenchyma fibers are also observed above the phloem. The pith thickens most noticeably, the diameter of which, compared to juvenile plants, increases more than ten times. Along with this, there is a slight increase in pith parenchymal cells, they are thin-

walled and highly vacuolated. The diameter of the latex ducts, which pierce through the entire basal part of the stem, almost doubles. It is worth noting that approximately 1/3 of the basal part of the stem deepens into the soil and forms from one to three additional roots.

The root system of immature plants is noticeably developed compared to juveniles. The tap root is characterized by a significant tenfold increase in diameter, while its length increases only 2.0–2.5 times. Thickening occurs due to highly parenchymatized xylem, the thickness of which, compared to the xylem of the tap root of juvenile individuals, increases approximately 25 times (Fig. 4B, Table 2). The main mass of

parenchymatized xylem consists of thin-walled isodiametric parenchyma cells $78.05 \pm 21.72 \mu\text{m}$ in diameter. The actual tracheal elements of xylem are scattered in small groups of 3–5 pieces among the thickness of these cells. The periderm is represented by a 10–11-layer of phellem, consisting of cylindrical tangentially flattened cells with thickened walls, as well as a multi-layered phellogen, the cells of which are very flattened (Fig. 4B, Table 2). Phellogen is represented by one layer of thin-walled tangentially flattened cells. The cortex parenchyma thickness also increases, among the isodiametric and thin-walled cells of which there are non-articulate latex ducts, as well as calcium oxalate druses.

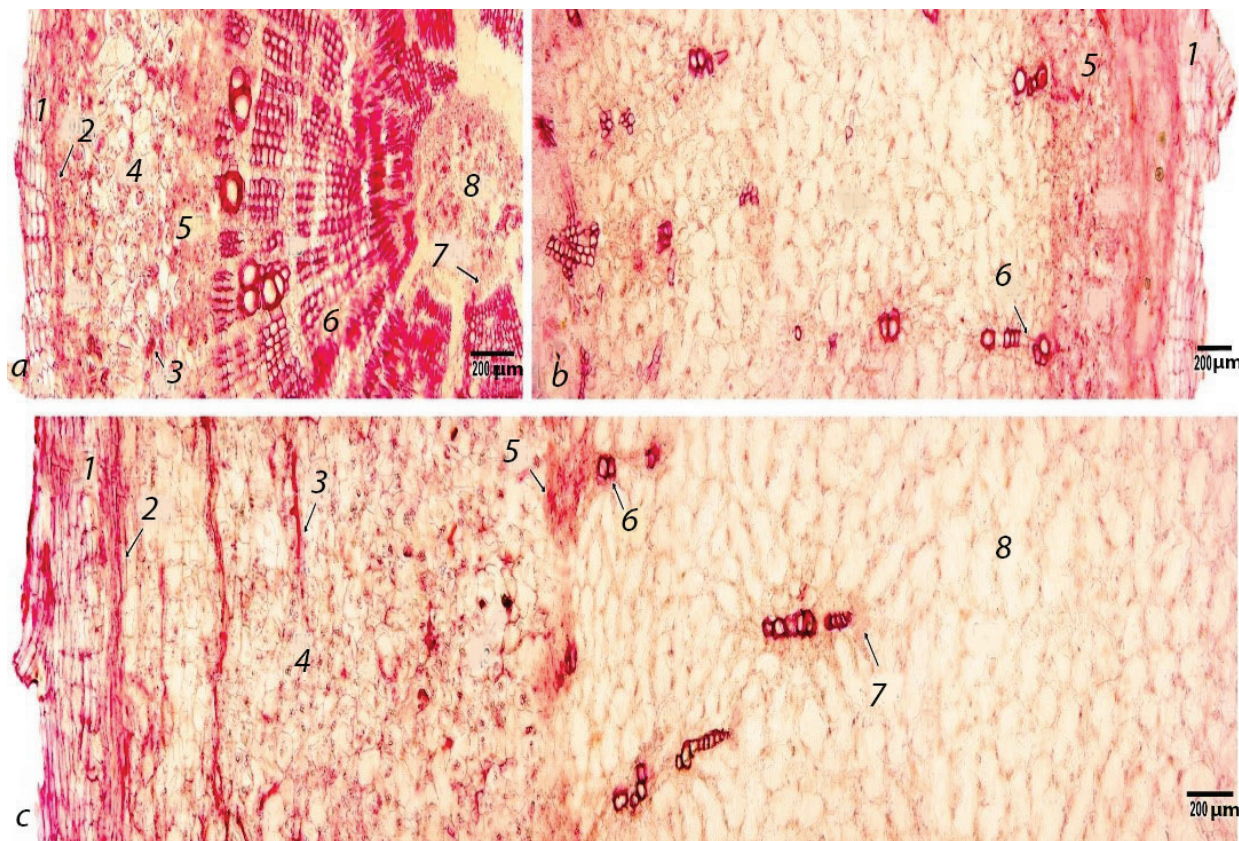


Fig. 4. Anatomical structure of immature plants of *Fockea edulis*: *a* – upper part of the stem, *b* – tap root, *c* – basal part of the stem: 1 – phellem, 2 – phellogen, 3 – latex cells, 4 – cortex parenchyma, 5 – phloem, 6 – xylem, 7 – intraxylary phloem; 8 – pith

Discussion

Analyzing the research data, it can be stated that representatives of *F. edulis* have a noticeably developed cylindrical hypocotyl, which is actively growing due to primary thickening already at the seedling stage. Juvenile plants develop a rather expanded basal part of the stem. The thickening of the basal part of the stem occurs according to the cortex-pith type, which is due to the strong growth of thin-walled parenchymal cells of the pith and cortex parenchyma, while these tissues are almost not developed both in the main root and in the upper part of the stem. Conductive tissues have a different nature of placement and development in different parts of juvenile plants. In the basal part of the stem, they are represented by vascular-fibrous bundles scattered over a long distance among parenchymal cells. Conductive tissues are represented by solid rings of xylem and phloem in the upper part of the stem and the tap root. The formation of intraxylary phloem zones in the upper and basal parts of the stem is a characteristic feature of this family. The basal part of the stem of immature representatives acquires a rounded shape due to further significant primary thickening and is clearly determined, compared to other parts of the plant. Its surface becomes quite rough due to the active growth and cracking of the cortex layer of the periderm. The conducting system retains its bundle-type structure, while the formation of vessels in the conducting bundles is not very intensive. Separate elements of xylem and intraxylary phloem go deep into the thickness of the pith, just like in *Adenium obesum* (Aviekin, 2017). This happens as a result of active

growth of the xylem parenchyma, the cells of which push individual conductive elements out of the main radial circle of the conductive bundles. Although the secondary thickening does not lead to a significant increase in its diameter, part of the stem is covered by the periderm and becomes woody due to the growth of the xylem. The thick and succulent radish-like root is formed by the growth of xylem parenchyma and forms a reservoir for the water and nutrients accumulation together with the thickened basal part of the stem.

The presence of chloroplasts in the phelloderm cells and partially in the cells of the outer layers of the cortex parenchyma enables the entire aerial part of the stem of *F. edulis* to conduct photosynthesis in the early stages of development. This is explained by the fact that representatives of this species lose their leaves in the places of their natural distribution during most of the year (Court, 1987).

Previously, we studied the first stages of development of *Petopentia natalensis*, which also has a similar morphological structure. When comparing the seedlings of *F. edulis* and *P. natalensis*, it can be stated that in the seedlings of *P. natalensis*, unlike *F. edulis*, the hypocotyl is not marked by noticeable thickening, its active development begins later, approximately in the second or third week after seed germination (Aviekin et al., 2021). The external form of the hypocotyl is individual for each species, but their development occurs as a result of the primary thickening of the cortex and the pith and generally has a similar anatomical organization. The cortex and phelloderm of seedlings' hypocotyls have chloroplasts in the surface layers of parenchyma cells. The root system also has a

similar primary type of structure. The presence of a secondary covering tissue (periderm) was noted in the hypocotyl of *A. obesum* seedlings, in contrast to the seedlings of *F. edulis* and *P. natalensis*. It may be an adaptation to the harsher conditions of existence in the places of *A. obesum* natural growth (Aviekin, 2017; Aviekin et al., 2021). The representatives of these taxa, starting from germination, develop excretory tissues in the form of un-articulated latex ducts, which penetrate the entire thickness of the cortex and pith parenchyma.

An expanded, succulent basal part of the stem is formed in the juvenile state of all three studied taxa, due to the active primary thickening of the cortex and the pith of the first hypocotyl internode. The upper part of the stem is a thin non-succulent liana in the representatives of *P. natalensis* and *F. edulis*, while in *A. obesum* the upper part of the stem is quite succulent, but much less pronounced compared to the basal part of the stem of the other two species. The bundle type of structure at the juvenile stage of development of *A. obesum* is preserved in the same way as in *P. natalensis* and *F. edulis* (Aviekin, 2017; Aviekin et al., 2021).

The most noticeable changes are observed in plants at the immature stage of development: an increase in the size of both the upper part of the stem and basal part of the stem, the periderm develops, and the root system undergoes noticeable changes. The upper part of the stem and basal part of the stem of *F. edulis* and *P. natalensis* plants are easily determined in the zone of the first cotyledon node. The upper part of the stem is formed in the form of a creeper. The bundle organization of conducting elements is preserved in the basal part of the stem of immature plants of *F. edulis* and *P. natalensis* (Fig. 5). In immature plants of *A. obesum*, a continuous ring of conducting elements is formed due to intervacular cambium.

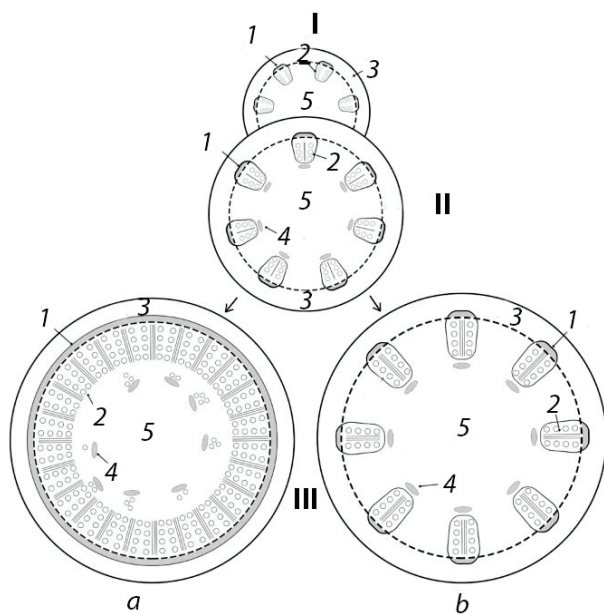


Fig. 5. Scheme of the conducting system formation of the stem basal part at different age states of the pregenerative period of ontogenesis: *a* – *Adenium obesum*, *b* – *Fockea edulis* and *Petopentia natalensis*; I – seedling, II – juvenile plant, III – immature plant; 1 – phloem, 2 – xylem, 3 – cortex parenchyma, 4 – intraxylary phloem, 5 – pith

A thick succulent radish-like root in *F. edulis* is formed by the growth of xylem parenchyma, while in *P. natalensis* a typical secondary root structure is formed, where most of the central cylinder is occupied by vessel xylem, which forms a polyarch stele. The formation of a radish-like root by the powerful xylem parenchyma at the immature stage of development also unites *F. edulis* with *A. obesum*.

Thus, in *F. edulis*, *P. natalensis* and *A. obesum*, which belong to different subfamilies of the Arocynaceae family, there is a convergent adaptation to arid climate conditions in the form of a combined a cortex and a pith thickening of the basal part of the stem. This adaptation is quite logical, since a significant part of succulent basal part of the stem is more protected by the soil, energy costs for transportation and accumulation of

water are reduced, and the formation of additional roots increases its absorption. In addition, a succulent tap root is also formed in *A. obesum* and *F. edulis*. Such a feature of root metamorphosis is characteristic of many succulent plants. In particular, primitive representatives of the Cactaceae family (*Pereskia*, *Peresciopsis*) were studied, which, with a weakly expressed succulent stem and the presence of non-succulent leaves, are characterized by the formation of a thickened tuber-like tap root in which water and starch accumulate. At the same time, according to J. Mauseth and J. Pate, the anatomical structure of the modified roots in many studied succulents is similar that found in immature individuals of *A. obesum* and *F. edulis*. That is, the function of storing nutrients and water is performed by xylem parenchymal cells, which are developed to a greater or lesser extent, depending on the type and features of natural conditions (Pate & Dixon, 1982; Mauseth & Stone-Palmquist, 2001). It should be added that succulents with a modified root system are found in arid and semiarid regions of almost all continents of the world (Ogbum & Edwards, 2010). Thus, the additional fact of convergent adaptation of succulent plants to arid conditions due to modifications of the tap root is confirmed.

Some researchers are inclined to the opinion that the adaptation of succulents to a lack of water initially occurred due to changes in the root system since this process is due to lower energy costs and the number of solutions aimed at protecting the succulent part of the plant from excess transpiration and various damages (Ihlenfeldt, 1985; Heam, 2013). In turn, more complex evolutionary forms of succulents are characterized by metamorphosis of the stem (*Ariocarpus* Scheidw., *Euphorbia obesa* Hook.) or leaves (*Echeveria* DC., *Haworthia* Duval, *Crassula* L.), which completely distinguishes them from typical mesophytes (Anderson, 2001; Eggli, 2001; Eggli, 2003). Therefore, the group of plants studied by us can be a transitional form between primitive and more developed succulents (between different types of plant adaptation to more or less arid natural conditions), and at the same time, they are characterized by individual strategies of adaptation to arid climate conditions.

Conclusion

It was established that the strategy of developing succulent traits in *Fockea edulis* at the organ level is characterized by the active development of the hypocotyl already at the seedling stage and the subsequent formation of an expanded basal part of the stem and a radish-like root. It was found that the formation of the expanded basal part of the stem in the studied plants occurs due to the combined cortex-pith thickening and subsequently the bundle type of conducting elements is preserved in *F. edulis*. Thickening of the tap root in *F. edulis* occurs as a result of the growth of xylem parenchyma. Comparing the features of the development of the vegetative parts of three representatives of the Apocynaceae family, we found that secondary thickening in the basal part of the stem occurs in two individual ways: in *Adenium obesum*, the cambium generates solid rings of phloem and parenchymatized xylem; in *Petopentia natalensis* and *F. edulis*, the bundle type of conducting elements is preserved. The thickening of the tap root in *F. edulis* is similar to that in *A. obesum*.

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