Journal of Cytology and Genetics 2022 VOL. 23 (NS): 99–106

RESEARCH ARTICLE

A COMPARATIVE STUDY ON STOMATAL CHARACTERISTICS AND PHOTOSYNTHETIC PIGMENTS IN WILD AND CULTIVAR OF *ZAMIOCULCAS ZAMIIFOLIA* (LODD.) ENGLER

CINTHYA CHRISTOPHER*, P. S. ASWATHY AND H. HARITHA

Department of Botany, All Saints' College, University of Kerala, Chackai, Thiruvananthapuram 695 007

*For correspondence. Email: cinthya_wilfred@

(Received 21 November 2022, revised accepted 8 December 2022

SUMMARY *Zamioculcas zamiifolia* (Araceae), an unusually drought resistant medicinal plant is native to tropical East Africa, and subtropical south-east Africa. It is described as a living fossil which may have evolved as early as 42 million years ago. In the present study, an attempt has been made to study the foliar morphology, stomatal variations, photosynthetic pigment estimation and absorption spectra analysis in wild and cultivar of this species. A strong negative correlation exists between the stomatal frequency and the area occupied by the stomata and between frequency of epidermal cells and the area occupied by the epidermal cells. Estimation of photosynthetic pigments revealed that the amount of chlorophyll *a* and *b*, total chlorophyll and carotenoid contents were high in the wild as compared to the cultivar. Stomatal study, estimation of photosynthetic pigments and absorption spectra analysis revealed that the wild is more efficient in its metabolic vigour as compared to the cultivar.

Keywords: *Zamioculcas zamiifolia,* stomata, photosynthetic pigments.

INTRODUCTION

Zamioculcas zamiifolia, commonly known as Zanzibar gem, Zuzu plant or Emerald palm, is a tropical perennial medicinal plant belonging to the family Araceae. It is a sole species of the genus *Zamioculcas* and native to eastern Africa, from Kenya South to northeastern South Africa. The plant is grown in dry grassland and often in stony ground and has several fleshy stalks bearing alternate pinnate leaflets and a very thick rhizome. The leaflets have the capacity to sprout new plants and form tiny rhizomes at their bases (Brown 2000). It is grown as an ornamental plant, mainly for its attractive glossy foliage. The plant contains an unusually high-water content of leaves (91%) and petioles (95%) (Moullec et al. 2015) and has an individual leaf longevity of at least 6 months (Chen & Henny 2003) which may be the reason for its survival under interior low light levels for 4 months without water.

Z. zamiifolia shows 2 defining attributes of crassulacean acid metabolism (CAM). It is the only CAM species described within Araceae and is the only documented nonaquatic CAM species.

It is postulated that CAM assists survival of *Z. zamiifolia* by reducing water loss and maintaining carbon gain during seasonal droughts characteristic of its natural habitat (Holtum et al. 2007).

Generally, in plants, intraspecific variations may be much evident in all aspects such as morphology, anatomy, cytology, palynology, biochemistry etc. Apart from genetic differences among individuals, much of the observed intraspecific variation is due to modifications during ontogeny. For example, depending on light conditions experienced during growth the leaves of a given individual may differ considerably in their photosynthetic response (Boardman 1977). There are many studies on photosynthetic rate of wild and cultivated crop species, like wheat (Evans et al. 1970), rice (Takano & Tsunoda 1971), barley (Chapin et al. 1989) etc. Most investigators reported that photosynthetic rate of some wild relatives of crop species was higher than that of the cultivated species (Cinthya Christopher 2000). In this backdrop, a comparative investigation on wild and cultivar of *Z. zamiifolia* on stomata and photosynthetic activity is significant and assumes importance. Hence, the present investigation is taken up to study the aspects in wild and cultivar of *Z. zamiifolia*.

MATERIALS AND METHODS

Field surveys were conducted to locate populations of *Z. zamiifolia* occurring in Kerala. During the survey, a wild and a cultivar of this species were collected and maintained in the Field Gene Bank of All Saints' College providing uniform environmental conditions. Stomatal characterization of leaf and leaflets were done. Five observations were scored for each character. Fresh mature leaves on 4th or 5th node from the shoot tip of the plants representing each accession, were collected and boiled in ethanol to remove the chlorophyll. The bleached leaves were kept overnight for incubation in 5% NaOH solution. The incubated leaves were washed in distilled water and the abaxial epidermis of the leaves peeled using forceps. The peeled epidermis was stained in 1% safranine, washed in distilled water, mounted in glycerine and observed under the microscope. The structure and types of stomata were determined as per the nomenclature and classification by Prabhakar (2004). The average value of the number of stomata, epidermal cells and sessile trichomes from 5 fields per leaf and 5 leaves per accession were calculated. Photomicrographs of the peels were taken using a Leica ICC50HD camera, attached to Leica DM 500 trinocular microscope. Slides as prepared for stomatal density are also used for determination of stomatal index by using the following formula:

$$\text{Stomatal index (\%)} = \frac{S}{S + E} \times 100$$

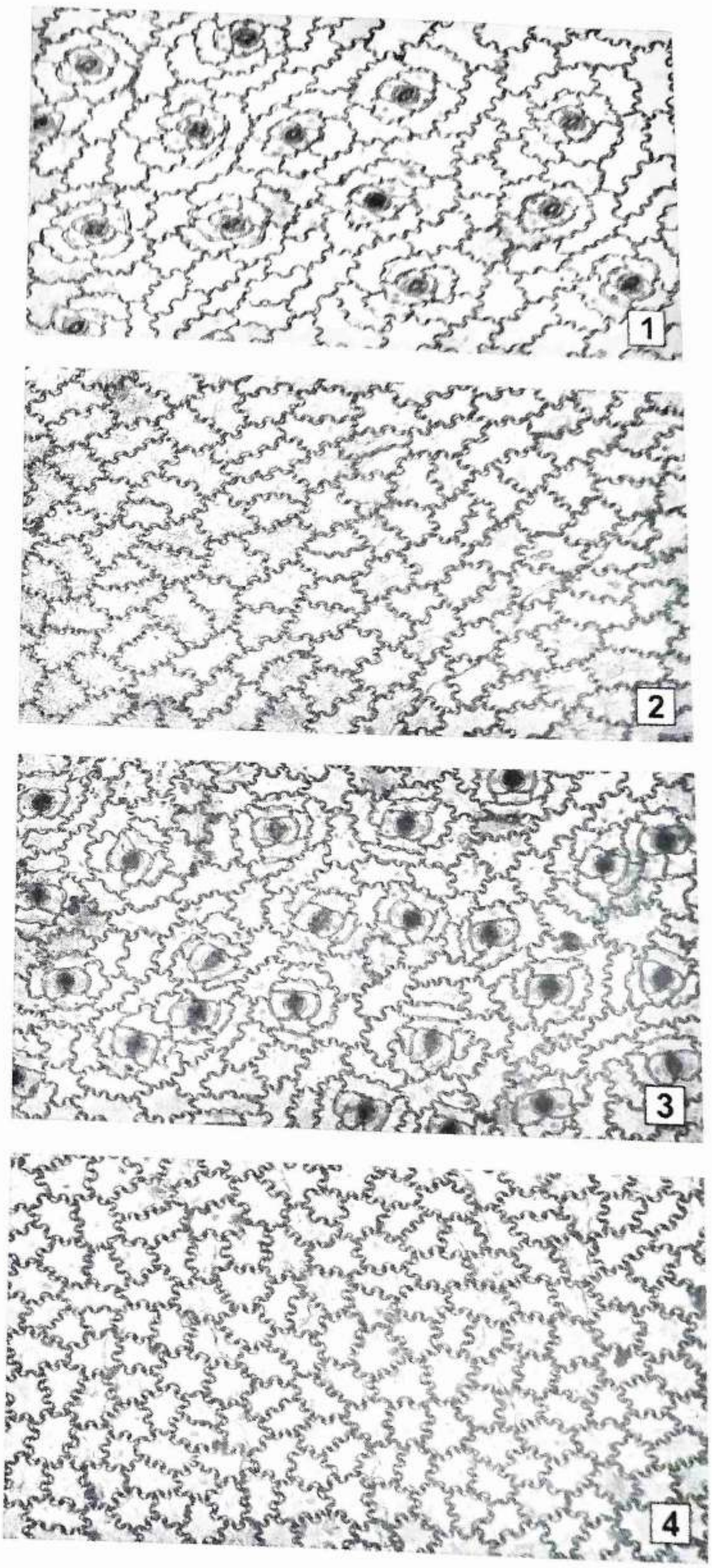
where, S and E are the number of stomata and epidermal cells respectively in microscopic field of view.

For the estimation of photosynthetic pigments, leaves are taken from the 5th node of the rachis. One g of fresh leaf tissue was taken and ground in 80% acetone. The ground mixture is made up to 50 ml by filtering it in cheese cloth using acetone until the supernatant became colourless. Optical density was noted spectro-photometrically at different wavelengths, 420 nm, 440 nm, 470 nm, 490 nm, 520 nm, 540 nm, 570 nm, 600 nm, 630 nm, 643 nm, 645 nm, 652 nm, 663 nm, 670 nm, 690 nm and 720 nm. Estimation of the pigments were done as per Arnon (1949) based on extinction coefficient with respect to the solvent in which the extraction of pigment was done. Absorption spectra analysis was also done by constructing a graph plotted against wavelength against optical density.

OBSERVATIONS

Stomatal characteristics in wild and cultivar of *Z. zamiifolia* were studied. In both populations, stomatal type is paracytic with 2 subsidiary cells aligned parallel to the 2 guard cells and 2 cells arranged at the poles of the guard cells. Number of stomata per unit area, area occupied by the stomata in unit area, number of epidermal cells per unit area, area occupied by the epidermal cells in unit area and the stomatal index were calculated. The abaxial epidermal peel showed distinct stomata distributed throughout the epidermis with subsidiary cells. The epidermal cells are characterized by undulating margins. Both populations lack stomata on the adaxial surface.

The number of stomata per unit area (stomatal frequency) was 11.16 and 22.46 in wild and cultivar respectively (Figs 1–4). Even though the stomatal frequency was high for cultivar, the



Figs. 1–4: *Z. zamiifolia* 1. Lower epidermis of the wild plant. 2. Upper epidermis of the wild plant. 3. Lower epidermis of the cultivar. 4. Upper epidermis of the cultivar.

area occupied by the stomata in the wild was 0.09 mm^2 while that of the cultivar is only 0.05 mm^2. Frequency of epidermal cell in unit area was also higher for the cultivar (240.43) as compared to that of the wild (145.27). The area occupied by each epidermal cell in unit area was lesser for the cultivar (0.004mm^2) while that of the wild is higher (0.006 mm^2). Stomatal index of the wild and the cultivar was estimated to be 7.13 and 8.54 respectively. So, in the present study, it is evident that a strong negative correlation exists between the stomatal frequency and the area occupied by the stomata and also between frequency of epidermal cells and the area occupied by the epidermal cells.

Estimation of chlorophyll pigment in the wild and the cultivar of *Z. zamiifolia* showed that the total chlorophyll content was high in the wild, averaging 1.56 mg/g, as compared to that of the cultivar with only 1.01 mg/g. The same pattern was observed in the fraction of chlorophyll *a*, chlorophyll *b* and carotenoids, which were 1.004 mg/g, 0.55 mg/g and 3.45 mg/g in wild and in cultivar, the corresponding values are 0.65 mg/g, 0.36 mg/g and 2.18 mg/g respectively. But the ratio between chlorophyll *a* and *b* was high (1.803) for the cultivar while that of the wild was 1.79, difference being very insignificant. However, carotenoid content in the 2 populations of the species showed considerable variation. Whereas one showed 3.45 mg/g while the cultivar showed only 2.18 mg/g.

The chlorophyll pigments in the 2 populations were analyzed with the help of absorption spectra in the range of wavelength from 420 to 720 nm. The pigment extract of wild one showed an absorption maximum of the blue region at the wave length, 440 nm with a narrow fall at 520 nm. The peak absorbance at the red region represented the wavelength of 670 nm with a broad base between wavelengths of 652 and 690 nm. The pigment extract of the cultivar also showed an absorption maximum of the blue region at the wavelength of 440 nm with a narrow fall at 520 nm. The peak absorbance at the red region represented the wavelength of 670 nm with a broad base between wavelengths, 652 and 690 nm (Table 1, Fig. 5). The pattern of absorbency in different wavelengths of light in the 2 populations was the same. The absorption spectra of

TABLE 1: Absorbency of pigments noted in different wavelengths of light in wild and cultivar of *Z. zamiifolia*.

Wavelength	Optical Density	
	Wild	Cultivar
420	0.81	0.53
440	0.95	0.61
470	0.63	0.40
490	0.34	0.23
520	0.10	0.08
540	0.09	0.07
570	0.10	0.07
600	0.13	0.09
630	0.17	0.11
645	0.21	0.13
652	0.27	0.17
663	0.44	0.28
670	0.47	0.31
690	0.10	0.07
720	0.03	0.03

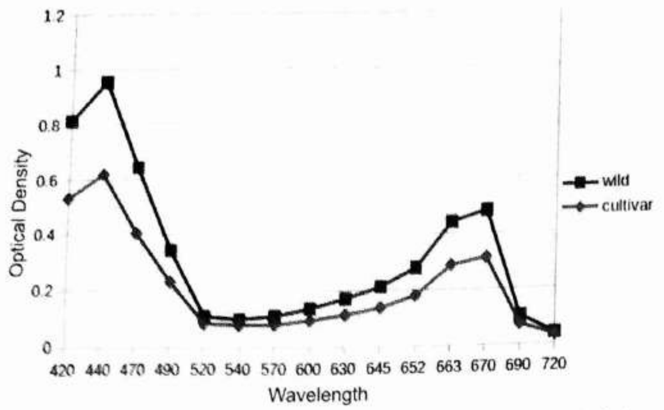


Fig. 5: Absorption spectra in wild and cultivar of *Z. zamiifolia*.

chlorophyll pigment in both the populations show that the wild one is more suited to the environment concerned than that of the cultivar, which is an evolved variety with low metabolic vitality.

DISCUSSION

Z. zamiifolia is grown as an ornamental plant, mainly for its attractive glossy foliage. The plant has air purifying qualities for the indoor environment. As an ancient plant, it has sustained substantial climate changes and attacks from millions of generations of pathogenic microorganisms (Moullec et al. 2015). Not only has it been described as a plant that is becoming or will become an important player in the foliage plant industry (Chen et al. 2002), but it was also listed among the Florida Plants of the Year in 2002. The ability of *Z. zamiifolia* to grow under low light condition, its tolerance to drought stress, its unique appearance, its low maintenance requirements and limited pest problems are characteristics that contribute significantly to its ornamental and landscaping value (Chen & Henny 2003).

The species has completely pinatisect leaves with unpaired pinnate leaflets in both wild and cultivar (subparipinnate). Leaf blade size is exceedingly diverse. The number of leaflets varies in the 2 variants studied. The distance between the leaflets also varies in the 2 variants. The wild has oblanceolate leaf whereas the shape of leaf in the cultivar is ovate. The average number of leaflets per rachis in the wild was 12 and that of the cultivar was 16. The petiolule is of much importance to the species as it is capable of regeneration. Plants are raised from the leaflets and the plantlets are developed from the leaf area with midrib. As the number of leaflets is higher and the arrangement of leaflets is more aesthetic, the cultivar is most preferred as indoor garden plant. The wild one is naturally adapted for propagation through leaflets as a small mechanical shock can remove the leaflet easily and regenerated easily.

The stomatal index was considered to be fairly constant within the leaves of a single species. Even though the stomatal number is determined by many extrinsic and intrinsic factors, the stomatal indices are quite constant and can be used in distinguishing different taxa (Poole et al. 1996). This matter was examined in the present material and found that the stomatal indices in the variants displayed striking variation. The stomatal indices of the wild and cultivar were 7.13 and 8.54 respectively. It is possible that this attribute may be genetically predisposed in the genotypes.

Photosynthetic capacity is closely linked to stomatal density (Xu & Zhou 2008). Moreover, photosynthetic potential might be enhanced with increased stomatal density in *Arabidopsis* by a modulating gas diffusion function, as reported by Tanaka et al. (2013). Recent studies (Zhao et al. 2015) had established significant negative correlations of stomatal density with photosynthesis and demonstrated that higher stomatal density reduced leaf photosynthesis. They argued that small stomata could maintain the pores opening with lower guard cell turgor pressures as compared to larger stomata. The higher stomatal density and reduced stomatal size responding to drought can effectively inhibit transpirative water loss and better water balance (Bosabalidis & Kofidis 2002).

It has been noted that in the wild *Z. zamiifolia,* even though the stomatal density and stomatal index are lower as compared to the cultivar, the photosynthetic efficiency may be high, which can be established by analyzing the chlorophyll content and absorption spectral analysis.

Estimation of chlorophyll pigment in wild and cultivar of *Z. zamiifolia* showed that the total chlorophyll content was high in the wild, averaging 1.56 mg/g, while in the cultivar the corresponding value was 1.01 mg g^{-1}. The same pattern was observed in the fraction of chlorophyll *a*, *b* and carotenoids, which were 1.004 mg/g, 0.55 mg/g and 3.45 mg/g in wild and in cultivar, 0.65 mg/g, 0.361mg/g and 2.189 mg/g respectively. But the ratio between chlorophyll *a* and *b* for the cultivar was 1.80 while that of the wild was 1.79, the difference being highly insignificant.

Carotenoid content in the wild and the cultivar showed considerable variation. Wild showed 3.458 mg/g while that of the cultivar was 2.189 mg/g. Thus the wild one is more benefited in this aspect as it showed an increase in the level of carotenoids.

The present study showed that the wild is photosynthetically more efficient than the cultivar and thereby indicating that the former is more active metabolically than the latter. It is in conformity with the earlier suggestion that wild plants have more vigour than the cultivars.

The chlorophyll pigments in the wild and cultivar were analyzed with the help of absorption spectra. The pigment extract of wild showed an absorption maximum of the blue region at the wavelength, 440 nm with a narrow fall at 520 nm. The peak absorbance at the red region represented the wavelength of 670 nm with a broad base between wavelengths 652 and 690 nm. The pigment extract of cultivar also showed an absorption maximum of the blue region at the wavelength, 440 nm with a narrow fall at 520 nm. The peak absorbance at the red region represented the wavelength 670 nm with a broad base between wavelengths 652 and 690 nm. The pattern of absorbency in different wavelengths of light in the 2 was the same (Fig. 5). Since the total chlorophyll, chlorophyll *a* and *b* were high in the wild, a relative increase in the quantity of the pigment was noticed in the absorption spectrum, as evidenced by an increase in absorbency.

Recent improvements in the techniques of isolating and extracting plant pigments, primarily photosynthetic pigments, as well as identifying the biological reactions to which they may be coupled by action spectra and absorption spectra are useful in biochemical and/or physiological research (Buchanan 1980). From the present study on the absorption spectra of chlorophyll pigment of wild and cultivar of *Z. zamiifolia*, it is evident that the wild one is more suited to the environment concerned than that of the cultivar, which is an evolved cultivar variety with low metabolic vitality.

Further investigation is necessary on the stomatal conductance, gaseous exchange, and other parameters to substantiate the photosynthetic efficiency in the wild and the cultivar. However, both wild and cultivar of *Z. zamiifolia* can be used well as indoor plants as they use up the carbon dioxide and give out oxygen during the night since both are CAM plants.

REFERENCES

ARNON D I 1949 Copper enzymes in isolated chloroplasts polyphenoxidase in *Beta vulgaris Plant Physiol* **24** 1–15

BOARDMAN N K 1977 Comparative photosynthesis of sun and shade plants *Annu Rev Plant Physiol* **28** 355–377

BOSABALIDIS A M & KOFIDIS G 2002 Comparative effects of drought stress on leaf anatomy of two olive cultivars *Plant Sci* **163** 375–379

BOWN D 2000 *Aroids: Plants of the Arum family* 2nd ed Timber Press Portland Oregon

BUCHANAN B B 1980 Estimation of chlorophyll and absorption spectra analysis *Ann Rev Plant Physiol* **34** 341–374

CHAPIN F S, GROVES R H & EVANS L T 1989 Response of growth photosynthesis and phosphate absorption to phosphorus stress in wild weedy and cultivated *Hordeum* species *Oecologia* **79** 96–105

CHEN J & HENNY R J 2003 ZZ a unique tropical ornamental foliage plant *Hort Technol* **13** 458–462

CHEN J, HENNY R J & MCCONNELL D B 2002 Development of new foliage plant cultivars *In* Janick J & Whipkey A (eds) *Trends in new crops and new uses* ASHS Press Alexandra

CINTHYA CHRISTOPHER 2000 *A comparative study on foliar anatomy and chemical characterization in Pandanus amaryllifolius Roxb and Pandanus odoratissimus Linn* M Phil Thesis University of Kerala

EVANS LT, DUNSTONE R L, RAWSON H M & WILLIAMS R F 1970 Wheat cultivation and utilization *Aust J Biol Sci* **23** 743–752

HOLTUM J M, WINTER K, WEEKS M A, TIMOTHY R & SEXTON T R 2007 Crassulacean acid metabolism in the ZZ plant *Zamioculcas zamiifolia* (Araceae) *Am J Bot* **94** 1670–1676

MOULLEC A L, JUVIK O J & FOSSEN T 2015 First identification of natural products from the African medicinal plant *Zamioculcas zamiifolia* –A drought resistant survivor through millions of years *Fitoterapia* **106** 280–285

POOLE I, WEYERS J D B, LAWSON T & RAVEN J A 1996 Variations in stomatal density and index implications for palaeoclimatic reconstructions *Plant Cell Environ* **19** 705–712

PRABHAKAR M 2004 Structure delimitation nomenclature and classification of stomata *Acta Bot Sinica* **46** 242–252

TAKANO Y & TSUNODA S 1971 Curvilinear regression of the leaf photosynthetic rate on leaf nitrogen content among strains of *Oryza* species *Jap J Breed* **21** 69–76

TANAKA Y, SUGANO S S, SHIMADA T & HARA-NISHIMURA I 2013 Enhancement of leaf photosynthetic capacity through increased stomatal density in *Arabidopsis New Phytol* **198** 757–764

XU Z Z & ZHOU G S 2008 Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass *J Exp Bot* **59** 3317–3325

ZHAO W, SUN Y, KJELGREN R & LIU X 2015 Response of stomatal density and bound gas exchange in leaves of maize to soil water deficit *Plants Soil and Climate Faculty Publications* 73