

Article

Seasonal Functional Partitioning of Carbohydrates and Proline among Plant Parts of the Sand Daffodil

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Abstract: The sand daffodil (*Pancratium maritimum*) is a perennial geophyte, widely distributed and grown in a wild stage along the Mediterranean seashores. The aboveground tissues of this geophyte are exposed to harsh, ambient conditions and its large inflorescences of remarkable beauty and fragrance expand during the drought season and carry particular ornamental worth. The ecophysiological principles underlining metabolic processes of this geophyte are poorly understood. The seasonal variation of soluble sugars, starch, and proline was investigated in individuals collected from patches of *P. maritimum*, therefore, monthly measurements were performed in bulbs, leaves, scapes, and petals during a year. It was found that (a) sugar content showed similar seasonal trends between bulbs and leaves, as well as between petals and scapes, (b) bulbs contained enhanced starch concentrations irrespective of season, (c) proline accumulation exhibited substantial seasonal fluctuations among the considered tissues and pronounced differences were detected between maxima in petals and leaves. A substantial increase in both sugar and proline content was evident in petals during the drought season. In leaves, the accumulation of proline and, to a lesser extent, sugars was negatively correlated to the precipitation of the Mediterranean study site. It seems likely that the astonishing flowering of *P. maritimum* is supported by large leaf and bulb reserves.

Keywords: aboveground; belowground; geophyte; Mediterranean; proline; sand daffodil; starch; sugars; summer blossoming

1. Introduction

Pancratium maritimum L. is a native geophyte to the Mediterranean region that belongs to the family Amaryllidaceae [1,2]. *P. maritimum* (sea or sand daffodil, sea or sand lily) grows naturally along the coastline of the Mediterranean region and blossoms during the hot and dry summer, at a time when simultaneously flowering plant taxa are scarce [3]. The expansion of its beautiful flowers coincides with the period of excessive tourism in coastal areas of the Mediterranean region; therefore, excess of floral sampling, tourist trampling, alteration, and dune erosion have seriously threatened populations of *P. maritimum* [4–8], which are expected to be further affected by sea-level rise and human-induced impacts [9,10]. Additionally, attention from the international scientific community has been given to the use of *P. maritimum* as a commercial, ornamental plant [11,12]. *P. maritimum* possesses an umbel-type inflorescence—which arises among the existing leaves and develops centripetally on a scape—that comprises several white scented flowers [13,14].

Abiotic, environmental stresses are major constraints to plant growth worldwide. In particular, in the drought coastal areas of Greece, plants encounter a combination of drought, elevated temperature, and salinity stress, which results in a greater impact on plant growth and productivity compared to individually stresses per se. Patches of *P. maritimum*, distributed at coastal dunes of Greece [15], are subjected to salt spray, low field capacity, high radiation, elevated temperatures, winds, and

sandblasting [16,17]. In Greece, *P. maritimum* has been known for at least 2500 years [18]; the beautiful lily frescos in the palace of Knossos in Crete and at Akrotiri on Santorini island dating back approximately 2500 years have been identified as sea daffodils [19–22]. Additionally, the sea daffodil has been described in detail in ancient texts, such as Theophrastus' treatise (3rd century BC) [23] and Dioscorides' codex (1st century AD) [24]. It is worth mentioning that *P. maritimum* was included in the 4th volume of the 1st edition of *Flora Graeca Sibthorpiana*—one of the rarest and magnificent botanical books ever written [25]—that was published in London in 1823; this archival, rare source has been digitized and is currently electronically accessed [2,26]. Therein, the original watercolor made by the artist Ferdinand Bauer (1760–1826) in Oxford [26] was based on specimens collected during a botanical expedition in Greece, in 1787 [27,28].

The etymology of the generic name *Pancratium* is derived from two Greek words, *pan* (παν) that means “all” and *cratos* (κράτος) that means “potent” [29], hence *Pancratium* means omnipotent; in English, the meaning of *maritimum* is growing by the sea [29].

Although several aspects concerning patterns of fruit and seed set within inflorescences, pollen, in vitro micropropagation, and genetic diversity of *P. maritimum*, as well as the extraction of natural substances from *P. maritimum* have been published [30–38], there is a scarcity of data on ecophysiological traits of the geophyte *P. maritimum* inhabiting maritime sands. The bulbs of *P. maritimum* remain protected below the soil surface, while the aboveground parts are seasonally exposed to ambient environmental conditions. The main objective of this study was to investigate seasonal fluctuations of carbohydrates and proline in above and belowground tissues of naturally grown *P. maritimum*, which according to the best of our knowledge have not yet been reported. The increasing understanding of the functionality of plant parts may provide a framework to evaluate integration of allocation trends and management options for the sand daffodil.

2. Materials and Methods

2.1. Site Description and Plant Material

The present study was carried out on bulbs, leaves, flowering stalks, and petals collected from patches of *Pancratium maritimum* L. grown naturally on sandy beaches, in Greece (38°37'16.8'' N 24°07'14.1'' E). Aboveground and belowground plant tissues were sampled from adult plants at monthly intervals from 15 January 2018 to 15 December 2018. It is noteworthy that the replicates were kept to a minimum due to the conservation status of *P. maritimum*.

Monthly mean temperature and monthly precipitation were obtained from the nearest meteorological enclosure to the research site (i.e., about 1 km distant), provided by the National Observatory of weather conditions in Greece. The climate of the study site is Mediterranean with a marked seasonality, typified by the alternation of a cold and wet period with a hot and dry period. During the study period, the mean air temperature of the cold (December–February) and the hot (June–August) season was 7.1 and 25.6 °C, respectively (Table 1); the average rainfall (Table 1) varied from 6.2 mm during summer (VI–VIII) to 73 mm in autumn (IX–XI), 131 mm in winter (XII–II), and 49.5 mm in spring (III–V).

Table 1. Mean monthly values of air temperature (T) and rainfall (R) obtained during the study period, from January (I) to December (XII).

Months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
T (°C)	5.4	8.6	10.5	14.8	18.3	24.3	27.0	25.5	21.8	17.0	13.2	7.3
R (mm)	155.6	98.0	95.6	30.6	22.2	10.2	5.6	3.0	25.2	56.0	137.6	139.8

Ten morphologically similar, adult plants of *P. maritimum* grown under ambient conditions were selected at random. Samples of expanded leaves, bulbs, petals, and flowering stalks (scapes) rising directly from the bulb were monthly harvested and immersed in liquid nitrogen while being transferred

from the field to the laboratory. The aboveground plant tissues (i.e., leaves, petals, and scapes) were oven dried at 60 °C for 48 h (petals and scapes) and 64 h (leaves and bulbs). The bulbs were first rinsed from suspended sand grains using distilled water and then they were oven dried. All samples were weighed and ground for the subsequent analyses using a Thomas Wiley Model 4 Mill (Thomas Scientific, Swedesboro, NJ, USA).

2.2. Soluble Sugars and Starch

Soluble sugar content was estimated spectrophotometrically [39]. Soluble sugars were extracted from dried leaf tissue (d.w.) in 80% ethanol (v/v), and the tissue residue was used to determine the starch content. Grounded samples were placed in 10 mL 80% ethanol (v/v), in a shaker, and the extracts were filtered using Whatman # 2 filter paper. Soluble sugar concentration was determined using a modified phenol-sulphuric acid method [40]. Quantitative determination of starch content was accomplished in sugar-free aliquots of dried tissues, which were solubilized in perchloric acid and the starch content was estimated using a modified anthrone method [41]. The values are expressed as mg g⁻¹ d.w.; D-glucose (Serva, Heidelberg, Germany) aqueous solutions were used for the standard curve.

2.3. Proline

Proline content was determined spectrophotometrically [42,43]. Dried samples were crushed into fine powder with liquid nitrogen and homogenized with aqueous sulphosalicylic acid (20 mL, 3% w/v), and the homogenate filtered through Whatman # 2 filter paper. A total of 2 mL of the filtrate reacted with acid-ninhydrin solution (2 mL) and glacial acetic acid (2 mL) in triplicate test tubes, which were heated at 100 °C for 1 h in a water bath and the reaction terminated in an ice bath. After cooling, the reaction mixture was extracted with 4 mL toluene, and homogenized in a vortex. The chromophore containing the toluene was aspirated from the aqueous phase, and the absorbance was measured at $\lambda = 520$ nm, using toluene as a blank sample. The proline content is expressed as $\mu\text{mol g}^{-1}$ d.w.; L-proline (Serva, Heidelberg, Germany) solutions were used for the standard curve.

2.4. Statistical Analysis

The results are presented as mean \pm standard error (SE). To determine differences in the ecophysiological traits during the studied period, a two-way analysis of variance (ANOVA) was performed on the presented parameters at $p \leq 0.05$, and the Duncan's multiple range test was applied for comparing the means. All statistical tests were performed using SPSS v23.0 (SPSS Inc., Chicago, IL, USA). Regression analysis was used to determine relationships among variables.

3. Results

3.1. Soluble Sugars

The highest soluble sugar content, among the considered plant parts, was detected in the flowering stalks (349.20 mg g⁻¹) in June (Figure 1), whereas the lowest in bulbs (96.23 mg g⁻¹) in July and August (Figure 1). Relatively elevated soluble sugar content was detected in bulbs from January to May and in expanded leaves of *P. maritimum* from December to June (Figure 1); thereafter the lowering of the abovementioned values is associated with constraints of the vegetative growth, as water deficit progresses during the dry period. Throughout the flowering period (June–August), elevated values of soluble sugar content (approximately 300 mg g⁻¹) were detected in the petals of *P. maritimum*, while those in the scapes gradually declined from 350 mg g⁻¹ in June to 194 mg g⁻¹ in August. Significant differences were found among seasonal values of leaf soluble sugar content ($p < 0.05$), but significant differences were not found in the sugar content of bulbs during autumn (i.e., from September to December) and from January to May ($p = 0.138$). Soluble sugar content in petals was significantly correlated with climatic parameters (Table 1), i.e., it is negatively coordinated with the precipitation ($y = -4.37x + 330.16$, $R^2 = 0.75$, $p < 0.05$) and positively with the temperature ($y = 12.49x - 17.09$,

$R^2 = 0.83, p < 0.05$). The soluble sugar content in scapes was significantly and positively affected by the temperature ($y = 9.26x - 150.02, R^2 = 0.99, p < 0.05$). Additionally, a positive correlation was detected between the soluble sugar concentrations in bulbs and leaves ($y = 0.92x + 89.49, R^2 = 0.62, p < 0.05$).

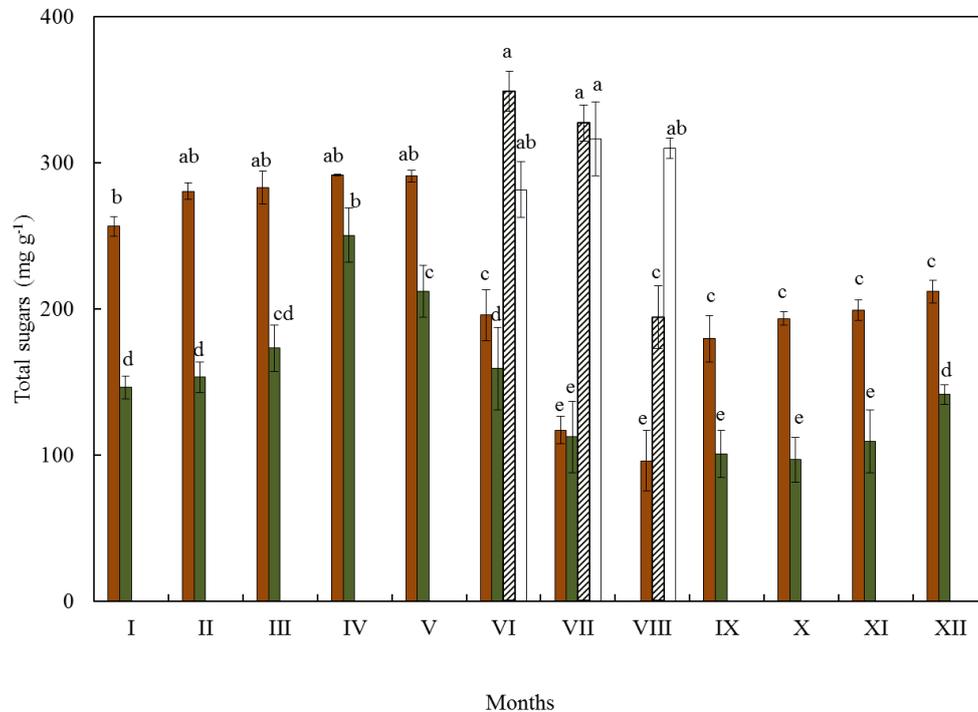


Figure 1. Sugar content in bulbs (brown bars), expanded leaves (green bars), scapes (dashed bars), and petals (white bars) of *Pancratium maritimum*, from January (I) to December (XII). Each column denotes means of five replicates \pm Standard Error. Significant differences ($p \leq 0.05$) of mean values are presented by different lowercase letters (a–e) on bars.

3.2. Starch

The highest starch content in bulbs of *P. maritimum* was observed in April and May (602 mg g^{-1}) when the climatic conditions were favorable for photosynthesis in the Mediterranean ecosystems, thereafter, starch content declined (Figure 2); intermediate values of starch content were detected in bulbs from June to January and the lowest value of starch content was observed in overwintering bulbs, in January (140 mg g^{-1}), while increased values of starch content were detected in bulbs from March to May. The starch content in leaves was significantly lower than that of the bulbs, throughout the year (Figure 2). The values of starch content in scapes were not different between June and July, while the starch of scapes decreased in August (Figure 2). Relatively low values of starch content in petals were not significantly different between June and August (Figure 2). Starch content of aboveground tissues, although 5–10-fold lower than that of bulbs, was negatively affected by the precipitation, i.e., in leaves ($y = -0.19x + 52.48, R^2 = 0.46, p < 0.05$), scapes ($y = -5.43x + 130.43, R^2 = 0.97, p < 0.05$), and petals ($y = -0.68x + 30.81, R^2 = 0.68, p < 0.05$). Regarding the seasonal values of starch accumulation, significant differences were found among tissues (Figure 3, Table 2).

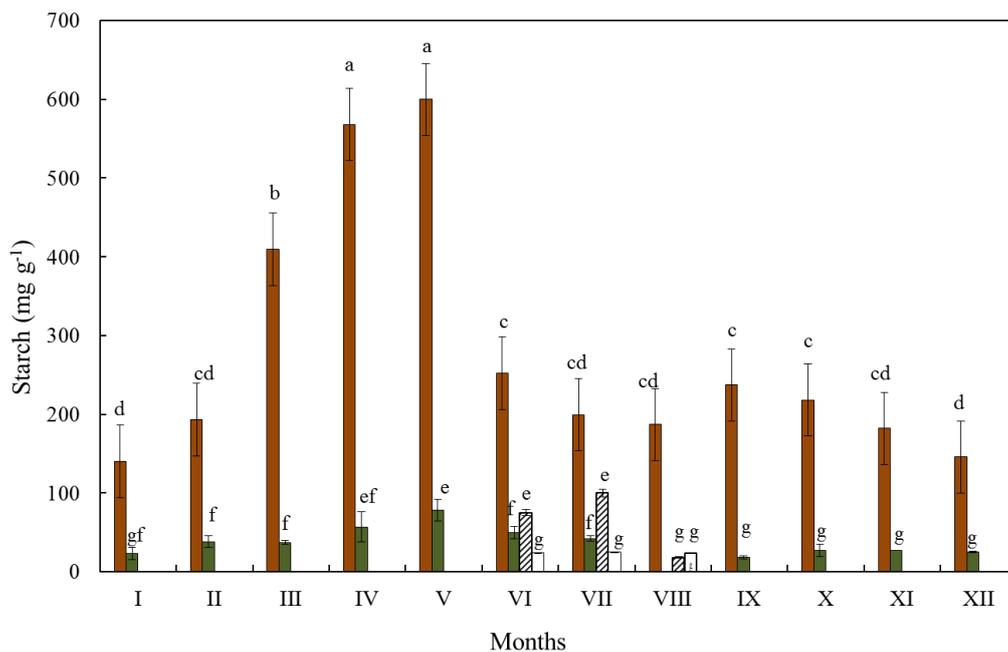


Figure 2. Starch content in bulbs (brown bars), expanded leaves (green bars), scapes (dashed bars), and petals (white bars) of *Pancratium maritimum*, from January (I) to December (XII). Each column denotes means of five replicates \pm Standard Error; Standard Errors smaller than the line thickness of the columns are not shown. Significant differences ($p \leq 0.05$) of mean values are presented by different lowercase letters (a–g) on bars.

3.3. Proline

Proline accumulation increased in leaves of *P. maritimum* from September to May, (Figure 3). In the senescent leaves in July, the lowest proline content was detected ($0.57 \mu\text{mol g}^{-1}$). Proline accumulation in the bulbs of *P. maritimum* declined from January ($4.68 \mu\text{mol g}^{-1}$) to June ($0.83 \mu\text{mol g}^{-1}$), while it increased from October ($1.85 \mu\text{mol g}^{-1}$) to December ($5.08 \mu\text{mol g}^{-1}$) (Figure 3). The accumulation of proline in the bulbs was low (Figure 3) from March to November, while it increased during the winter (from December to February). The highest value of proline content was detected in petals in July ($22.80 \mu\text{mol g}^{-1}$), during the drought stress. The highest values of proline content in scape-samples were also detected in July ($3.78 \mu\text{mol g}^{-1}$), but they were approximately 6-fold lower than those of petals; nevertheless, concerning proline accumulation, a significant positive correlation was detected between petals and scapes ($y = 0.24x - 1.78$, $R^2 = 0.92$, $p < 0.05$). Additionally, enhanced values of proline accumulation were detected in the petals of *P. maritimum* in June ($14.24 \mu\text{mol g}^{-1}$) and August ($12.32 \mu\text{mol g}^{-1}$). Regarding the seasonal values of proline accumulation, significant differences were found among tissues (Figure 3, Table 2). Proline accumulation in the leaves was negatively affected by the precipitation ($y = -0.12x + 8.07$, $R^2 = 0.81$, $p < 0.05$). In the bulbs, proline was positively coordinated with the precipitation ($y = 0.02x + 0.92$, $R^2 = 0.73$, $p < 0.05$) and negatively affected by the temperature ($y = -0.16x + 4.89$, $R^2 = 0.62$, $p < 0.05$). The accumulation of proline in both scapes ($y = 0.98x - 23.04$, $R^2 = 0.90$, $p < 0.05$) and petals ($y = 3.38x - 70.03$, $R^2 = 0.66$, $p < 0.05$) was positively affected by temperature.

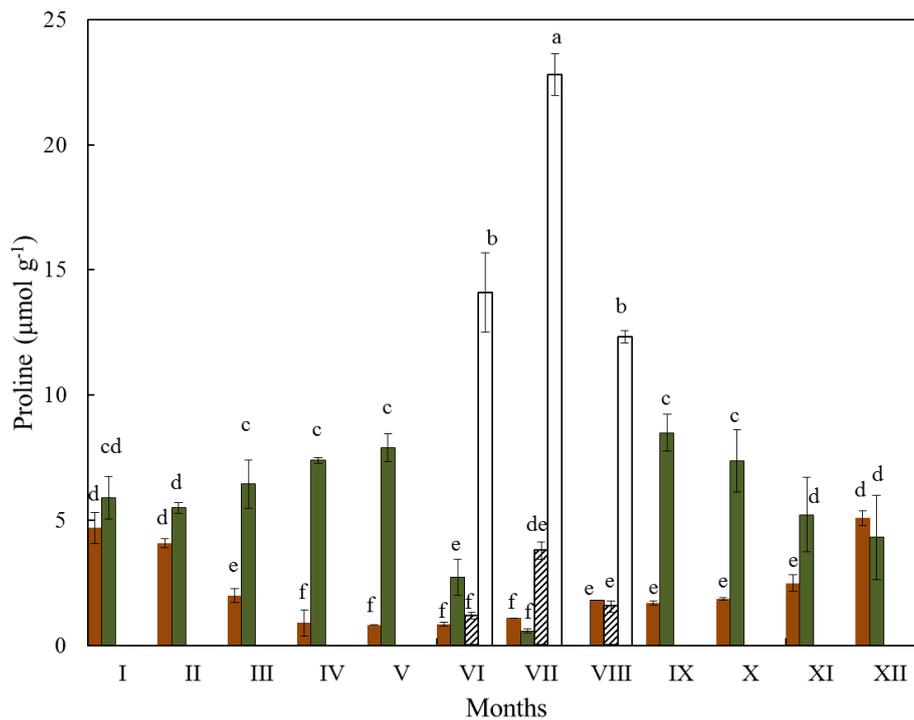


Figure 3. Proline content in bulbs (brown bars), expanded leaves (green bars), scapes (dashed bars), and petals (white bars) of *Pancratium maritimum*, from January (I) to December (XII). Each column denotes means of five replicates \pm Standard Error; Standard Errors smaller than the line thickness of the columns are not shown. Significant differences ($p \leq 0.05$) of mean values are presented by different lowercase letters (a–f) on bars.

Table 2. A two-way analysis of variance (ANOVA) was performed on ecophysiological traits among different tissues; statistically significant differences at $*p < 0.05$.

Source	Parameter	MS	F	Source	Parameter	MS	F
Tissues	Sugars	28,131.71	8.18	Substances	Leaves	55,575.67	*56.14
	Starch	128,342.21	10.93		Bulbs	251,016.60	25.35
	Proline	169.35	28.56		Petals	79,213.60	620.21
			Scapes		64,789.33	22.54	

4. Discussion

Geophytes must allocate resources to storage, vegetative, and reproductive tissues, in order to ameliorate their survival, development, and reproduction [44]. In natural species, allocation strategies reflect trade-offs between survivorship risk and subsequent fitness benefits, being therefore crucial aspects to a species' ecophysiology [45]. It is well known that the geophyte growth strategy is associated with temporal separation of the vegetative and the flowering phases [46]. *P. maritimum* exhibits a leaf longevity that ensures photosynthetic carbon gain for a prolonged period throughout a year, while its flowering coincides with the duration of the drought season in Mediterranean ecosystems. The enhanced soluble sugar concentration in the expanded leaves of *P. maritimum* occurs concomitantly with the elevated photosynthetic rates obtained in this species during spring [47]. In plants energy in the form of sugars is transported from the leaves to sites of active growth [48]. It is likely that sugars, imported from the leaf source region, are partitioned among non-photosynthetic sinks, i.e., such as bulbs and petals [48]. The subsequent, seasonal lowering of sugars in leaves of *P. maritimum* is linked to the increased sugar content in its floral tissues. It is known that the mobile elements are moved to growing regions; our results of soluble sugars and proline accumulation indicate a strong coupling

between vegetative and reproductive growth. The enhanced accumulation of soluble sugars in the bulbs of *P. maritimum*, from January to May, may be in agreement with the concept that enhanced sugar concentration is a prerequisite for efficient transport of soluble sugars [49]. Additionally, the elevated soluble sugar content in bulbs preserves water uptake from a drying soil.

A key to ornamental suitability in landscapes is flower quality, time-initiation of flowering, and flower duration [50]. Flowering of *P. maritimum* constrained to the warmest period of the year, is in part probably related to pollination [14,51]. However, the maintenance of petal expansion—during a 3-month dry period—seems to be ameliorated by adjustment via osmotic agents, such as proline and soluble sugars, translocated through plant tissues [52–54]. It has been argued that reproductive organs are constructed from resources either recently acquired, or previously stored by the vegetative parts [48,55]. In fact, elevated accumulation of soluble sugars and proline in petals (from June to August) may result to a reduction of their osmotic potential, which is expected to sustain their turgor and avoid the harmful effect of desiccation, during the dry period. Additionally, the pronounced proline accumulation in expanded leaves of *P. maritimum* from September to May indicates the protective role of proline in leaves exposed to saline environment [56]. Low proline content in bulbs of *P. maritimum* from early spring to late autumn indicates a timely nitrogen supply to the expanding foliar and floral tissues [52–54], which is also supported by the positive correlation between bulb proline content and precipitation ($y = 0.02x + 0.92$, $R^2 = 0.73$, $p < 0.05$).

Starch is mainly stored in bulbs of *P. maritimum* and the elevated values from March to May coincide with elevated photosynthetic rates [47]. Geophyte underground storage organ reserves are constantly being allocated to other plant parts, such as foliar and floral tissues; starch is the carbon source most likely used exclusively for sprouting of leaves and inflorescences [45,57]. Although starch content in leaves was substantially lower than that of bulbs, a positive correlation was detected between bulbs and leaves ($y = 7.62x - 7.01$, $R^2 = 0.68$, $p < 0.05$).

Our results reveal different both magnitude and partitioning of starch, sugars and proline in the above and belowground tissues of *P. maritimum* grown under field conditions; this suggests that distribution is a major determinant of the life cycle of this geophyte, linked to a blossoming that is not affected by the severity of the dry season, because its flowers that open sequentially remain expanded for 3 months.

In the current global agenda of agronomy, issues such as biodiversity loss, salinity, water depletion, and human-induced environmental activity pose new challenges for researchers. In this context, native-plant responses to abiotic stresses may represent promising sustainable aspects for ornamental plant production (under unfavorable environments), which reflects the human need for ornamental plant consumption as part of the life [58]. Further research is required in order to understand allocation patterns and the integrated response of *P. maritimum* to multiple abiotic stresses and its astounding capacity to acclimate its flowering during the dry summer concomitantly with the cessation of the foliar growth.

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