ORIGINAL ARTICLE

'Variations in phenological and functional traits in *Thapsia garganica* populations in Al Jebel Al Akhdar, Libya'

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Abstract

Thapsia garganica is a herbaceous perennial in the Apiaceae, distributed around the Mediterranean and traditionally used in North Africa as a remedy for arthritis, herpes, hair-fall, hypertension, rheumatic, eczema and scabies. *T. garganica* is the source of thapsigargin: known for killing cancer cells. Seven populations were studied in sites differing in aspect and elevation within Al Jebel Al Akhdar in Libya. We studied the within-population size distribution of individuals, the timing of the phenological events, and vegetative and reproductive traits. *Thapsia garganica* showed high flexibility of functional traits and shifts in the timing of phenological events in response to elevation and aspect (north- or south-facing slopes). Local soil properties, together with effects due to altitude and aspect, could be direct causes of the observed differences in terms of population size and phenological traits. The phenological patterns detected also provide insights into the species response to climate change. Despite this phenotypic flexibility, and good growth in sites at 300–700 m a. s. l. on south-facing slopes, most sites in this study have an unstable size-structure, with few small individuals. This raises concerns about the long-term persistence of the other populations of this species in Al Jebel Al Akhdar.

Résumé

Thapsia garganica est une vivace herbacée de la famille des Apiacées présente sur le pourtour méditerranéen. Elle est traditionnellement utilisée en Afrique du Nord comme remède contre l'arthrite, l'herpès, la chute de cheveux, l'hypertension, les affections rhumatismales, l'eczéma et la gale. *T. garganica* est une source de thapsigargine, connue pour détruire les cellules cancéreuses. Sept populations situées sur plusieurs sites répartis au sein du district d'Al Jebel Al Akhdar en Libye, présentant chacun des expositions et altitudes différentes, ont été étudiées. Nous avons étudié la répartition des tailles, la chronologie des événements phénologiques ainsi que les traits végétatifs et reproductifs des individus au sein de chaque population. *T. garganica* a présenté une grande variabilité en termes de traits fonctionnels et d'évolution au cours des différents stades phénologiques, en réaction à l'altitude et l'exposition (pentes exposées au nord ou au sud). Les propriétés du sol local ainsi que les effets provoqués

par l'altitude et l'exposition peuvent constituer des causes directes des différences observées en termes de taille de population et de traits phénologiques. Les tendances phénologiques constatées fournissent également des renseignements sur la réaction de cette espèce face au changement climatique. Malgré cette variabilité phénotypique et une croissance satisfaisante sur les sites situés entre 300 et 700 mètres au-dessus du niveau de la mer sur les pentes exposées au sud, la plupart des sites ayant fait l'objet de la présente étude présentent une structure de taille inégale avec quelques individus de petite taille. Cela suscite des inquiétudes concernant la persistance à long terme des autres populations de cette espèces au sein du district d'Al Jebel Al Akhdar.

1 | INTRODUCTION

It is important to understand the effect of environment variables on the performance of populations in terms of population structure and trait trade-offs, in order to predict the response of species to climate change and to identify suitable conservation strategies (Csergő et al., 2017; Hegazy, Barakat, & Kabiel, 2006; Hegazy & Lovett-Doust, 2018; Sutherland et al., 2013). Local conditions within a heterogeneous landscape could promote convergence of the reproductive phenology of different species within plant community to coincide with favourable environmental conditions (Gugger, Kesselring, Stöcklin, & Hamann, 2015; Hall & Willis, 2006; Hegazy, Kabiel, Hosni, Badawi, & Lovett-Doust, 2019; Verhoeven, Poorter, Nevo, & Biere, 2008). At the species level, divergent reproductive phenology can be adaptive in different slope aspects (e.g. Hegazy, Kabiel, Boulos, & Sharashy, 2010). Within Al Akhdar mountainous landscape, this behaviour is well documented for other species, as *Juniperus phoenicea* L. and *Arbutus pavarii* Pamp. (Kabiel, Hegazy, Lovett-Doust, Al-Rowaily, & Al Borki, 2015 and Kabiel, Hegazy, Lovett-Doust, Al-Rowaily, & Al Borki, 2016; Hegazy, Kabiel, Al-Rowaily, Lovett-Doust, & Al Borki, 2016).

Among intraspecific adaptive strategies in herbaceous perennials are summer and winter dormancies that ensure persistence in the unfavourable summer or winter seasons (Gillespie & Volaire, 2017). Summer dormancy is a physiological state allowing stress avoidance and is considered as an efficient 'escape strategy', seen in several Mediterranean herbaceous perennials subjected to hot dry summers, where the development of dormant underground buds is comparable to seed dormancy in annual species (Balachowski, Bristiel, & Volaire, 2016; Crabbé, 1994; Dennis, 1996; Graeber, Nakabayashi, Miatton, Leubner-Metzger, & Soppe, 2012; Hoffman & Parsons, 1993). Dormancy in vegetative tissues is an adaptive response to ensure survival under severe drought conditions where meristem becomes temporarily insensitive to growth-promoting signals and activity ceases (Lang, Early, Martin, & Darnell, 1987; Rohde & Bhalerao, 2007). The process of dieback of above-ground tissues precedes dormancy and starts with the progressive senescence of vegetative branches during the fruiting phenophase until early seed dispersal, followed by senescence of the reproductive stalks accompanying the dispersal process, followed by complete dieback. Dieback is controlled by photoperiod and temperature conditions that trigger phytohormonal and chemical signals within the plant (Gillespie & Volaire, 2017; Volaire & Norton, 2006). Dormancy has been shown to be effective in enhancing survival in other summer dormant species (Nie & Norton, 2009).

Thapsia garganica or 'deadly carrot' is unpalatable and poisonous to grazing animals (El-Mokasabi, 2014b; Weitzel, Rønsted, Spalik, & Simonsen, 2014). It is an important medicinal plant that belongs to the stenomediterranean chorological type, and was recorded in the heterogeneous landscape of Al Akhdar mountain by several investigators who studied the distribution (Hegazy, Kabiel, Boulos, & Sharashy, 2011 and Hegazy et al.,2016) and the traditional use of the species (El-Mokasabi, 2014a and El-Mokasabi, 2014b). Among the traditional uses of *T. garganica* in the eastern Mediterranean region of Libya, cited by El-Mokasabi, (2014a) and El-Mokasabi (2014b), are arthritis, herpes, hair-fall, hypertension, rheumatic and scabies. In Algeria, the species is used as an anti-inflammatory treatment for eczema (Boudjelal et al., 2013).

Four phenylpropanoids (potent cytotoxins) and a thapsigargin analogue have been isolated from the fruits (Liu et al., 2006). The resin is extracted from *T. garganica* root bark (French, 1971). Micropropagation is used due to the importance of thapsigargin in killing cancer cells, due to the difficulty of inducing seed germination and seedling establishment of *T. garganica* (Jäger & Nyman, 1993; Jäger et al., 1993; Makunga, Jäger, & Staden, 2003 and Makunga, Jäger, & Staden, 2005; Pickel et al., 2012; Andersen, López, Manczak, Martinez, & Simonsen, 2015, Huang, Wang, & Wang, 2018). In this study, we aimed to explore the adaptive strategy that permits the species to persist in different slope aspects and climate and soil types associated with a wide range of altitudes in Al Jebel Al Akhdar, despite the difficulties of inducing seed germination (Andersen et al., 2015; Makunga et al., 2003). This investigation could provide insight to predict species' strategies in response to the projected

climate change and could guide strategies for optimal conservation management.

2 | MATERIAL AND METHODS

2.1 | Study species

Thapsia garganica L. (Arabic name: Derias; Family: Apiaceae) is distributed around the Mediterranean Sea and grows in various sites within the Al Akhdar Mountain (the Green Mountain) landscape, in eastern of Libya (Hegazy et al., 2011 and Hegazy et al., 2016; Boudjelal et al., 2013). The species is an herbaceous perennial that shows complete summer dormancy: a dieback phenomenon where the whole above-ground shoot system dries up. The plant perennates over the dry season and grows a single above-ground shoot system each year from the root system. The vegetative shoot consists of several branches originating from a discoid underground stem. Leaves are compound with dissected leaflets. The species is a scabies hemicryptophyte having one to three leafless floral axes per individual supported by lateral vegetative branches at the base. The vegetative shoot is generally much smaller than the flowering or fruiting stalks bearing the inflorescences or infructescences. The inflorescences are compound umbels where each umbellule carries fifteen yellow flowers. The fruits are winged capsules containing two mericarps each of them with one seed. The colour of the fruits changes from green to brown upon maturation.

2.2 | Study area

Al Akhdar Mountain is composed of ancient marine limestone sediments and is the most vegetated part of Libya where the diversity of species, life forms and phenophase durations are sensitive to elevation above sea level (Hegazy et al., 2016). Vegetation and species richness varied with altitude; however, temporal and spatial variations in climatic conditions remain the main determinant of vegetation types (Hegazy et al., 2011). The climate along Al Akhdar Mountain is influenced by elevation, aspect and distance from the Mediterranean Sea (Kabiel et al., 2015). Along the mountainous landscape, shrubs and trees dominate the coastal plains and low and mid-elevations, while the mountain top is dominated by herbs and chamaephytes. The greatest species richness is recorded at mid-altitudes, and the same type of vegetation is seen on the north-facing (more humid) and south-facing (more arid) slopes but at a higher altitude on the south-facing slope (Hegazy et al., 2011). Ambient average monthly temperatures range from 20.8°C at low elevations, to 18.1°C at mid-elevations, to 17.2°C at high elevations along the north-facing slope. The amount of annual rainfall ranges from 267 mm at low elevations, to 360 mm at mid-elevations, to 528 mm at high elevations along the north-facing slope. Climate data were obtained from nearby weather stations along the mountain in Derna (32°45′59″ N, 22°37′56″ E), Al Merj (32°29′19″ N, 21°50′01″ E) and Al Beida (32°45′58″ N, 21°44′28″ E), for records on low, mid and high elevations, respectively (LNMC, 2012).

2.3 | Plant data

A preliminary field survey led to the selection of seven populations of *T. garganica* for our study, four of them along the north-facing slope, and the other three on the south-facing slope (Table 1). The selection depended on the presence of well-defined population along the north-facing and south-facing slopes. The study populations were classified into altitudinal zones: Alt 1 =< 50 m a.s.l.: one population located in the coastal dunes on the north-facing slope; Alt 2 = 300–500 m a.s.l., and Alt 3 = 500–700 m a.s.l.: populations at mid-altitude (mid-mountain), two on the north-facing slope and two on south-facing slope; and Alt 4 => 700 m a.s.l.: two populations, one on the mountain top on the north-facing slope, and the other at the highest elevation on the south-facing slope (Table 1). The phenological pattern of *T. garganica* was recorded throughout 2016–2017 at each site. Each week, starting from March to November, the phenological stage (sprouting and

TABLE 1 Locations of the study sites

Altitudinal zone	Elevation	Aspect	Location	Site	Soil texture	Site I.D.
North-facing slope						
Alt 1	28	Coastal dunes	32°53′25.0″ N, 21°56′19.3″ E	Susa	clay	N1
Alt 2	445	Mid-mountain	32°39′16.6″ N, 21°33′54.7″ E	EL-Wardia	clay to clay loam	N2
Alt 3	506	Mid-mountain	32°43′52.5″ N, 21°35′26.7″ E	Massa	clay loam	N3
Alt 4	839	High-mountain (Mountain top)	32°38′21.0″ N, 21°47′21.7″ E	Sidi AL-Hemery	clay loam to silty loam	N4
South-facing slope						
Alt 2	473	Mid-mountain	32°28′58.0″ N, 21°22′28.7″ E	Marrow	loam	S2
Alt 3	686	Mid-mountain	32°33′32.5″ N, 21°35′42.6″ E	Qandulah	clay loam to silty clay loam	S3
Alt 4	816	High-mountain	32°36′31.6″ N, 21°44′51.4″ E	Slanta	clay	S4

Note: Populations in the north-facing and south-facing slopes within four altitudinal zones: Alt 1 < 50 m a.s.l.; Alt 2 = 300–500 m a.s.l.; Alt 3 = 500–700 m a.s.l.; and Alt 4 => 700 m a.s.l.

vegetative growth, flowering, fruiting, seed dispersal, senescence of vegetative shoot, senescence of reproductive shoot and dormancy of perennating buds) was recorded in each population. The start of a phenological stage was determined when at least ten per cent of the population was involved (visual observations).

During the fruiting phenophase in each population (site), fifty individuals were selected for the measurements of vegetative (plant dimensions: height and two diameters, number of branches) and reproductive (number umbellules) traits. Five individuals were sampled for determination of fresh and dry weights of plant tissues. Only the above-ground tissues were harvested leaving behind the perennating buds and their associated roots for conservation purposes. *Thapsia garganica* seedlings were rarely observed and showed vegetative growth with a volume of shoot ≤ 0.0156 m^3, throughout the growing season.

2.4 | Soil data

Three soil samples were taken from each site to a depth of 20 cm within a 50 cm circle around *T. garganica* plants. To characterise the water availability and mineral nutrition among populations, the physical and chemical analyses of soil were performed: the texture, in terms of coarse sand, fine sand, silt and clay fractions, was determined, organic matter content was estimated (through loss of mass on ignition), as well as the concentrations of calcium, magnesium, sodium and potassium (Allen, Grimshaw, Parkinson, Quarmby, & Roberts, 1974; Jackson, 1973).

2.5 | Data analyses

The density of *T. garganica* individuals per 100 m^2 and the contribution of size classes to each population were calculated. The whole population was included in the census. Individuals within each population were classified into four size classes in terms of volume: A =< 0.05 m^3, B = 0.05–0.1 m^3, C = 0.1–0.3 m^3, D => 0.3 m^3. This was based on the volume of the vegetative shoot at the flowering stage when maximum vegetative growth has been completed for the season. The volume of the vegetative shoot was calculated as $(\pi/6(R_1R_2R_3)$, where R_1 is the height of the vegetative shoot, and R_2 and R_3 represent the major and minor diameters (http://www.onlin econversion.com/object_volume_ellipsoid.htm). Each population was characterised in terms of plant density and size class distribution. The fresh and dry weights (oven-dry at 70°C until constant dry) of the leaves, stems and infructescences (reproductive stalks) were determined and their moisture content calculated. The allocation, in terms of the ratio of reproductive to vegetative dry mass, was estimated. The means, standard errors and coefficient of variations of the measured traits were calculated for each population.

The generalised linear model was used to test the effect of elevation (altitudinal zones), and slope aspect (north-facing vs. south-facing slopes) and the interaction between these parameters on the

traits of the study plants. As a discrimination tool for the populations studied, principal component analysis (PCA) was performed on the data for soil physical and chemical properties, and plant traits, using the CANOCO 4.5 software (ter Braak, 2008).

3 | RESULTS

3.1 | Population structure

The number of individuals per 100 m^2 differed among populations (Figure 1a). The highest density (5.8 individuals) was recorded on the north-facing slope at Alt 2, while the lowest value (3.52 individuals) was seen at the same altitude on the south-facing slope. The highest
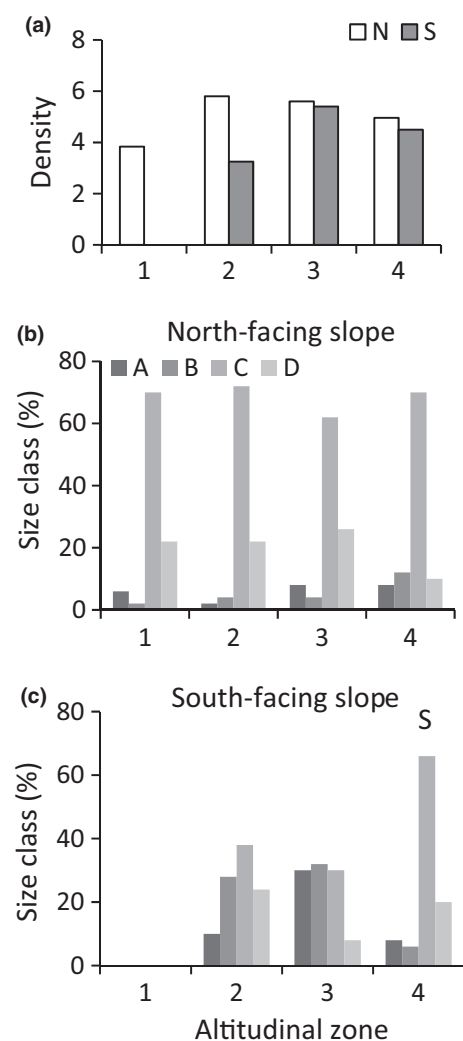
FIGURE 1 Density of *T. garganica* individuals per 100 m^2, in the seven study populations (a), and the size class distribution in populations on the north-facing (b) and south-facing (c) slopes, at different altitudinal zones. *N* = north-facing slope, S = south-facing slope. Altitudinal zones are as follows: 1 =< 50 ma.s.l.; 2 = 300–500 m a.s.l.; 3 = 500–700 m a.s.l.; 4 => 700 ma. s. l. Size classes are as follows: A =< 0.05 m^3, B = 0.05–0.1 m^3, C = 0.1–0.3 m^3, D => 0.3 m^3

FIGURE 2 The three phenological patterns of *T. garganica* observed in the study region. Phenophases: 1 = sprouting and vegetative growth, 2 = flowering, 3 = fruiting, 4 = seed dispersal, 5 = senescence of vegetative shoot, 6 = senescence of reproductive shoot, and 7 = dormancy of perennating buds. Altitudinal zones are as follows: 1 = < 50 ma.s.l.; 2 = 300–500 m a.s.l.; 3 = 500–700 m a.s.l.; 4 => 700 ma.s.l
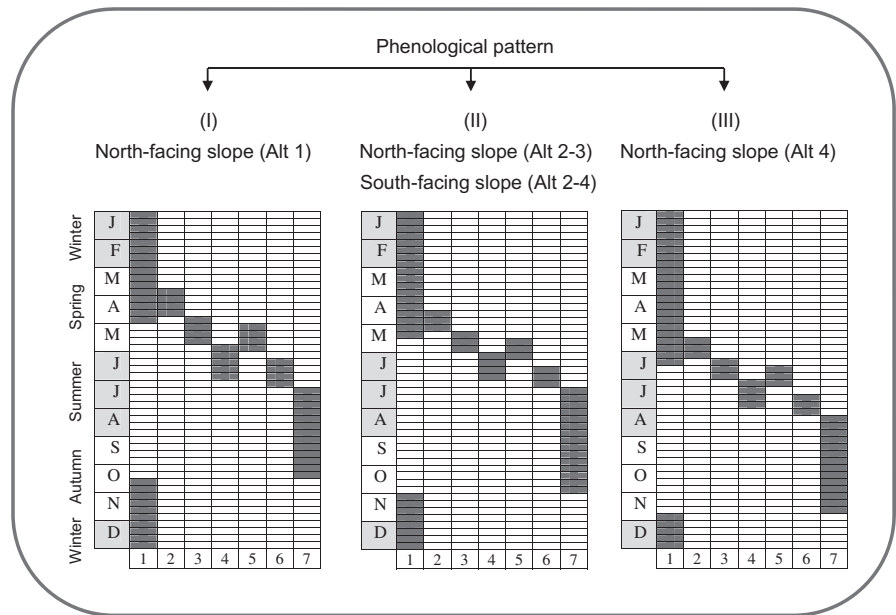
density on the south-facing slope was seen at Alt 3 (5.4 individuals), with 4.5 individuals at Alt 4. On the other hand, the lowest density on the north-facing slope was seen in the coastal dune site (Alt 1) with 3.83 individuals per 100 m², contrasted with values ranging from 5.8–4.9 at higher altitudinal zones.

The size class distribution of populations on the north-facing slope was unimodal with size class C being dominant (62%–72% contribution) at all elevations (Figure 1b). The second most frequent was size class D (10%–26%). The smaller size classes were of very low abundance (2%–12%) in all populations. Populations on the south-facing slope showed two distinct size class distributions (Figure 1c). Populations at Alt 2 and 3 had balanced distributions with more plants in the small size classes. Size class A contributed 10 and 30%, and size class B reached 28 and 32% in populations at Altitudes 2 and 3, respectively. However, the distribution of size classes in the population at Alt 4 resembled those on the north-facing slope with 66% contribution of size class C, contrasting with contributions of 38 and 30% in size class C, in populations at Alt 2 and 3, respectively, on the south-facing slope.

3.2 | Phenology

Three phenological patterns were visually observed, as shown in Figure 2: (a) Pattern I, in the coastal dunes at Alt 1, on the north-facing slope; (b) Pattern II, on the south-facing slope (Alt 2, 3, and 4) and mid-altitudes of the north-facing slope (Alt 2 and 3); (c) Pattern III, on the mountain top on the north-facing slope (Alt 4).

Plant population belonging to phenological pattern I started sprouting by mid-October, in mid-Autumn (Figure 2). Vegetative shoots persisted to the end of April. Then, by early May, senescence began, during late spring, with complete senescence of vegetative shoots by the end of the month. Flowering started in late March, during early spring, followed by fruiting in late April. Seed dispersal

began in late May accompanied by progressive senescence of reproductive stalks. Complete senescence of aerial parts (dieback of all above-ground vegetative and reproductive shoots) was achieved by early July, leaving the hidden dormant perennating buds. Dormancy extended for 13 weeks, during summer and autumn.

Plant populations characterised by phenological pattern II started sprouting and vegetative growth by early November, in late autumn (Figure 2). The green branches remained until mid-May where senescence began, followed by senescence of vegetative branches in early June. Flowering began in mid-April, during mid-spring, followed by fruiting in early May. Seed dispersal started in early June, continuing until the end of the month, during early summer. Complete senescence of aerial parts was achieved by early July, as in pattern I, followed by 15 weeks of dormancy, during summer and autumn.

Plant population characterised by phenological pattern III started sprouting by late November, in the end of autumn (Figure 2). Vegetative shoots persisted until mid-June. Senescence of vegetative shoots ended by early July. Flowering started in mid-May, in late spring, followed by fruiting in early June, during early summer. During mid-summer, seed dispersal started in early July, and complete die back ended in early August. Dormancy extended for 15 weeks, mostly during autumn.

3.3 | Vegetative and reproductive traits

The GLM analysis revealed the significant effect of elevation and aspect (north- or south-facing slope) on variation in the numbers of branches and umbellules per individual (Table 2). The interaction between elevation and slope aspect is significant only for the number of umbellules per individual (Table 2).

In populations on the north-facing slope, the number of branches per individual declined from 9.7 and 10.2 at Alt 1 and 2, respectively, to 7.4 and 7.5 at Alt 3 and 4, respectively, with the greatest number

of branches seen at Alt 2 (Figure 3a). These values showed low variability (a coefficient of variation (c.v.) of 0.19 and 0.14) for populations at Alt 1 and 2, respectively. The c.v. increased to 0.3 at Alt 3 and 4. On the south-facing slope, comparatively more branches per individual were seen, with 13.4, 8.9 and 11.2 at Alt 2, 3 and 4, respectively, with the greatest value at Alt 2 (Figure 3b). However, plants on the south-facing slope showed greater variability; the c.v. ranged from 0.3 at Alt 4 to 0.6 at Alt 2.

On the north-facing slope, plants had more umbellules per individual in the population at Alt 1 with a maximum of 322 umbellules, contrasting with values ranging from 130 to 249 at higher elevations with c.v. values around 0.2 (Figure 3c). On the south-facing slope, the maximum number of 356 umbellules was seen in the population at Alt 3 with a coefficient of variation of 0.15 (Figure 3d). Lower values were seen at Alt 2 (149 umbellules) and Alt 4 (246 umbellules) with low variation within these populations.

Populations on the south-facing slope generally had more branches and more umbellules than the corresponding populations at the same altitudinal zone on the north-facing slope (Figure 3a–d).

The GLM analysis showed no statistically significant effect of aspect on the ratio of reproductive to vegetative shoots; however, the effect of elevation and the interaction between elevation and slope aspects were significant (Table 2). The ratios of reproductive to vegetative tissues, on a dry weight basis, was highest at Alt 1 on the north-facing slope reaching 29.29; that is, reproductive branches weighed about 30 times as much as the vegetative branches (Figure 3e). Low variability was noted (c.v. = 0.23) within that population. In other populations on the north-facing slope, the ratios of reproductive to vegetative shoots were lower, but increased from Alt 2 (5.9) to Alt 3 (17.6) with coefficient of variation ranging from 0.33 to 0.43. On the south-facing slope, the highest ratios of reproductive to vegetative shoots (21, c.v. = 0.28) were seen in the population at Alt 3, decreasing to 10.52 (c.v. = 0.36) and 4.63 (c.v. = 0.48) at Alt 4 and 2, respectively (Figure 3f).

The GLM analysis revealed the significant effect of the elevation and aspect and the interaction between them, on the variation in the moisture content of leaves, stems and reproductive stalks (Table 2). The moisture content of leaves on plants in the coastal dune population (Alt 1, north-facing slope) was higher (73.9%) than in the population at Alt 2 (55.5%) on the north-facing slope (Figure 4a). In contrast, similar values (47 and 41% in Alt 1
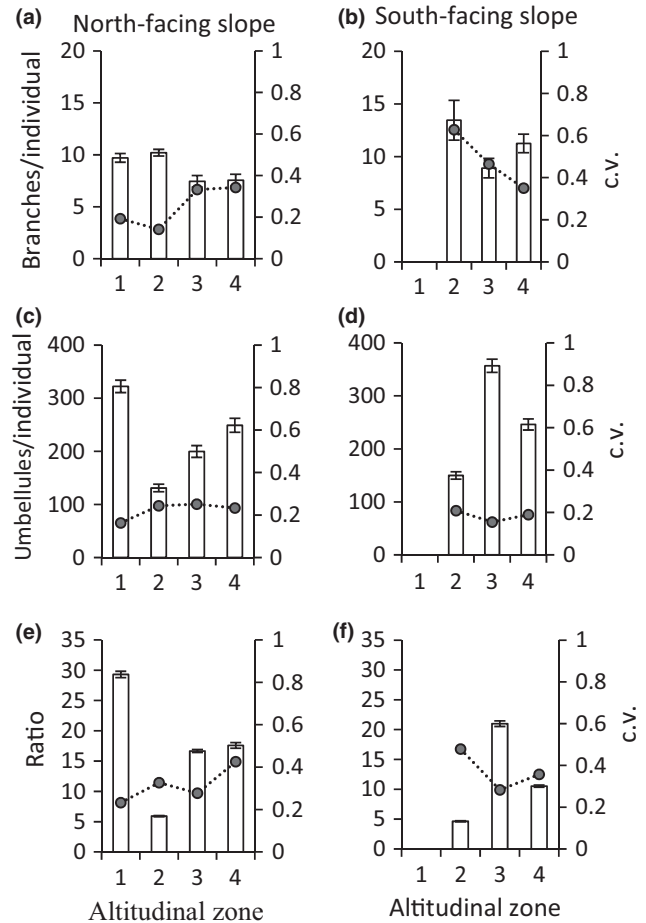
FIGURE 3 The number of branches (a and b), and the number of umbellules (c and d) per individual, in populations of *T. garganica*, at different altitudinal zones on the north-facing and south-facing slopes. The ratios of reproductive to vegetative dry weights are shown in (e) and (f). Altitudinal zones are as follows: 1 =< 50 ma.s.l.; 2 = 300–500 m a.s.l.; 3 = 500–700 m a.s.l.; 4 => 700 ma. s. l. Error bars represent standard errors of the means. Dashed lines represent the coefficient of variation (c.v.) in each population

and 2, respectively) were seen in terms of the moisture content of stems although the variability of stem moisture content was high with the c.v. reaching more than 40% in both populations (Figure 4c). The moisture content of reproductive stalks reached 78.2% in the coastal population, contrasted with lower values in

Trait	Elevation	Aspect	Elevation* Aspect
Number of branches per individual	18.14*	14.16*	1.71
Number of umbellules per individual	309.36*	46.25*	69.67*
Ratio of reproductive to vegetative shoots	266.29*	7.89	14.91*
Leaf moisture content (%)	80.46*	17.71*	94.24*
Stem moisture content (%)	22.57*	1.64*	64.95*
Reproductive stalk moisture content (%)	61.76*	12.32*	13.11*

TABLE 2 GLM of the dependence of the vegetative and reproductive parameters of *T. garganica* on the elevation (altitudinal zones) and the slope aspects (north-facing and south-facing slopes), and the interaction between them
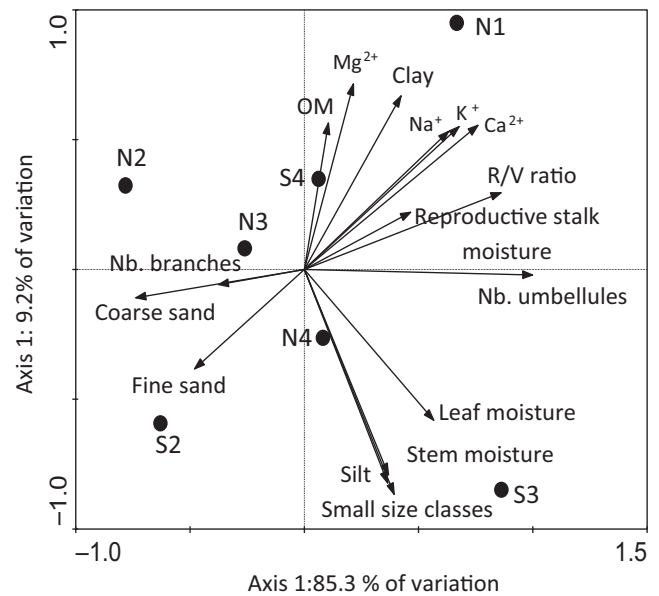
Note: Chi-square values are shown, and a star would indicate a significant effect on the same parameter at *p* ≤ .05.
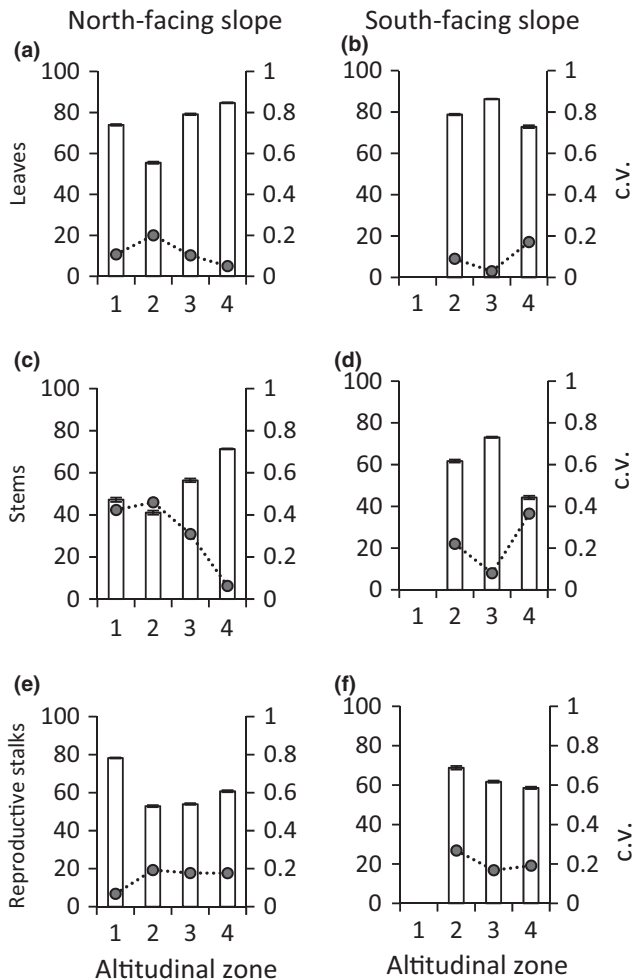
FIGURE 4 The moisture content (%) at the fruiting phenophase, of the leaves, stems and reproductive stalks of *T. garganica* on the north-facing and south-facing slopes, at different altitudinal zones. Altitudinal zones are as follows: 1 =< 50 m a.s.l.; 2 = 300–500 m a.s.l.; 3 = 500–700 m a.s.l.; 4 => 700 m a. s. l. Error bars represent standard errors of the means. Dashed lines represent the coefficient of variation (c.v.) in each population

FIGURE 5 Ordination biplot of the first two principal components of a PCA carried out on the study populations and the matrix of the soil parameters and plant traits. Each point represents a study population where the symbol represents the slope aspect followed by the altitudinal zone. Slope aspect: north-facing slope = N, south-facing slope = S; Altitudinal zone: 1 =< 50 m a.s.l.; 2 = 300–500 m a.s.l.; 3 = 500–700 m a.s.l.; 4 => 700 m a. s. l. Components of the soil physical analysis and the concentration of organic matter are indicated as percentages; the concentrations of the elements in soil as ppm. R/V ratio = ratio of reproductive (reproductive stalks) to vegetative (leaves and stems) tissues, on a dry weight basis. Small size classes are represented by their contribution to the populations

the other populations at higher elevations on the north-facing slope (Figure 4e).

Populations at Alt 2–4 on the north-facing slope showed a progressive increase in moisture content from lower to higher elevations ranging from 55.4% to 84.7% in leaves, 41.1 to 71.3% in stems and 52.9 to 60.7 in reproductive stalks at Alt 2 and 4, respectively (Figure 4a,c and e). In contrast, populations at Alt 2–4 on the south-facing slope had the highest values for moisture content at Alt 3 in terms of leaves (86.3%) and stems (73.1%), and at Alt 2 for reproductive stalks (68.7%, Figure 4b,d and f).

3.4 | Soil parameters and plant traits

From lower to higher elevations, the soil texture varied from clay to clay loam to silty loam on the north-facing slope (Table 1). While on the south-facing slope, the soil texture varied from loam to clay loam to silty clay loam (Table 1).

The matrix with the soil physical and chemical variables, and plant traits showed variations among the study populations that were mostly reflected on Axis 1 accounting for 85.3% of the variation (Figure 5). Most obviously, N1 had the highest concentrations of sodium, potassium and calcium ions; this makes sense as the site is closer to the Mediterranean Sea (Figure 5). The previous soil parameters, together with high contents of clay and organic matter, were highly correlated with the ratio of reproductive to vegetative tissues, the moisture content in the reproductive stalks and the number of umbellules per individual (Figure 5).

The ordination of the soil and trait variables among the study sites showed another trend where S3 was mostly associated with silt content and had the greatest correlations with the contribution of small size classes and stem and leaf moisture contents. In another context, S3 shared with N1 a high correlation with the number of umbellules per individual. On the other side of the ordination plot, N2 and S2 showed the greatest correlation with the number of branches per individual which was associated with greater coarse and fine sand contents.

4 | DISCUSSION

The presence of populations of *T. garganica* at a wide range of elevations and aspects enhances its adaptive potential within the Al Akhdar mountainous landscape. Local populations contrast in terms of phenological patterns and trait variations. Populations on the north-facing slope showed variations in phenology and functional traits along the altitudinal gradient, where temperatures tended to decrease while precipitation and soil moisture availability tended to increase. As a result, populations at lower elevations experience a more severe and extended summer drought than other populations along the elevation gradient (Vasek & Sauer, 1971; Körner, 2003; Hegazy et al., 2011 and Hegazy et al., 2016). In contrast, populations on the south-facing slope experienced harsher conditions than those on the north-facing slope (Hegazy et al., 2011 and Hegazy et al., 2016). However, populations on the south-facing slope and those at mid-altitudes on the north-facing slope showed similar phenological pattern suggesting the species has a wide phenological amplitude where phenological patterns changed only in response to very different environmental conditions. These populations showed variations and trade-offs among vegetative and reproductive traits. The effect of microhabitat on trade-offs between vegetative and reproductive organs is well documented (e. g. Olsson & Ågren, 2002).

Populations showed two distinct phenological groups: (a) populations showing a significant shift in the timing of the phenological events: the coastal and mountain top populations on the north-facing slope and (b) populations showing similar timing of phenological events despite experiencing different elevations and aspects. The latter populations showed intermediate timing of phenological events. In both cases, variations in population structure and functional traits in *T. garganica* populations are largely affected by local conditions. Clearly, the coastal dune population, experiencing a more xeric environment, was placing more emphasis on sexual reproduction, as compared to the other populations that allocate more time and biomass to vegetative growth. Similarly, Matthews and Mazer (2016) showed that higher average minimum temperatures promoted flowering, and higher precipitation tended to delay flowering. Dahlgren, Zeipel, and Ehrlén (2007), similarly, found that higher soil temperatures were the most important cause of earlier shoot emergence and flowering time. Indeed, earlier flowering and earlier dormancy are the direct reflection of the higher aridity in Pattern I (Ofir & Kigel, 2003). Similarly, the correlation between earlier flowering and earlier emergence has been reported by several authors, for example Sola and Ehrlén (2006), and directional selection imposed by climate change, and favouring earlier flowering was reported by Anderson, Inouye, McKinney, Colautti, and Mitchell-Olds (2012). Such shifts in reproductive phenology and life history traits on the inter- and intraspecific level following increased global warming are inevitable and have been well documented by several authors (e. g. Gugger et al., 2015; Hegazy et al., 2010). Flowering phenology is often found to be highly responsive to local conditions (Anderson et al., 2012).

We suggest that contrasting soil properties, together with variations in environmental conditions associated with altitude, are responsible for the observed variations in population traits. In this context, the soils in the coastal dune population tended to have higher clay content, with higher sodium, potassium and calcium content. Elevated potassium in soil tends to support early sprouting, which supports our findings (Dahlgren et al., 2007). In addition, this population had the greatest moisture content of reproductive stems, which, in theory, would favour higher reproductive success. Indeed, the coastal dune population showed greater reproductive output and a higher ratio of reproductive to vegetative aboveground biomass. On the other hand, the population showed lower density of individuals and predominance of large size classes. All of these findings enforce the superior performance of plants in terms of sexual reproduction, in part because early flowering in this population may reduce the storage resources available in the underground tissues for subsequent vegetative growth (Olsson & Ågren, 2002). Moreover, it is argued that unimodal size distribution, dominated by an intermediate size of individuals, is indicative of life history strategy rather than of population trends, and that it does not reflect population decline (Bin et al., 2012; Wright, Muller-Landau, Condit, & Hubbell, 2003). And, although unimodal species are characterised by lower seedling recruitment as shown here in *T. garganica*, this could be compensated for by lower mortality (Bin et al., 2012). The situation reflects the stresses of the more arid environment where plants must initiate vegetative growth early, and a large portion of biomass is allocated to sexual reproduction (Dunne, Harte, & Taylor, 2003). Unfortunately, juvenile recruitment is low, so unless the survivorship of perennating individuals is superior, the net result will be a declining population in these coastal dunes. The threat of population decline due to low recruitment in *T. garcanica* populations is raised in other sites even within theoretically balance structured populations. Despite high contribution of small size classes, they are not likely to be all younger, but rather adult individuals, with low vegetative growth and high reproductive output.

The differences among populations on the north-facing and south-facing slopes regarding the density and vegetative and reproductive growth is mainly dependent on local conditions associated with variation in altitudes and physical and chemical characteristics of soil that play a major role in the variation in water storage capacity and infiltration and transport rates in soils in these populations. On the other hand, the photosynthetic flowering stems in *T. garcanica* contribute to a great extent in the growth of the species at all sites. A photosynthetic reproductive structure can make a significant contribution to a plant's carbon gains (Aschan, Pfanz, Vodnik, & Batič, 2005). And, this clearly shows the independence of the reproductive tissue from the direct limited capacity of the vegetative tissue and nutrients reserved in the rootstock in providing dry matter for reproduction. In this case, even individuals with few branches were able to produce large reproductive stalks; for example, in the coastal dune population, the reproductive

biomass is approx. 30 times greater than the biomass of vegetative shoots, on a dry weight basis. In fact, local conditions such as early flowering and an extended growing season in the coastal dune population are major determinants of population reproductive output.

5 | CONCLUSIONS

The study species, *T. garganica*, buffers itself from environmental heterogeneity by flexibility as it has an effective adaptation strategy involving trait variations that can be adjusted to meet local conditions. Variations in the environmental variables imposed by different altitudes and slope aspects, together with local soil properties, could be considered the proximate causes of the observed variations in population traits in Al Jebel Al Akhdar.

In most of the region, the smallest size class, that most likely to include new juvenile individuals, is relatively small. Although populations of perennial species can, indeed, persist, ultimately the recruitment of juveniles is critical to long-term population survival. It would be useful to carry out long-term demographic studies to assess juvenile recruitment and population age structure (as distinct from size structure), in order to assess the likely response of this species to the ongoing increase in aridity as climate change proceeds.

The species has not yet been evaluated by the IUCN; however, it is subjected to collection for medicinal use and medicinal research. From a conservation perspective, we recommend that the status of this medicinal species—which is increasingly wildcrafted in the region—would be assessed in North Africa. Further investigations of seed germination and recruitment are needed to evaluate the regeneration potential of the species in nature.

As we could not emphasise that small plants that either did not flower were seedlings that arose that year, or is it possible that they were just individuals that were growing poorly (that could have been older), this point could be pursued in the future through greenhouse studies. Moreover, raising the species under controlled conditions is needed to better evaluate dry matter allocation in aerial and underground parts, and to confirm that the species is not capable of clonal propagation.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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