



## Article

# Wild Grapevine (*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmelin) Hegi)—Novel Species to the Israeli Flora

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**Abstract:** The wild grapevine, *Vitis vinifera* subsp. *sylvestris*, grows naturally throughout the northern hemisphere, including the Mediterranean region. Wild grapevines have also been observed sporadically across the southern Levant and are considered a non-native feral plant. Nevertheless, no formal characterization has been conducted for wild grapevines in this region; thus, its taxonomical assignment remains elusive. Previously, we have shown that the wild grapevine populations growing in northern Israel are genetically separated from the feral domesticated forms. This work aimed to comprehensively describe the morphological, anatomical, and ecological traits of wild grapevines naturally thriving in two distinct habitats in Israel. The dioicous nature of the wild grapevine, the flower and pollen morphology, and the characteristic *Sylvestris* fruit and seed morphology, in addition to the occurrence of the natural germination of seeds in close vicinity of the mother plant, have all led to the conclusion that these plants belong to *Vitis vinifera* subsp. *sylvestris* and should be included in the Flora Palaestina. These findings, combined with the recently published genetic evidence for these populations, significantly advance our understanding of the species' ecology and the importance of its preservation.

**Keywords:** Israeli flora; morphological characterization; *Sylvestris*; *Vitis*; *Vitis vinifera* subsp. *sylvestris*; wild grapevine species persistence



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## 1. Introduction

The *Vitaceae* family mostly includes shrubs and woody lianas that climb using leaf-opposed tendrils. Most grape cultivars belong to the *Vitis* genus, which consists of 83 subordinate taxa [1] that primarily prevail in the northern Hemisphere, including North America and East Asia. The Eurasian species of common grapevine, *Vitis vinifera* subsp. *sylvestris* (C.C. Gmelin) Hegi [2] (hereafter, *Sylvestris*), is the best-known species, as it is the ancestor of most of the grapes cultivated today [3–5]. The cultivated form, *V. vinifera* subsp. *sativa* (hereafter, *Sativa*), is one of the most notable perennial crops, cultivated across 7.3 million hectares worldwide [6]. The distinction between these two subspecies is mainly based on the morphological differences in their reproductive organs, as, while the wild grapevine is dioecious, *Sativa* is a hermaphrodite [5,7,8].

Wild grapevine (*Sylvestris*) grew abundantly in natural habitats in Europe until the mid-nineteenth century, when the invasion of pests and pathogens from North America, including phylloxera and powdery and downy mildews, caused a decrease in their

populations [8]. Later, accelerating urbanization processes and extensive anthropogenic land use dramatically damaged the natural habitats of the wild grapevine (*Sylvestris*), reducing its distribution range and endangering its persistence [9,10]. While the *Sylvestris* populations shrank, the cultivated grapevine (*Sativa*) flourished throughout Europe and the eastern Mediterranean region, where, by the end of the 19th century, according to Post's seminal work, it was "cultivated everywhere in numerous varieties, but nowhere strictly spontaneous" [11].

The southern Levant was considered a region beyond the distribution range of the wild grapevine; thus, early studies of Israeli vegetation during the 20th century considered all grapevine plants as *Sativa*, i.e., feral populations. Indeed, feral grapevines may emerge due to vineyard abandonment or neglect, wherein the previously cultivated grapevines begin to proliferate without human intervention. Over time, the seeds originating from these plants may germinate and be mistakenly observed as wild grapevines, adopting similar climbing growth habits. Because of the mixture of both feral and real wild subspecies in the same habitat, and the false assumption that *Sylvestris* does not grow in such a way down south, the *sylvestris* species was not considered to be native to the land. The first indication of wild grapevine in the southern Levant region was in 1994, discovered in surveys in the Upper Galilee region, along the banks of the Jordan River [12,13]. Unfortunately, the available records of these surveys lack the necessary description and exact location of the observed plants. For these reasons, until 2004, the species was not included in wild flora records in this region [14]. Over the years, more evidence of indigenous southern Levantine *Sylvestris* has accumulated. Fossil pips, pollen, and wood of wild grapevines were discovered in archaeological sites from the Lower Paleolithic Gesher Benot Ya'aqov (780,000 BP) through the Upper Paleolithic Ohalo II (23,000 BP) [15–19]. These sites are located around the area of the Hula Lake, the Sea of Galilee, and the Jordan River, in high geographical proximity to the populations observed in the 1994 survey.

Recently, a dedicated comprehensive survey of grapevines in Israel uncovered new populations of hermaphrodite and dioecious plants [20]. Their genetic analysis, with SSR markers and morphological characterization, identified a marked separation between the hermaphrodite accessions and the dioecious groups, with the latter displaying unequivocal wild grapevine characteristics (leaf, bunch, and berry shape, and growth habits). Moreover, the dioecious wild grapevine (*Sylvestris*) accessions show a genetic split between two distinct subgroups, in accordance with ecogeographic divergence. The dioecious populations, occurring primarily along the main streams in the Upper Galilee region and around the Sea of Galilee, marked the southern edge of the distribution range of the wild grapevine [21].

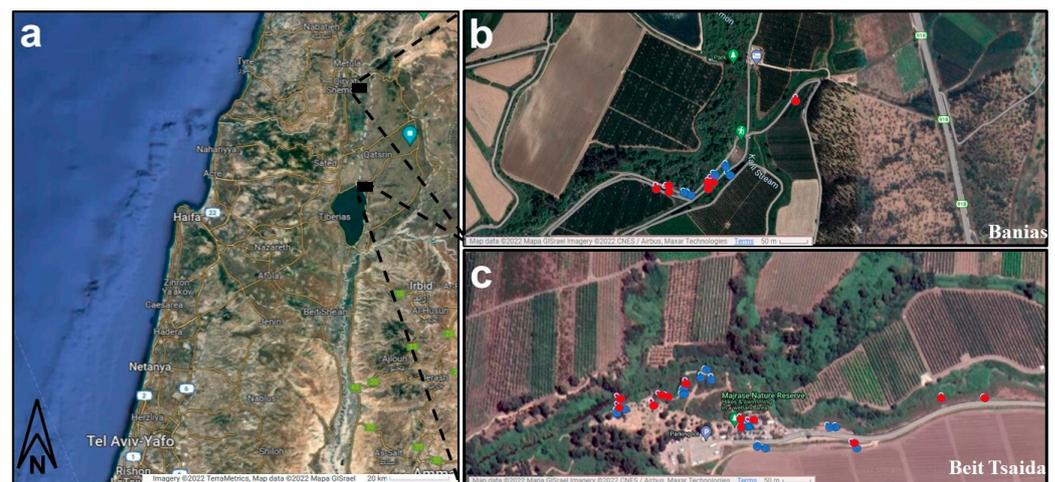
An in-depth analysis of whole-genome sequencing data from these accessions has further supported the previous observations that wild grapevine grew naturally in the southern Levant for millennia [22]. In fact, these wild grapevine accessions were identified as the progenitors of the domesticated indigenous varieties from the Levant, which genetically contributed to some of the European varieties [22,23]. These conclusions were further supported in a recent comprehensive genomic study of more than 3500 accessions, which provided unequivocal evidence for the contribution of southern Levantine *Sylvestris* populations to domesticated grapevine species around the world [24]. The *Sylvestris* populations from Israel (E1) were identified as the genetic source of the table-grape group (CG1) [24]. This group later genetically contributed to the most well-known wine grape varieties used in modern times worldwide.

Despite the number of studies and amount of evidence for thriving wild grapevine populations in Israel, there remains a considerable knowledge gap with regards to the evolution, geographic distribution, ecology, and domestication of this species. This work provides a systematic and comprehensive morpho-anatomical characterization of the wild grapevine population that spread between the two different sites in Israel, including a thorough characterization of the male and female flowers, the fruit and seed morphology, the distribution dynamics of the male and female individuals in each group, and natural regeneration by spontaneous germination. The findings clearly affirm that the inspected

wild grapevine populations were accurately assigned to the protologue and type specimens of *Vitis vinifera* subsp. *sylvestris*.

## 2. Materials and Methods

**Plant material and research area:** The sampling was carried out during the spring (May 2022), when the plants were in full blossom, which enabled the identification of developing pistils and stamens in the dioecious (unisexual plants) or monoecious plants. The samples were collected in northern Israel, where stable populations of wild grapevines, denoted here as *Sylvestris*, had been previously observed [21]. Thirty-two wild grapevine accessions were collected from the Beit Tsaida site, located next to the Sea of Galilee ( $32^{\circ}53'09.2''$  N  $35^{\circ}38'34.6''$  E), and fourteen accessions were collected from the Banias River site in the Upper Galilee region ( $33^{\circ}12'12.3''$  N  $35^{\circ}38'27.6''$  E), from an area of about 8000 m<sup>2</sup> in each location (Figure 1). The geographical positions of the wild grapevine accessions were georeferenced using the free application Google Maps (Google, Inc., Mountain View, CA, USA). Branches with young and mature leaves, as well as inflorescences, were collected from each accession. A subsample of each accession was dried and prepared for Herbaria deposition, and the remaining parts were fixed and stored in FAA solution (formaldehyde: acetic acid: 70% ethanol, 1:1:20) for histomorphological inspection (samples were stored at Ariel University). The exsiccata of all accessions were stored at Tel Aviv University (voucher specimen numbers from TELA4443 to TELA4450).



**Figure 1. Study sampling sites.** (a) Satellite map of the northern part of Israel. The black squares indicate the location of the two study areas. (b) An aerial photo of the Banias area and (c) an aerial photo of the Beit Tsaida area. Blue and red dots represent male and female wild grapevine accessions, respectively. The map was created with Google Maps App. (Mapa GISrael).

**Analysis of growth variation between male and female individuals:** The growth variation between male and female populations was examined by assessing the variation in internode length and diameter, using a digital caliper and a normal scale. This investigation encompassed three male and three female accessions, measuring three branches from each plant and ten internodes from each branch. The plants were collected from Ariel University's Grapevine Germplasm collection, where plants are maintained under uniform growing conditions (common garden) to ensure uniform irrigation, soil, climate, and irrigation conditions [25].

### 2.1. Morphological Characterization

**Leaf:** The leaf morphology of the dry herbarium specimens was examined. The length of the petiole and the length and width of the leaves in their greatest extension were measured. The ratio between leaf length and width and the length ratios of the blade to the petiole were then calculated. The shade differences between the abaxial and adaxial sides of

the leaf lamina were recorded in an effort to determine whether the leaves were concolorous or discolorous. Leaf form was described according to the glossary in Kafkafi [26].

**Seed and berry:** To obtain healthy berries and seeds of optimal size for characterization, plants that were cultivated under ideal conditions at the Ariel University's Grapevine Germplasm collection, located at Ariel University, were studied. Approximately 60 seeds and 100 berries from each site were used for morphological characterization. Berry diameter and seed length and width were measured using a Sparkfun electronics digital caliper (0–15 mm), and the mean and standard deviation are presented herein (Table 1). In addition, the morphological descriptors of the International Organization of Vine and Wine (OIV) [27] were used to describe the morphological traits of the seeds and berries.

**Table 1.** Comparison of the main morphological features of wild grapevines collected at Beit Tsaida and Banias, Israel.

	Beit Tsaida	Banias
Abaxial/adaxial leaf color contrast	Concolor	Discolor
Blade/petiole length ratio	1.72 ± 0.92 *	1.09 ± 0.3 *
Blade shape	Not constant	Trilobate
Blade size ratio	0.8 ± 0.11 <sup>ns</sup>	0.76 ± 0.093 <sup>ns</sup>
Color of berry skin (OIV 225)	Blue black (6) #	Blue black (6) #
Berry shape (OIV 223)	Globose (2) #	Globose (2) #
Berry diameter (mm)	10.04 ± 0.90 *	11.34 ± 0.97 *
Seed shape	Ellipsoid	Ellipsoid
Seed length (OIV 242)	Very short (1) #	Very short (1) #
Seed length (mm)	5.38 ± 0.45 *	5.10 ± 0.42 *
Seed width (mm)	3.66 ± 0.29 <sup>ns</sup>	3.68 ± 0.23 <sup>ns</sup>
Observed ♀/♂ ratio	0.88	1

Measured values are mean ± SD; # numbers indicate OIV description parameters [27]; \* indicates significant differences between the two locations ( $p$ -value < 0.01); ns: non-significant.

**Flower:** Male and female flowers were placed on a glass microscope slide, with or without black paper wrap, and illuminated with a white LED light. Images were captured with a Nikon SMZ25 stereomicroscope (Nikon Ltd., Tokyo, Japan) equipped with a camera (Nikon DS-Ri2). Sixty digital photomicrographs (resolution: 4908 × 3264 pixels) were taken at different focal planes, at ~50 µm intervals, and compiled into a single image using ND2-NIS elements software version 5.02.02, with an extended depth of focus (EDF) patch (Nikon Instruments, Tokyo, Japan). The images were then transformed into a single high-quality focused image using dedicated software.

**Pollen:** Dried anthers with pollen grains were coated with gold using a sputter machine (Quorum Q-150T ES). The prepared samples were then imaged with a Field Emission Scanning Electron Microscope (FE-SEM) (Tescan Ultra-High-Resolution MAIA 3), with a beam voltage of 1.0 kV and an SE detector.

**Tissue histology:** Tissue samples of male and female flowers were fixed in FAA, as described above, embedded in paraplast, sectioned to a 12-µm thickness with a rotary microtome (SLEE medical GmbH, Nieder-Olm, Germany) and stained with safranin-alcian blue [28]. The slides were photographed under an Olympus SZX7 stereomicroscope (Tokyo, Japan) equipped with a camera (Pixelink USB 3.0 version PL-D795CU, Ottawa, ON, Canada) using the PixelINK Capture program.

## 2.2. Statistical Analysis

A statistical analysis was carried out on each dataset to compute the mean, standard deviation, and minimum and maximum values. The normality of distribution was evaluated for each variable. In cases where a normal distribution was ascertained, a  $t$ -test was executed. Cases of non-normal distribution were studied by applying a Wilcoxon 2-sample test. A significant difference, defined by a  $p$ -value < 0.05, served as the basis for inferring equal variances. To explore the morphological attributes of the leaf and petiole

measurements, a principal component analysis (PCA) was performed on the correlation matrix. All statistical analyses were performed using JMP<sup>®</sup> Pro 16.0.0 software [29].

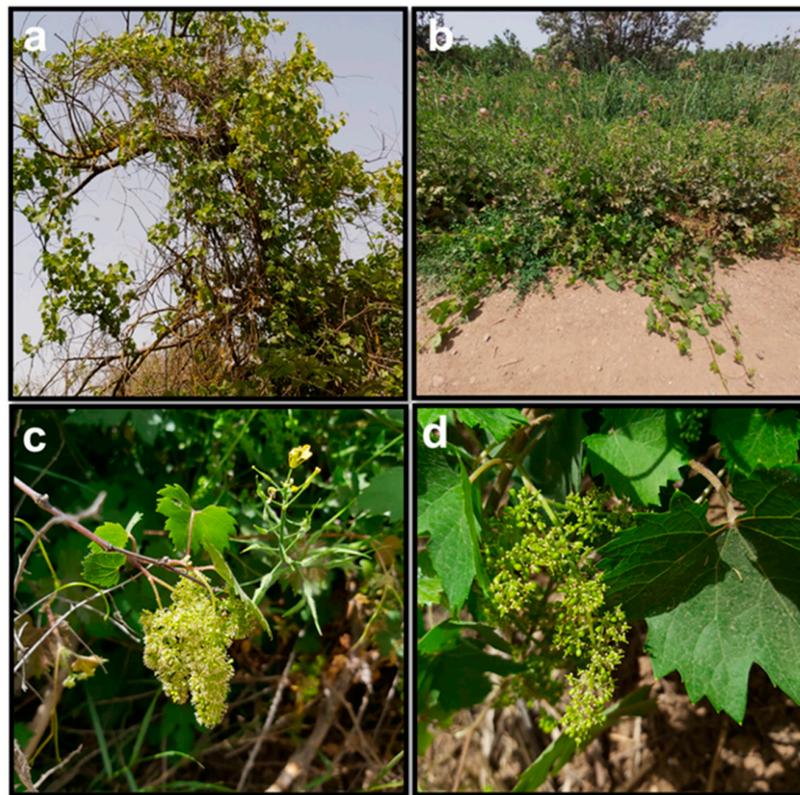
### 3. Results and Discussion

**Sylvestris growth habits in wild habitats:** This study focused on the two sites where stable and large grapevine populations had been previously reported [21]: the northern site at the Baniyas River and the southern site at Beit Tsaida (Figure 1). Both of the sites are located on alluvial soils within protected natural reserves (Baniyas and Majrase). The sampling was performed randomly along the water streams where grapevines grow. Though the sampling areas were of equal size (circa 8000 m<sup>2</sup>), more individuals were found at the Baniyas site than at the Beit Tsaida site. In total, forty-six accessions were collected, with thirty-two wild grapevine accessions originating from Beit Tsaida and fourteen accessions from the Baniyas River. The variation in the population size of the wild grapevine accessions between the Beit Tsaida and Baniyas River areas can likely be attributed to factors such as differing riverbank sizes, micro-climatic conditions, wildfire frequencies and intensity, nutrient availability, ecological characteristics, fluctuation in the water level of the Sea of Galilee, and varying levels of site disturbance due to anthropogenic activities [21]. Notably, the Baniyas River area exhibited a reduction in species diversity due to pronounced anthropogenic activities [30].

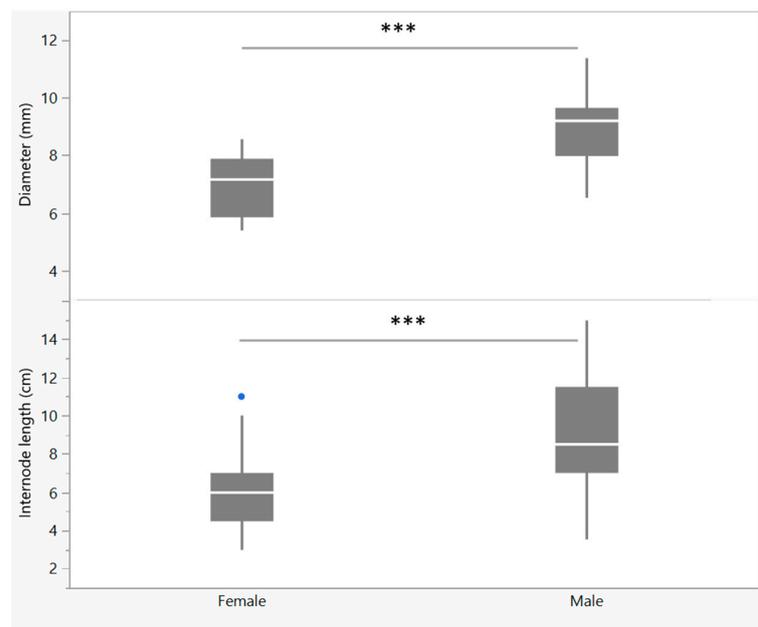
All *Vitis* plants at both of the sampling sites were dioicous, supporting their identification as true *Sylvestris*. The ratio between the male and female plants was close to 1, with a slight dominance of male accessions (Table 1). This finding strengthens the assumption that the examined populations are wild, with no bias toward the fructiferous female form [31].

The growth habits varied between the male and female grapevines at both locations. The male grapevines were taller and tended to climb to the tops of trees, while the female grapevines were generally shorter and tended to prostrate in a tangle of low vegetation (Figure 2a,b). The male grapevines were characterized by multiple and densely packed flower clusters, usually located near the top of the vine (Figure 2b), while the females produced fewer and smaller flower clusters (Figure 2c), which were located lower along the stem. The male climbing habits can be attributed to the wind-pollination strategy [9], and the low stature of the females may be required to structurally support the heavy bunches of fruits. The sexual dimorphism observed in the plant height and inflorescence position seem to be adaptive to the dioecy and pollination by wind and insects, i.e., to a cross-pollination reproductive system. The taller growth of the male population aligned with their significantly longer internode length and wider stem diameter, as compared to the female accessions grown in a common garden (Figure 3).

In the current survey, the grapevines grew in deep uncultivated soils very close to flowing sweet water streams, as was previously reported [21]. The male and female plants were spread randomly. At both of the sites, the wild grapevines grew in proximity to fig trees (*Ficus carica*), plane trees (*Platanus orientalis*), and holy raspberries (*Rubus sanguineus*), a plant community typical to water-rich habitats along the Mediterranean basin. Interestingly, the observed *Sylvestris* female plants tended to grow between the spiny holy raspberry plants, which, apparently, provide protection during flowering against herbivores that are abundant in these regions, including wild boar (*Sus scrofa*) [32] and mountain gazelle (*Gazella gazella*) [33], which commonly feed on grapevine shoots and leaves in cultivated vineyards. Spiny vegetation has been suggested to have played a vital role in protecting wild vines from both wild and domestic animals [34,35]. Overall, the observed populations at both sites had a distinctive appearance, with a significant polymorphism in the leaves, and clusters of small, greenish-yellow flowers, which developed into black grape berries. Their growth habits and woody stems make them hardy plants, providing cover, and serving as a habitat for various animals sharing the same natural environment [36,37].



**Figure 2.** Growth habits of *Sylvestris* plants in northern Israel. (a) Male grapevine climbing on a tree in Beit Tsaida. (b) Female grapevine by the side of the road in Baniyas. (c) Male inflorescence and (d) female inflorescence from Beit Tsaida.



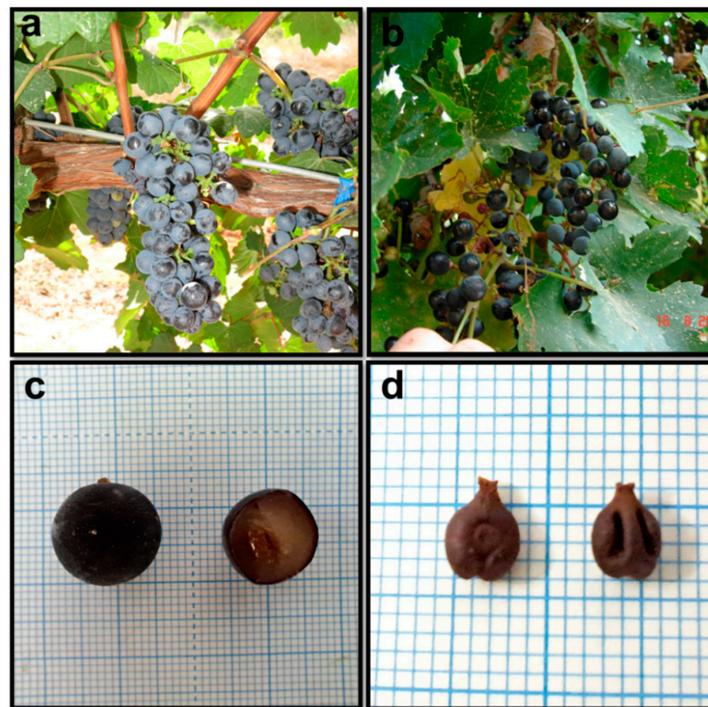
**Figure 3.** Differences in internode length and diameter between male and female wild grapevines. Box plot comparison of the internode length (cm) and diameter (mm) of three male and female wild grapevine accessions from a common garden. The horizontal white lines in the graph represent the median values. The boxes indicate the range between the 25th and 75th percentile of each group's distribution of values. Observation that falls outside of this range is denoted by a blue dot. \*\*\*  $p$  value:  $< 0.001$ .

#### 4. Morphology

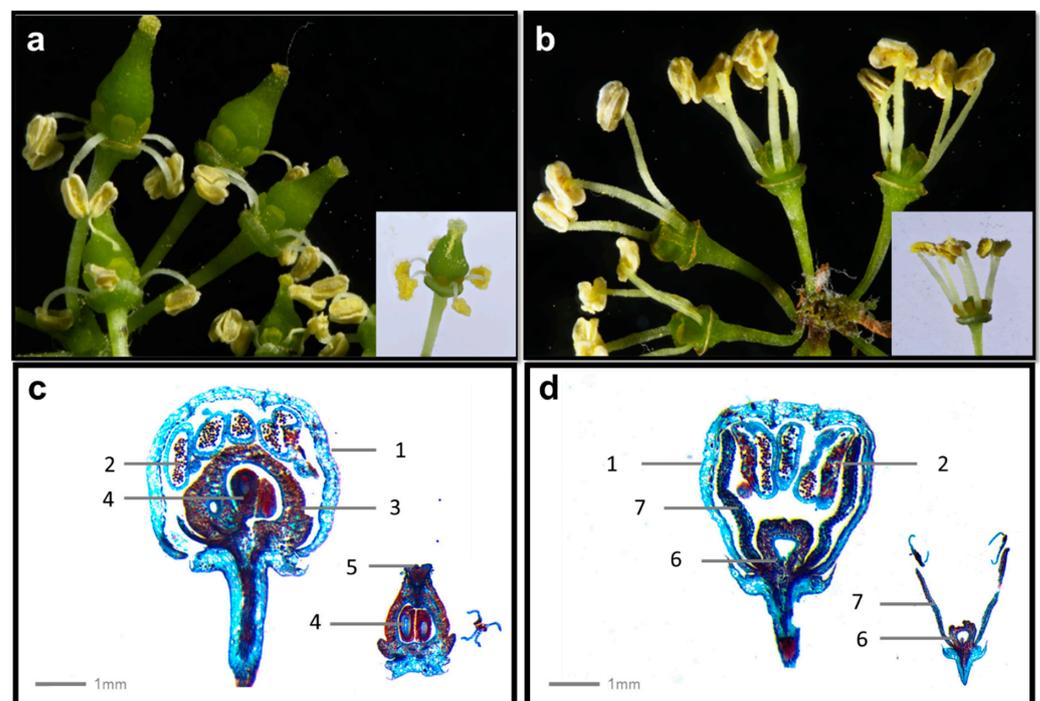
**Leaf morphology:** The leaf shape and morphology were highly polymorphic, ranging from reniform, with weak lobation, to cordate with pronounced lobation. No significant correlation was observed between the blade (length—width) ratios ( $p$ -value > 0.05) and the sampling location (Table 1), presumably due to the wide variation in leaf length. The blade—petiole-length ratio of the wild grapevine population was higher in Beit Tsaida ( $p$ -value < 0.001) than in Baniyas. The dorsal surface of the wild grapevine leaves from Baniyas was hairy, in contrast to the grapevine leaves from Beit Tsaida [21]. This resulted in a shade contrast, with the leaves in the Baniyas populations being discolored, while the Beit Tsaida populations had concolor leaves. The PCA of the leaf and petiole data revealed a very weak separation between the two populations, where the first two principal components explained 89.2% of the variation in the analysis (Supplementary Figure S1). The variation in the PCA was explained mainly by the leaf length (43%), followed by the leaf—petiole length ratio (23%), which further support the above-mentioned statistical analysis and corroborate the slight separation of the populations derived from a greater number of samples and other OIV attributes in our prior study [21]. Taken together, it is reasonable to conclude that the sampled populations do not represent discrete entities, but rather constitute a single population that underwent adaptive changes in response to diverse environmental conditions across the different geographic regions, thereby giving rise to specific morphological characteristics.

**Berry and seed morphology:** The growing conditions have a dramatic effect on the number and size of grape berries and seeds. To obtain healthy, optimal-sized berries and seeds for inspection and characterization, cuttings were sampled from plants at both of the sites and were grown under optimal conditions at the Ariel University's Grapevine Germplasm collection, at Ariel University [25]. When they were cultivated at the university, the fruit bunches were well-grown and dense (Figure 4a), while the bunches were sporadic when grown in the wild (Figure 4b). A broad range of polymorphism in the cluster shapes was observed among the samples, yet most of them had sparse clusters of tiny, typically black, berries, each typically with 2–3 seeds (Figure 4c). The berry diameter was significantly different ( $p$ -value < 0.001) between the populations, with Baniyas *Sylvestris* berries being larger than those of Beit Tsaida (Table 1). In both populations, the berry skin was thin, and the pulp was juicy and sweet, with high acid levels, which were, however, lower than those reported for European *Sylvestris* grapes [20]. The seed length differed significantly between the sampling sites ( $t$ -test,  $p$ -value < 0.001), and was longer in Beit Tsaida (mean = 5.38, sd = 0.45) (Table 1). These seed values correspond to the previously recorded *Sylvestris* varieties [38,39].

**Flower morphology:** It is well established that flower development, and, more specifically, that of the reproductive organs, is the main feature that distinguishes the differences between wild and domesticated grapevines [8]. The female flower includes an ovary and reflexed rudimentary/atrophied stamens that angle downwards, while the male flower displays upright stamens and a reduced pistil, without a style or stigma, and a rudimentary/atrophied ovary at the base (Figure 5). These features are clearly distinctive to *Sylvestris* plants, while the *Sativa* forms, also found occasionally growing feral, all have a hermaphrodite phenotype [20]. Grapevine flowering in the studied areas occurred in the month of May, giving rise to fruit in the female individuals in the summer (August). In the present analysis, the flowers of the wild grapevines were small, greenish-yellow, and arranged in panicles. The individual flowers had a diameter of around 5 mm and contained five petals fused at the base. *Vitis vinifera* subsp. *sylvestris* is the wild ancestor of the cultivated grapevine varieties of *Vitis vinifera* [24]. In the case of *Sylvestris*, the presence of both male and female plants in wild populations requires crossbreeding for the formation of fruit, while the domesticated types are hermaphroditic and can self-pollinate [40]. The hermaphroditic nature of the *Sativa* types was an essential step of the domestication process, leading to stable yields and full bunches, due to better pollination and the possibility of planting only reproductive plants without the need for male pollinator plants [8].



**Figure 4.** Morphological traits of wild grapevine berries and seeds collected from northern Israel. Female grapevine bunches (a) from Ariel University's Grapevine Germplasm collection, at Ariel University, and (b) from the wild (Beit Tsaida). (c) A whole grape berry next to a sectioned berry (Accession number 22, from Ariel University's Grapevine Germplasm collection). (d) Ventral (right) and dorsal (left) sides of the seeds (Accession number 189, from Ariel University's Grapevine Germplasm collection) on graph paper with 1-mm squares.



**Figure 5.** Female and male flowers of wild grapevines and their histological sections (a) Female and (b) male wild grapevine specimens at flowering. Histological sections of closed and open (lower right) (c) female and (d) male flowers. Parts of the flower: 1—Petal, 2—Anther, 3—Ovary, 4—Ovules, 5—Stigma, 6—Atrophied ovary remnant, and 7—Anther filament.

The histological sections of the female grapevine flower showed a single ovary (Figure 5c), a style, and a stigma, which comprised the pistil. The ovary was located at the base of the flower and contained ovules that eventually developed into seeds, if fertilized with pollen (Figure 5c). The structure of the male flower was distinctly different from that of the female flower (Figure 5). The male grapevine flower, sampled at the same location, consisted of an atrophied ovary and stamens that were arranged in a tight cluster at the base of the flower. The stamens produced and released pollen grains that were transferred by wind and/or insects to the female flowers, allowing for fertilization and fruit production [41].

**Pollen grain morphology:** A SEM scan was utilized to image the pollen of the *Sylvestris* accessions sampled at the experimental vineyard (Figure 6a,b). In the male flowers, the pollen grains exhibited tricolpate morphology (with three furrows) and were ellipsoidal in shape (Figure 6b). In the female flowers, the pollen grains were inaperturate and spheroidal in shape (Figure 6a). Additionally, the pollen grains found on the anthers in the female flowers appeared collapsed or exhausted, while being potent in the male flowers, further supporting dioecy. The morphology of the grapevine pollen grains was consistent with previous findings in studies comparing sterile and fertile pollen grains in *Sylvestris* [42,43].

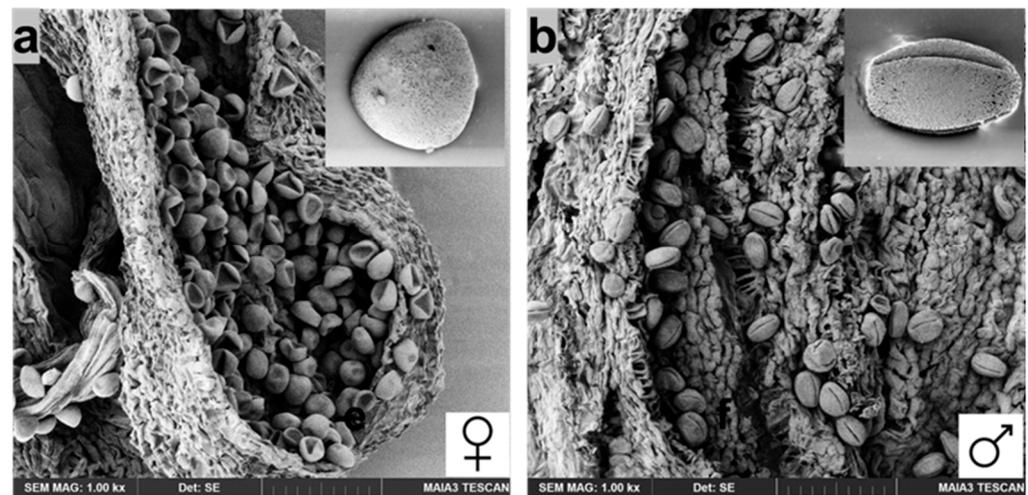
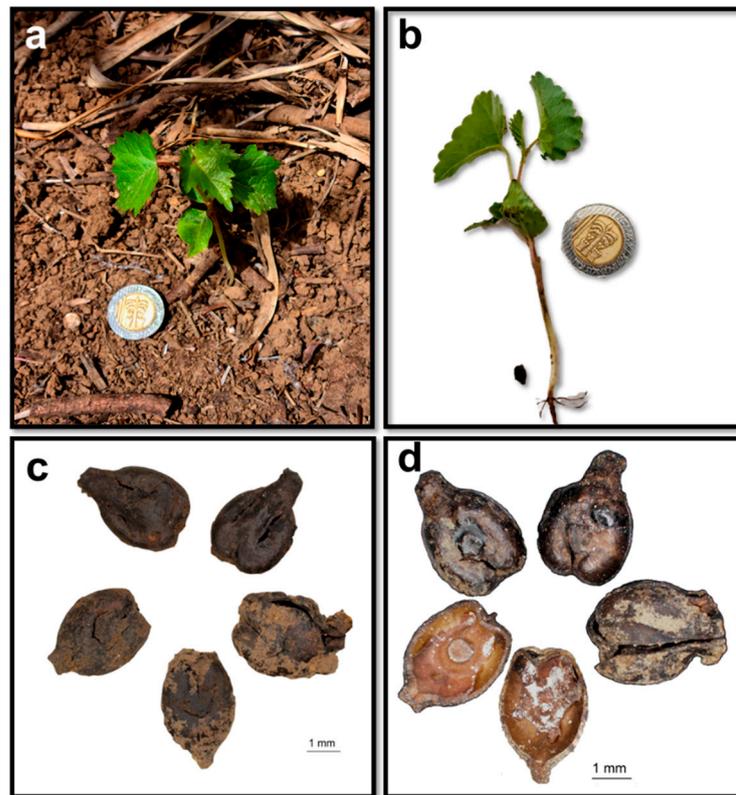


Figure 6. Scanning electron microscope images of pollen grains produced by (a) female and (b) male flowers of wild grapevine (*Vitis vinifera* subsp. *sylvestris*) inside the anther. The scale bar is 50  $\mu$ m. Enlarged single pollen grains are presented on the top right of each image.

**Natural seed germination:** A thorough survey of the *Sylvestris* habitats in the wild was conducted in search of the natural germination of seedlings. In the Baniyas area, five seedlings were found, all beneath female plants (Figure 7a). This germination habit was abundant, indicating its success under the ecological conditions of this specific natural habitat. The young plantlets were carefully removed from the soil, including the remnants of their outer integuments (Figure 7c,d). The grapevine seeds were clearly identifiable, despite being slightly damaged and soiled. To the best of our knowledge, this is the first time that the natural germination of *Sylvestris* in its natural habitat has been recorded. The occurrence of natural *Sylvestris* germination in a natural habitat provides strong evidence for the spontaneous nature of the population and its persistent strength as a stable population—as an indigenous plant in Israel. Due to the growth habits of *Sylvestris* inside of a dense bush of spiny raspberry plants, young seedlings were not identified in the Beit Tsaida area.



**Figure 7.** *Vitis vinifera* subsp. *sylvestris* germination in the field. (a) Naturally germinated grapevine seedling found in the Baniyas area of north Israel under female grapevines (coin diameter 23 mm), and (b) the seedling following its extraction from the soil, including the remnant integument (coin diameter 23 mm). (c,d) Wild grape seeds (c—ventral side, d—dorsal side), which were carefully dug out and removed from the ground.

To summarize, previously identified grapevines growing in the wild in the northern part of Israel were considered to be feral *Sativa* plants, and there was no confidence as to the occurrence of *Vitis vinifera* subsp. *sylvestris* in Israel. This was likely due to the failure to carry out a comprehensive survey, and the minimal description of this population by the surveyors, who provided only brief notes on the species [12,13]. The present work has systematically described the habitats, growth habits, morphology, and anatomy of widely spread wild grapevine populations growing in two distinct habitats in north Israel. All of the findings, including the dioecious nature of the wild grapevine plants, the sexual dimorphism between the male and female plants, the characteristic traits of their flower development, pollen, berry size and morphology, seed structure, and the spontaneous regeneration of the population from seeds, together with our previous genomic findings, showing a clear separation of these populations from feral *Sativa* accessions [20,22], all lead to the conclusion that wild grapevine populations grow naturally in Israel. The results indicate that wild grapevines occur in natural habitats located within the region of their ancient area of appearance during the Pleistocene, as corroborated by archaeological findings [24].

This botanical description clears up the previous uncertainty as to the definition of these populations, and is of particular significance in light of the emerging notion that these wild populations are representatives of the core population from which the cultivated grapevine was first domesticated, circa 11,000 years ago [24]. These emerging new data emphasize the significance of the conservation of the environmental conditions and biodiversity of the Sea of Galilee, the prevention of drastic water level fluctuations, and the preservation of the stream banks and forest habitats of the Upper Galilee region, as the main habitats of this important population.

## 5. Conclusions

The research presented here supports the persistence of the wild grape species in the Israeli flora, extending the southern edge of its global distribution. As a result, Israel can be confidently added to the map of the native distribution of wild *Vitis vinifera*, and this plant can be included in the Flora Palaestina.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae9090998/s1>, Figure S1: PCA (principal component analysis) biplot analysis performed on 46 *Sylvestris* accessions, using the leaves and petioles measured data.

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