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A Study on the Characteristics of Buds and Flowers in Pomegranate: Differences among Cultivars

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Abstract: The growing cultivation of pomegranate and the interest of consumers in the nutraceutical properties of the fruit have not yet spurred similar increases in the knowledge base of some important botanical and physiological aspects of this species, such as bud differentiation. The aim of this research was to study the bud morphology of four pomegranate cultivars ('Comune S. Giorgio', 'Wonderful', 'Ki-Zakuro', and 'Haku Botan') to better clarify the bud differentiation process. In all four cultivars, the number and the position of the buds were variable, from a typical two buds per node to a single bud or more (3). Two types of buds were observed: small and thin on the twigs (short, medium, and long) and large and round on the spurs; large buds were also observed on twigs but in a reduced number. Flowers showed differences among the cultivars, with a larger number of petals in 'ornamental' cultivars because of the petaloidy. Hermaphrodite flowers were larger and longer than the staminate flowers and also more abundant. Moreover, flowers developing from a flower bud were longer than flowers developing from mixed buds. The presence of an undifferentiated meristem, starch grains in the protective scales, and two small transition leaves were observed in all the types of buds from summer to the end of endodormancy in all cultivars. However, a dome-shaped meristem was observed in a few flower buds. Our observations indicate pomegranate behaves as a species for warm, sub-tropical, and tropical areas more than for temperate ones.

Keywords: flower bud; mixed bud; bud differentiation; microscopic observations; μCT; starch grains

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1. Introduction

Although pomegranate (*Punica granatum* L.) is cultivated worldwide in various climatic conditions, FAO (Food and Agriculture Organization) data on production and cultivated areas are lacking. Estimates suggest 5–7 million metric tons of production, mainly concentrated in the Middle East and India [1]. In Italy, pomegranate is not among the leading fruit crops, but its cultivation increased significantly in the last few years. Recent data (2021) of harvested fruits report a production area of 1249 ha with a total harvested production of 18,697 t of fruits [2]. In the Puglia region, southeastern Italy, pomegranate cultivation is spread over 365 ha with a production of 6502 tons [2]; it is becoming an important fruit crop compared to only a few years ago and more hectares have been recently planted. Although the growing cultivation and the increasing interest of consumers in the nutraceutical properties of the fruit, i.e., the antioxidant activity [3], information about some important botanical and physiological aspects of the species, i.e., bud differentiation and flower characteristics, are lacking compared to other fruit tree species.

Buds are important organs for the vegetative and reproductive growth of trees. They are classified by position: terminal, lateral, or adventitious; function: vegetative, reproductive (flower), or mixed; activity: prompt or dormant; cover: scaly or naked [4]. Pomegranate buds are tiny (0.1–0.3 cm) compared to other fruit species, have a brownish-green color, and are generally turnip-shaped [1]. Larger-sized buds are generally located in the top part

of brachiblasts (spurs), whereas smaller-sized axillary buds are on twigs of the current year with different lengths [5] or on secondary (thorny) twigs, where they are even smaller [6]. Two types of dormant scaly buds in 'Rabbab'-e-Neyriz' have recently been described: narrow buds with sharp scales and large buds with oval-shaped scales; narrow buds develop only vegetative shoots, whereas large buds can develop either reproductive or vegetative shoots [7]. The buds on the secondary growth are lateral and appear on the axils of the leaves (of different twigs), whereas the terminal buds may produce a thorn, grow into a flower or clusters of flowers, or simply fall off. Consequently, pomegranate growth mainly occurs through the activity of these lateral buds [8].

Pomegranate flowers are almost sessile, actinomorphic, bisexual, and heterostylous with a brightly colored hypanthium and nectaries between the stamens and the ovary base [9]. Flowers may be hermaphrodite or staminate (with often intermediate types), bell-shaped (staminate), or more vase-shaped (hermaphrodite), with their ratio varying among cultivars and from year to year [1,10]. Hermaphrodite flowers have a perfect female (stigma, style, ovary) and male (filaments and anthers) structure and are often defined as 'fertile', 'vase- shaped', or 'bisexual' flowers. They are long-styled, have urceolate (pitcher-like) calyx, and a larger, well-developed ovary with a large stigma at the anthers' height or often higher, thus allowing self-pollination as well as pollination by insects. Staminate flowers are smaller, with a campanulate (bell-shaped) calyx, and with an underdeveloped female structure. Male flowers typically drop and fail to set fruit and when they set fruit the quality is poor [11–14].

Bud differentiation of pomegranate has been scarcely investigated compared to other fruit crops. A study carried out on 'Wonderful' reported that flower organogenesis was not evident before bud break in spring [13], but, to our knowledge, information on other cultivars or about the evolution of the different buds is still lacking.

The aims of this research were: (1) to study the morphology of buds and flowers on twigs and spurs of four pomegranate cultivars with different characteristics; (2) to understand the mechanism behind the bud differentiation towards vegetative or reproductive activity (or both) in the different types of twigs and spurs. This information could help in a better management of pruning operations.

2. Materials and Methods

2.1. Experimental Site and Cultivars

The trial was conducted in 2021 and 2022 at the pomegranate repository located at the "P. Martucci" Experimental Station (University of Bari 'Aldo Moro', DiSSPA, Fruit Tree Unit), in the countryside of Valenzano in the Bari province, Puglia region, southeastern Italy, 41°1′35.155″ N, 16°54′6.624″ E. The climate of the area is a typical Mediterranean climate, with dry and hot summers and rains occurring mainly in winter and spring (500–600 mm). Among the several genotypes grown in the repository, 4 cultivars were selected for this study on buds and flowers: 'Comune San Giorgio' (Italian ecotype), 'Wonderful', 'Haku Botan' (white skin fruits), and 'Ki-Zakuro' (ornamental). In this repository, trees are trained to vase and spaced at 4 × 4 m. The orchard is subjected to the cultural practices commonly adopted for pomegranate trees in the region and weed management is carried out through mowing (no use of herbicides at all).

'Comune San Giorgio' yields large fruits with a green-yellow-pink peel and pinky, sweet, and juicy arils with hard seeds; thorns are average. 'Wonderful' is the worldwide cultivated pomegranate thorny cultivar yielding medium to large red fruits; arils are sweet-tart to tart, or even sour, depending on climate; seeds are moderately hard and small. 'Haku Botan' is a yellow-white, thornless, sour pomegranate cultivar with white flowers and can be used as ornamental; fruits are small to large, white to yellow; both the arils and seeds are small, and the seeds are medium-hard to hard. 'Ki-Zakuro' is a 'double flower' cultivar because all the stamens are modified into petals and used as an ornamental cultivar for its beautiful white and orange/red flowers [1].

Three plants per each cultivar were used in this trial and the different types of twigs and spurs were tagged for the successive observations and samplings of both the buds and flowers.

2.2. Buds Observation, Collection, and Analysis

The buds on the different tagged twigs and spurs of the pomegranate cultivars were observed and photographed in the field for their position, number per node, shape and size, and evolution (vegetative, mixed and flower). Buds were collected from June 2021 until and throughout the endodormancy period, up to ecodormancy before bud break, every 14 days from different twigs (short, medium, and long twigs) and spurs, placed in labeled plastic bags and carried to the laboratory to be examined. At each sampling date, three twigs for each type and three spurs were collected for each cultivar (one per plant) and 6 buds per cultivar were detached and examined from each type of twig and spur. In the lab, the fresh buds were observed under a stereomicroscope (Nikon SMZ800, Tokyo, Japan), either entire or sectioned. Successively, the buds were cross- and transverse-sectioned using a steel blade, stained with 0.05% toluidine blue, and observed under an optical microscope. The slides were viewed with a bright field microscope (Nikon Eclipse Ci-L, Tokyo, Japan) attached to a digital C-mount camera TP3100 and a pc monitor to examine the sections better. In addition, some buds were collected in late October 2021 and stored in vials without any treatment except for air drying for a few days. The 3D morphological studies were carried out on these buds at the Micro X-ray Lab (www.microxraylab.com) of the University of Bari 'Aldo Moro' by high-resolution X-ray micro-computed tomography (μ-CT) using a SkyScan 1272 μ-CT instrument (Bruker Nano GmbH, Berlin, Germany).

2.3. Flowers Observation, Collection, and Measurements

The flowers developing from buds on tagged twigs and spurs were observed and photographed in the field for type (hermaphrodite or male) and number (single or in clusters). Flowers were collected during the main flowering flush, in particular 24 per cultivar for the different twigs and spurs (8 per plant). Six flowers per cultivar were also collected at the beginning of flowering for the different twigs and spurs (2 per plant). Flowers were selected as follows: hermaphrodite flower from a flower bud; hermaphrodite flower from a mixed bud; male flower from a flower bud; male flower from a mixed bud. Flowers were placed in labeled plastic bags and carried to the laboratory for successive measurements of the different tissues. The following measurements were conducted in the lab for each flower: number of sepals, number of petals, number of anthers, diameter and height of the flower, diameter and height of the ovary, length of the style, and area of the stigma.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed with XLSTAT-Pro software (Addinsoft, Paris, France) at the 0.05 P level. The assumptions of variance were verified with the Levene test (homogeneity of variance) and the Lillefors and Shapiro–Wilk tests (normal distribution). The mean values obtained for the different factors were statistically separated by using the REGWQ test at the 0.05 P level.

3. Results and Discussion

3.1. Field Observations: Buds and Flowers

The observation in the field indicated that the smaller and thinner buds were generally vegetative, whereas the rounded and bigger ones could be either flower or mixed buds, thus containing either flowers or reproductive shoots (Table 1). Our results agree with that reported for Iranian pomegranate cultivars, with narrow buds developing shoots and large buds prevalently originating flowers or mixed shoots [7]. Buds of pomegranates are generally described as small (0.2 cm), brownish-green and turnip-shaped [15], with larger-sized buds located at the tip of the spurs and smaller-sized axile buds of vegetative sprouts [5].

Table 1. Observations in the field of buds and flowers.

Cultivar	Bud/Node [1]			Bud Evolution [2]			Bud Size [3]		Flower Type [4]		Flower [5]	
	1	2	3	V	M	F	Sm	B	H	M	S	C
Comune S. Giorgio	•	••••		•••••	•••	•	•••	•	•••	••	•	•••
Wonderful	•	••••	•	•••••	•••	•	•••	•	••••	•	•	•••
Haku Botan	••	••••		••••	•••	••	•••	••	••••	•	••	•••
Ki-Zakuro	••	••••	•	••••	•••	••	•••	••	••••	•	•••	•••

[1] Bud per node: 1, 2, and 3 indicate the number of buds per each node. [2] Bud evolution: V for vegetative, M for mixed and F for flower. [3] Bud size: Sm for small and B for big and large buds. [4] Flower type: H for hermaphrodite and M for male. [5] Flower number per bud: S for single and C for cluster. The number of dots (•) indicates the occurrence of that type of parameter.

When considering the different types of buds, the vegetative narrow buds, in particular in vigorous cultivars such as 'Wonderful' and 'Comune S. Giorgio', developed primary shoots often with abundant secondary shoots having a terminal thorn (Figure 1), whereas the mixed buds developed shoots of different lengths: spurs (<1 cm), short (2–5 cm), medium (5–10 cm), and long (>10 cm) with single (Figure 2) or grouped flowers (Figure 3). The narrowest and smallest buds developed only into vegetative shoots, but large buds not only developed into vegetative shoots, but could also give rise to reproductive shoots and flowers [1]. In pomegranate, the buds on the secondary growth are lateral and appear on the axils of the leaves, whereas the terminal buds may produce a thorn, grow into a flower or clusters of flowers, or simply drop [8].

A **B**

Figure 1. A vegetative bursting bud with developing growing secondary shoots (**A**) and a lignified secondary shoot with a terminal thorn and bursting buds (**B**) in 'Comune S. Giorgio'.

Figure 2. A mixed bud developing a medium shoot with a terminal single flower in 'Wonderful'.

A **B**

Figure 3. Mixed buds developing a long shoot in 'Haku Botan' (**A**) and a short shoot in 'Comune S. Giorgio' (**B**).

Flower buds were much fewer in number and developed a single flower (Figure 4) and resulted more abundant in 'Haku Botan' and 'Ki-Zakuro' (Table 1).

In all four cultivars, the number and the position of the buds were variable (Table 1), from a typical two buds per node at 90° orientation (Figure 5) to a single or more (generally 3) buds per node (Figure 6). Few cases of three buds per node were observed in the cultivar 'Wonderful', but this number was also observed in the other cultivars, such as 'Haku Botan' (Figure 6), but to an even lesser extent. To our knowledge, the position and number of buds per node have never been described before for pomegranate. However, it resembles that of species of *Prunus* genus (sweet cherry, plum, almond, apricot).

A **B**

Figure 4. A flower bud developing a single red flower on a spur of 'Comune S. Giorgio' (**A**) and a single white flower on a spur of 'Haku Botan' (**B**).

A

B

C

Figure 5. Two buds per node with a rotation of 90° at each successive node for Ki-Zakuro (**A**), Comune S. Giorgio (**B**), and Wonderful (**C**). The scale bar represents 2 cm.

The internodes were of variable length with occasionally an irregular pattern (Figure 6A) along the entire length of the twig.

Hermaphrodite flowers were bigger and more abundant than the male flowers for all the examined cultivars and more male flowers were observed in 'Comune S. Giorgio' (Table 1, Figure 7). In pomegranate, flower development can follow a path of either male or hermaphrodite flowering. Hermaphrodite flowers are capable of producing fruit, and high ratios of female:male flower types will promote higher production. Female flowers are vase-shaped and fertile with a normal ovary, whereas male flowers are bell-shaped and drop without fruit set [11–13]. The number of vase-shaped flowers is the major factor affecting the fruit set capacity and shows a positive correlation with the bearing capacity of the cultivar [16,17]. The total number of flowers and the ratio of hermaphrodite to male flowers also vary with season, plant age, position within the plant and on the twig, and the environmental conditions, including the horticultural practices [1]. Modified cultural

practices, including the application of chemical treatments to promote flowering, may be considered as ways to improve flower production, i.e., hermaphrodite flowers [17,18]. The percentage of vase-shaped flowers was reported as 43–66% for Israeli cultivars [19], 78–86% in the Turkish 'Hicaznar' cultivar [20], and 53–80% for the local Indian cultivars [21].

A **B**

Figure 6. A single bud at each node for Ki-Zakuro (**A**) and more buds (3) per node for Haku Botan (**B**). The scale bar represents 2 cm.

A **B**

Figure 7. A hermaphrodite vase-shaped flower (**A**) and a male bell-shaped one (**B**) in 'Comune S. Ggiorgio'. The bar represents 1 cm.

3.2. Microscopic Analysis of the Buds

Two types of dormant buds have been observed on all the twigs and spurs of the four cultivars: small and thin buds (≈1500–2000 µm long and ≈1000–1300 µm wide), more abundant in number, and large and round buds (≈2000–2500 µm long and ≈1800–2000 µm wide) to a lesser extent, as also recently reported for Iranian pomegranate cultivars [7].

While narrow buds developed into vegetative shoots, large buds could develop into either vegetative shoots or reproductive structures as observed in spring in the field on the tested trees. Smaller buds (≈800–1000 µm long and ≈400–600 µm wide) were measured on the secondary shoots with a terminal thorn more abundantly in the 'Wonderful' and 'Comune S. Giorgio' cultivars. These smaller buds only developed vegetative shoots.

In all the examined cultivars, the smaller, less developed, and narrower buds, with sharp scales developed on the twigs of different lengths and secondary shoots, whereas the more rounded, larger buds, with generally oval-shaped scales, developed prevalently on the spurs. However, some large and rounded buds were also located on the twigs, although to a lesser extent. The observations at the microscope indicated no reproductive primordia from summer until the full endodormancy stage (Figure 8) in both types of buds (mixed and flower). Small transition leaves were observed (Figures 8B and 9A), but no flower primordia developed in the season for all the cultivars, though a dome shape was observed only in a few flower buds on spurs (Figure 9A), thus indicating the successive evolution towards a flower. In other fruit tree species (sweet cherry, peach, apricot, grape, etc.) flower differentiation begins in summer during the growing season of the first year and complete the differentiation in the second year in spring [22]. In these latter temperate fruit crops, flowers are initiated in summer or autumn prior to endodormancy, and flower primordia are more distinguishable and even flower anomalies are clearly visible during endodormancy [23], or even the number of inflorescences in grape [22]. In contrast, in tropical and sub-tropical species, floral initiation may occur just before anthesis, in 14–28 days [1,13]. Cultural practices can affect plant metabolism and alternate the number and the ratio of the different buds/flowers [24–26].

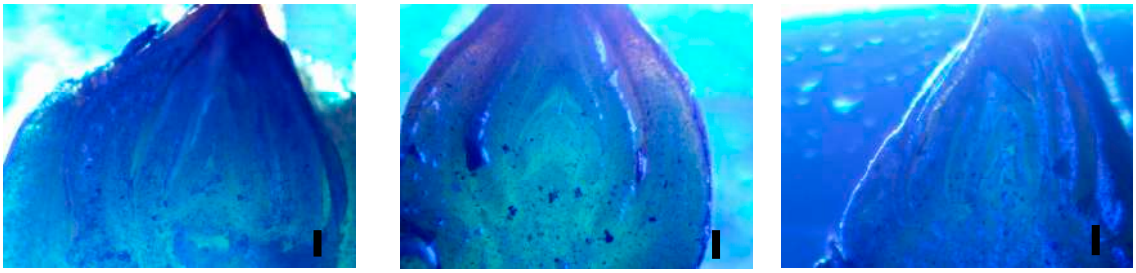

Figure 8. A cross section of a bud in summer (**A**), leaf fall (**B**), and full endodormancy (**C**) in 'Haku-Botan'. The bar represents 200 µm.

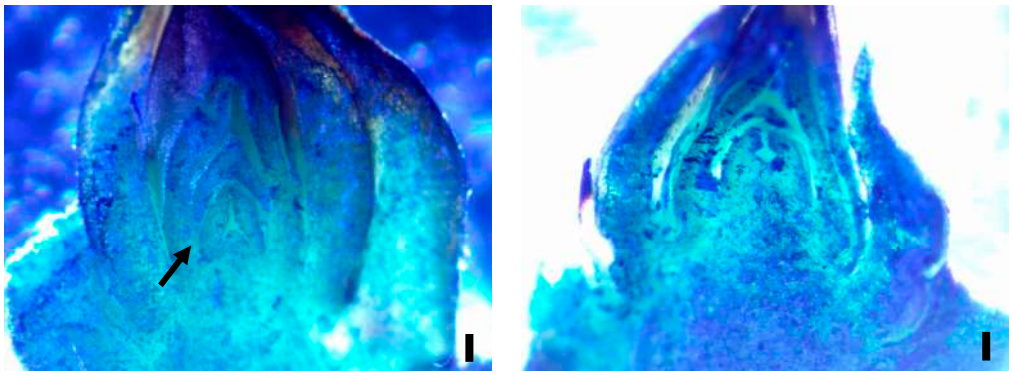

Figure 9. The two transition leaves are visible (arrow) with a dome-shaped meristematic apex indicating the successive evolution towards a flower ((**A**), Ki-Zakuro), and a still undifferentiated and flat meristematic apex ((**B**), Haku Botan). The bar represents 150 µm.

Although it is not precisely clear when pomegranate floral induction occurs, the microscopic observations only showed undifferentiated meristems at endodormancy stage (and earlier at paradormancy) in almost all the sectioned buds with the exception of the meristematic dome visible in few flower buds on spurs, a preparation for the successive spring differentiation. Pomegranate floral induction, as observed in our trial, seems similar to species of warm and tropical areas or of the Mediterranean basin, such as *Ficus carica* [27], or olive [28], where the differentiation starts at the end of the ecodormancy period just prior to bud break. According to [13], in 'Wonderful', flower organogenesis was not visible until spring, when a floral meristem was observed in dissected buds. Under tropical conditions, flower bud development occurs at different times of the year. The time from the initiation of flower bud growth to anthesis may range from 14 to 28 days, depending on the cultivar and climatic conditions [10]. Moreover, flowering can occur from the last week of March through the second week of May, with various flushes on the same tree [29]. Flower bud development in Indian cultivars completes within 20 to 27 days [21]. Among 24 pomegranate genotypes grown under a semi-arid climate, the time required for bud development varied between 14 days (for 'Patiala') and 27 days (for 'Japanese Dwarf') [30]. In the semi-arid tropics of western India, flower bud development in 'Ganesh' took 20.4 days, which was higher than 'Bhagawa' (19.3 days), 'Mridula' (17.5 days), and 'Arakta' (16.2 days) [31].

Meristems in dormant buds of 'Wonderful' are undifferentiated, and flower organogenesis is not evident prior to bud break in spring. In early spring, however, a floral meristem with primordia developed along the margins of a central reproductive apex [13].

Pomegranate flowers have been reported to occur on both old wood (2–3-year-old spurs) and current year growth shoots, with flowering varying with cultivar and season and generally occurring 1 month after bud break [14,18].

Our observations noticed no differences since all the sectioned buds showed undifferentiated meristems or just a transition towards a meristematic dome (only in flower buds on spurs), but no primordia were present in the buds until the ecodormancy stage, either mixed or flower buds.

Not only the bud development, but also the bud dormancy is shown to vary with cultivar. Bud dormancy (defined as the time between abscission of more than 50% of the leaves, and the appearance of vegetative buds or more than 10% new growth) among 24 genotypes grown under a semi-arid climate ranged from 56 days in 'Sur Sukkar', to 66 days in 'Kazak Anar' and 'Kali Shirin', up to 67 days in 'Gul- e- Shah' [30].

3.3. Lab Observations of the Flowers

The measurements of the flowers carried out in the lab showed significant differences among the cultivars and the types of flowers and buds (Table 2). With regard to the different parts of the flower, the number of sepals was six on average, with up to eight sepals in 'Haku Botan' (Figure 10) and no differences were noticed for both types of flower and bud. This seems to be a very stable parameter of the pomegranate flower. The number of petals was similar to that of sepals, i.e., 6, for 'Comune S. Giorgio' and 'Wonderful', but 'Ki-Zakuro' and 'Haku Botan' showed extra-petals ('double flower') with almost 50 petals per flower because of the modification of stamens into petals [14]. In ornamental cultivars the presence of extra petals is common, such as in the cultivars 'Multiplex', 'Chico', 'Peniflora', 'Rubra Plena', and 'Variegata', where this petaloidy is useful for decorative purposes. Petals persist for about 6–7 days in the ornamental cultivars and only 1–3 days in the commercial cultivars [10]. With regard to the petals, hermaphrodite and male flowers had similar numbers (Table 2).

Table 2. Morphological parameters of the flowers of the different cultivars [1].

Treatment	Number of Sepals	Number of Petals	Number of Stamens	Flower Height (mm)	Flower Width (mm)	Style Length (mm)	Stigma Area (mm^2)	Ovary Width (mm)	Ovary Height (mm)
Cultivar									
'Comune S. Giorgio'	5.9a	5.9b	192.2a	26.8a	12.2c	5.2a	0.7b	2.5b	3.5a
'Wonderful'	5.8a	5.7b	168.6b	24.1a	13.2c	4.5a	0.4b	2.0b	2.0b
'Haku-Botan'	6.3a	44.4a	134.9c	27.0a	19.6a	4.7a	0.6b	2.3b	1.4b
'Ki-Zakuro'	5.9a	47.4a	121.9c	26.3a	17.1b	2.7b	4.2a	3.8a	1.8b
Flower									
Hermaphrodite	6.0a	22.8a	164.7a	31.1a	17.6a	8.2a	1.7a	3.7a	3.4a
Male	6.0a	25.9a	149.4b	21.6b	13.4b	1.0b	1.0b	1.5b	1.1b
Bud									
Mixed	6.0a	22.6a	157.3a	24.7b	14.6a	4.2a	0.8b	1.9b	1.9a
Flower	5.9a	26.0a	155.9a	27.2a	16.1a	4.5a	1.8a	3.1a	2.4a

[1] Letters within the columns indicate significant differences ($p < 0.05$) for each parameter for cultivar, flower, and bud according to REGWQ test.

Figure 10. Sepals, petals, anthers of a flower of 'Haku-Botan'. The bar represents 1 cm.

The number of stamens ranged from a minimum of 85 in 'Ki-Zakuro' up to 300 in 'Wonderful', in the range of what has been previously reported [5]. In general, a higher number of stamens was recorded for the first flowers to open, mainly from flower buds, in a range of 200–300, whereas in successive flowerings the number was lower and almost stable in the range of 100–200. In 'Ki-Zakuro' and 'Haku Botan', the lower number of stamens is a consequence of their evolution in petals (petaloidy) and this development of petal-like organs has occurred repetitively throughout angiosperm evolution [32]. Perhaps these two cultivars are a kind of relic in the path of pomegranate evolution. Hermaphrodite flowers had more stamens than male ones (Table 2), maybe because the limited development of these latter flowers influenced not only the ovary but also the male components, thus even reducing the number of stamens.

Hermaphrodite flowers were larger (17.6 mm vs. 13.4 mm) and longer (31.1 mm vs. 21.6 mm) on average than the staminate flowers. Moreover, flowers developing from a

flower bud were longer than flowers developing from mixed buds (considering the central flower). When also considering the other flowers of the clusters, flowers from flower buds resulted always bigger in size.

A difference in weight between the two types of flowers was also reported in a recent paper [7], with an average weight of 4.8 g (hermaphrodite) with respect to only 1.7 g (staminate) [10]. The four cultivars showed the same size of the flowers for the length, whereas some significant differences were measured for the width, with cultivars 'Haku-Botan' and 'Ki-Zakuro' having larger flowers, possibly an evolution of their ornamental habit (attractive purpose).

The style was similar among the cultivars with the only exception of 'Ki-Zakuro', which possessed a modified female structure with almost no style and a large ovary structure. The style was longer in hermaphrodite flowers with an average length of 8.2 mm and up to 15–16 mm, whereas in staminate flowers the average style was 1.0 mm long, and always shorter than 2 mm. The staminate flowers often did not present the style, and only a small ovary was located at the bottom of the flower. The area of the stigma was in the range of 0.4–0.7 mm^2 with the exception of 'Ki-Zakuro' (4.2 mm^2). The stigma area was larger in the hermaphrodite flowers (1.7 mm^2) with respect to staminate ones (1.0 mm^2) and a similar difference was measured between flower buds and mixed ones, with a larger stigma in the flowers developing from flower buds. The stigma is papillate and covered with exudates and a different stigmatic area was noticed among the different flowers.

The size of the ovary was similar for the four examined cultivars, with only 'Ki-Zakuro' having the largest one (although not a true ovary but more as a modified structure), and 'Comune S. Giorgio' having the highest ovaries (Table 2).

3.4. X-ray Analysis

The μ-CT renderings of endodormant buds showed protective scales with starch grains, recognizable as higher density (brighter) clusters (Figure 11). These grains are localized either in the scales or at the base of the bud. These grains are used as thermal insulation to face the winter low temperatures (in the scales after hydrolysis) or even as a C source at bud break (and earlier) for the differentiation process and the first stages of growth of the shoot/flower. The presence of starch grains in dormant buds has been reported for other tree fruit species, such as peach, to support the differentiation of the various tissues of a flower bud, i.e., xylem, anthers, ovary [33]. In sweet cherry, starch accumulates in flower buds (in particular in the ovary) during endodormancy, supporting physiological activity in the flower primordia during this period [34]. Starch accumulated in sweet cherry followed the same pattern than chilling accumulation and reached a maximum at chilling fulfilment. This starch subsequently vanished during ecodormancy concomitantly with ovary development before bud break. Moreover, starch has been reported in flower primordia at this developmental stage also in sour cherry [35]. The beginning of starch accumulation occurred concomitantly with the beginning of chilling accumulation in two sweet cherry cultivars [34] and the maximum peak of starch accumulation corresponded with the accomplishment of chilling requirements. The accumulation of starch in pomegranate buds may reflect the chilling requirement, and the starch is fundamental for the endodormancy stage. Moreover, the more abundant starch grains at the base of the big bud on the spur (11 A) may suggest their use for supporting the differentiation of the flower tissues, as reported for peach [33], with respect to the fewer starch grains in the small bud (11 B) possibly evolving towards a vegetative shoot.

The X-ray survey also showed two small transition leaves and a meristem with no reproductive organs visible, thus confirming the observations at the optical microscope.

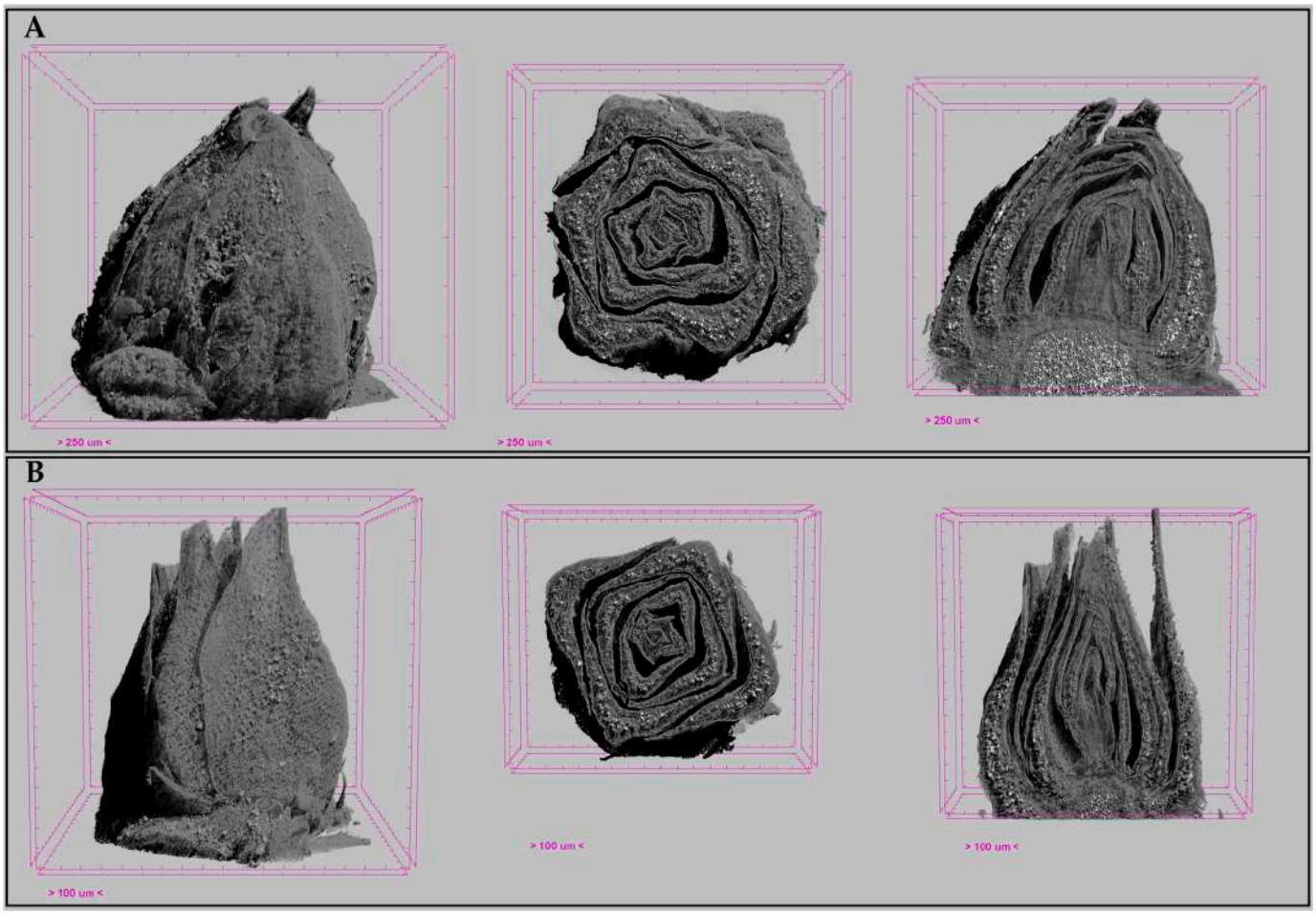

Figure 11. μ-CT 3D micrographs of "Comune S. Giorgio" bud from a spur (**A**) and "Wonderful" bud from a twig (**B**). For both buds, the full external view (left panel), the top view of the central cross section (middle panel) and a transverse section (right panel) are shown. The white brighter dots indicate the presence of higher density particles, e.g., starch grains.

4. Conclusions

In the four pomegranate cultivars, field observations reported two types of buds: smaller and thinner buds developing vegetative shoots, and rounded and bigger buds developing into either flowers or reproductive shoots. The number and the position of the buds was variable, from a typical and frequent two buds per node at 90° orientation to a single or even more (generally three) buds per node, these latter being more rarely detected. Hermaphrodite flowers were bigger and more abundant than the male flowers for all the examined cultivars, but more male flowers were observed in 'Comune S. Giorgio'.

From summer until winter endodormancy (December–January), undifferentiated meristems were observed in all the buds. Small transition leaves were also observed, but no flower primordia developed in the season for all the cultivars, though a dome-shaped meristematic apex was observed only in few flower buds on spurs indicating the possible flower differentiation early in spring. However, no differences were noticed among the four cultivars at all samplings, and the presence of undifferentiated meristem in the buds until endodormancy indicates that the pomegranate behaves as a species for warm, sub-tropical, and tropical areas more than for temperate climates.

Differences were observed for the flowers. Hermaphrodite flowers were larger and longer than staminate ones, with the flowers originating from flower buds being longer than the ones from mixed buds. 'Ki-Zakuro' and 'Haku-Botan' showed extra petals ('double flower') with almost 50 petals per flower because of the modification of stamens into petals,

compared to the average 6 petals of the other cultivars. Female structures were bigger in hermaphrodite flowers and in flower buds.

The X-ray micrographs clearly showed starch grains in the protective scales and at the base of the bud. These grains are used as thermal insulation (in the scales after hydrolysis) or even as a C source for the differentiation of the various tissues and the first stages of growth of the shoot/flower.

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