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TO PHENOLOGY OF SEEDLINGS OF THE WILD POMEGRANATE (*PUNICA GRANATUM, PUNICACEAE*)

Process of germination of the seeds of the wild pomegranate, characteristics of germ morphology, leaf arrangement and branching of the juvenile plants of the wild pomegranate (*Punica granatum* L.) sprouted in the laboratory and warm-house conditions were examined. Separate stages were observed directly *in situ*.

Ստեփանյան Ն. Պ. Վայրի նռան (*Punica granatum, Punicaceae*) ծիլերի ֆենոլոգիան: Ուսումնասիրվել են վայրի նռան (*Punica granatum*) սերմերի ծլումը, ծիլերի մորֆոլոգիայի առանձնահատկությունները, վայրի նռան՝ լաբորատոր եւ ջերմոցային պայմաններում աճեցված յուվենիլ բույսերի տերեւների դասավորությունը եւ ճյուղավորումը, ինչպես նաեւ որոշ ֆենոլոգիական փուլեր անմիջապես *in situ*:

Степанян Н. П. К фенологии сеянцев дикого граната (*Punica granatum, Punicaceae*). Изучались процессы прорастания семян дикого граната (*Punica granatum* L.), особенности морфологии проростков, листорасположение и ветвление у ювенильных растений дикого граната, проращиваемых в лабораторных и тепличных условиях, а также отдельные фенологические фазы непосредственно *in situ*.

Introduction

The majority of studies on *Punica granatum* L. (*Punicaceae*) are related to cultivars, they have been very few studies on the wild pomegranate. Data on the wild pomegranate can mainly be found in articles elaborating cultural sorts. This can be explained by several factors: cultural pomegranate has more often been in the focus of attention because of its practical value; besides, the area of wild pomegranate growth is relatively small and occupies a narrow region from the Anatolia on the West to the N-W India on the East, while the area of cultivated pomegranate, and consequently – of the research centers investigating it makes up a broad circle (from N 41° to S 41° width) around the globe sphere and at places, even substantially goes beyond that circle.

The dominance of studies on the cultural pomegranate could be partly explained by the circumstance that studies of cultivars are often easier to implement: particularly phenological observations, i.e. studies of the dynamics of germination, branching, buds formation, flowering, fruiting, type of pollination and other seasonal phenomena are rather difficult to execute in field conditions. During studies of cultivars it is possible to conduct large sampling in the same conditions, which, naturally, is easier to carry out in a garden or experimental station, than *in situ* – in a hardly accessible place, in wilderness.

Although the available data on cultivars are very important for understanding the wild pomegranate's nature and can be used, in some cases – can even be extrapolated on the wild populations, it is obviously necessary to conduct a special study of the wild pomegranate. The aim of this research is to fill in the existing gap to extent possible and to investigate the early stages of the wild *P. granatum* ontogenesis.

Material and method

Phenological investigation on the germs and juvenile plants of the wild pomegranate were executed. Seed material was collected on the territory of southern Armenia in the 4 populations: Meghri region, near Shvanidzor, stony slopes, with *Paliurus spina-christi* Mill., 38°55'–38°56' N, 046°21'–046°22' E, h 625–814 m; Meghri region, near Nrnadzor, dry stony slopes, crevices in rocks, 38°55' N, 046°26' E, h 783–816 m; Kapan region, near Nerkin And, Shikahogh reserve, open mixed forest, 39°01'–39°02' N, 046°29' E, h

Wild pomegranate growth areas on the territory of Armenia

950–966 m; Goris region, near Vorotan river, dry slopes, 39°26' N, 046°23'–046°24' E, h 749–830 m.

We examined the process of wild pomegranate seed germination, characteristics of germs morphology, leaf arrangement and branching of the juvenile plants of the wild pomegranate, that were sprouted in laboratory and greenhouse conditions. In addition, phenological observations were conducted directly *in situ* in the indicated regions and also in the north-eastern regions of Armenia (map).

Results

Germination of the seeds collected in the end of October, started after dormant period. These results do not concur with the contended in literature data that pomegranate seeds "do not need any dormant period and post-ripening for germination" (Rozanov, 1961: 59). All the seeds of the wild pomegranate, sown in late autumn, have not sprouted in any temperature conditions and water treatment and sprouted only in spring, on a par with seeds stored at room temperature and sowed in the March–April.

Generally, the character of stratification depends on the history of the species. Thus, for the species appearing in a cold and dry climate, a deeper dormant period – often with obligatory freezing, is necessary (Sapankevich, 1961). It is assumed that the formation of *P. granatum* took place at the Tertiary (Levin, 2007), when warm moist climate dominated on territory of its growth. That is why it should be expected that there will be no dormant period or, at least; sprouting after dormant period, however without freezing. At the present time, pomegranate grows in regions with seasonal temperature and moisture perturbation. For the seeds of such a plants existence of a dormant period is typical (Melikyan and others, 1980). According to our observations, seeds grew after period of a rest without freezing, that fits partly with historical, partly with the current conditions of the growth of pomegranate.

Often observed the above-ground germination of pomegranate's seeds, during which seed coat appears on the surface – a feature specific for the majority of dicotyledons and also gymnosperms (Eames, 1961). But sometimes the seed coat remains under the ground and only cotyledons, convoluted in a tense small tube come to surface. We noted a broad interval of a pomegranate seed germination terms – from 3 weeks to several months, germination of some seeds took place even after the 18th months after sowing. According to our observations on the even-aged seeds of the wild pomegranate, big variance in the germination period was noted for the seeds from all populations under observation, for seeds, collected from one tree and even from one fruit. This means that heterospermy (heterogeneity of seeds from one fruit) on germination terms has been evident. According to Levin's (1981) data on cultivars with different periods of seed germination, in addition to heterospermy, the difference could also be due to the age of seeds and difference in the solidity and structure of seed coat in different sorts and forms. Apparently, the expansion of germination term for *P. granatum* is not only a feature typical to some sorts or populations, but is also a specific feature and could be observed as a form of biological adaptation allowing the species to survive. If, in adverse weather conditions, some part of the plantlets die, the another plantlets that were sprouted much later, have chances to continue their growth.

Very high germinating capacity was observed: germinated even seeds sown in autumn, over which later another plants were transplanted. One of such seeds leaked among plentiful and branched growth of another plant 6 month after seeding. Some time later the germ threw off the «moss-cap» from sclerificated part of the seed (remains of sclerificated mesotesta), seed leaves evolved, a seedling began to grow, but soon after relocation leaves began to yellow starting from apical parts and the seedling died. Another seed germinated among dense sprouts 18 months

Fig. 5. Spiral galaxy NGC4321.

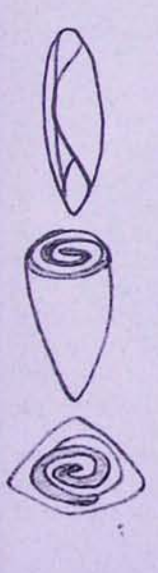

Fig. 1. A view of a seed with removed exotesta (a, b – according to Gaertner, 1788; c – according to Niedenzu, 1898).

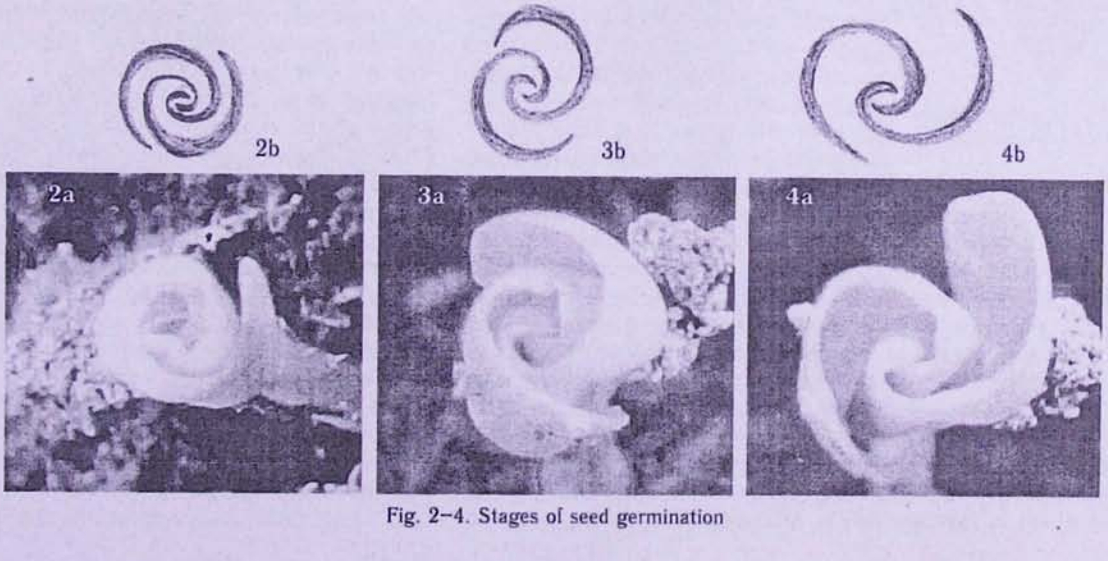

Fig. 2–4. Stages of seed germination

after seeding. This seedling had abnormal long hypocotyl – 6 cm, that left upconvoluted seed leaves, which were covered by sclerenchymated «moss-cap». In cases when the seed germinated not in such extreme conditions the length of this caulicle is 12 times shorter – about 0,5 cm. The seed leaves did not manage to develop and the sprout slowly died.

All the remaining seedlings successfully continued their growth. After rootlet's and hypocotyl's development, during several days the unfolding of seed leaves takes place (fig. 1). After dropping of the «seed moss-cap» first we can see seed leaves convoluted in a tense tube, which repeat their arrangement in the seed where they jointly spirally intorted (the same principle of seed leaves arrangement is typical for some another species, as *Metrosideros splendens, Tristaniopsis laurina (Myrtaceae), Lumnitzera racemosa, L. coccinea (Combretaceae)*). Then two spirals began to unwind, during which seed leaves moved from a vertical to horizontal plane (fig. 2–4).

As we can see in the comparison of figures 1–5, the form of seed leaves at unfolding is astonishingly similar to the form of the spiral galaxy (the more widespread type of galaxies, to which also our galaxy Milky Way belongs). Generally speaking, the «spiral tendency» is a universal principle incidental to both natural worlds – organic and inorganic, as, for example, spiral twist of DNA molecule, shells of many mollusks, history development on a spiral etc. (Araratyan, 1967, 1979; Shafranovskij, 1985). In the plant's structure it is possible to specify a spiral alongwith the pairs of opposite leaves settle down, arrangement of petals and stamens in a flower. But in the case described above – unwinding of the spiral convoluted seed leaves of pomegranates, this uniform micro- macrocosmical principle acts particularly clearly and visually.

Fig. 6. Seed lobe

Seed leaves of *P. granatum* are repandous, 1,3–1,7×1,5– 2,1 cm, with a smooth surface. As seen in fig. 6., the lobes of the seed leaves are somewhat dissymmetric. Seed leaves in the pair are of a similar size. These data partly differ from the ones available at T. D. Vishenskaya (1996: 23): "after unwinding seed leaves are broadly- oblong, reach 3,25–5,5×3–4,5 mm, are blunt, entirely or more or less emarginated with unequal shares, ... in the seed the smaller share is turned inside bigger one". Unlike this author, we did not observe seed leaves of such a size, as well as, any discrepancy in the size of leaves.

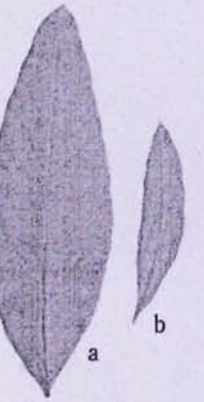

Fig. 7. Leaves of pomegranates seedlings

2–3 months after germination the seed leaves began to fall, but for many observed seedlings they remain quite long – up to 7 months. Thus, seed leaves function not only as a storage organ at early stages of seed germination, but also rather long time remain as a photosynthetic organ. The first true leaves are often narrow-lanceolate, always smooth-edged.

Both germination and the further development of seedlings proceeds very differently depending on expositions: seeds in the conditions of southern and eastern exposition, that is, with most well warmed up soil, start to germinate first, much later germinate seeds in the conditions of north-western exposition. Accordingly, we can differentiate juvenile plants of a pomegranate by growth rate: more intensive growth was observed among seedlings in southern, then eastern, western, least – at northern exposure. By the end of the vegetative period (in the end of October) some seedlings reach 30–32 cm at length, others – only 4–6 cm. The maximum length of leaves in the first year is 5,2 cm among seedlings in the conditions of southern (fig. 7a) and 2,8 cm – in a the north-western exposition accordingly (fig. 7b).

The most intensive growth occurs in the hottest months of summer – July–August. Thus, the most actively developed seedling of the pomegranate has internodes' growth at the beginning of vegetation (in April–May) – 0,7 cm, in July–August – 1,8 cm length. The same growth dynamics was also noticed among cultivars – the average daily growth of seedlings increases sharply in July and reaches a maximum in the first decade of August (Levin, 1981). Obviously for the adult plants of *P. granatum* the situation varies – so, it is noticed for cultivars, that the most intensive growth of sprouts occurs in spring-and-summer months, «slowing a little down in the hottest season» (Rozanov, 1961: 61).

It is necessary to notice that juvenals of a pomegranate survive very well even in the conditions of extremely intensive illumination. For example, if we compare them with seedlings of another subtropical plant – *Citrus nobisis* Lour. *subsp.*, planted nearby, the growth of the which in the conditions of a southern exposure is strongly inhibited, high formation of sclerenchyma, development of numerous speckles is observed. In the conditions of a north-western exposition the shoot of a mandarine was much more in growth (35 cm and 55 cm accordingly). With a pomegranate, on the contrary, the shoot developed in the conditions of high insolation exceeded the length of the shoot developed in more shaded conditions by 8 times.

The leaf arrangement of *P. granatum* is crosswise-opposite, the node is open – the base of the leaf occupies quite a small part of the circle of a stem. Usually the leaves in the pair are located strictly against each other (fig. 8a), sometimes – with displacement in 1–2 mm (fig. 8b), but among adult plants there can also be a distance between pair leaves of 1 cm (fig. 8c). The latter, obviously, could be considered as an atavistic feature, as alternate leaf arrangement is more primitive and phylogenetic primary (Araratyan, 1944, 1967; Takhtajan, 1966). Atavistic lines, in general, rather frequently are observed among the leaves of adult plants of a pomegranate (Stepanyan, 2008).

The first true leaves are located against the seed lobes cross-wise (fig. 9). During the period of growth some mixtion could be observed – the angle of divergence between each pair of leaves is a little above 90° (approximately at 3°–4°), and after 10–12 internodes there is a displacement at approximately 40°. However it is possible that there were a deviations from the specified sizes due to the inequality of lighting.

The length of internodes ranges from 0,3–2,3 cm among juveniles up to 7 cm among adult plants. Besides, there are sprouts with extremely shortened internodes, which develop from the axillary buds. The pair of second-order leaves, which are arranged cross-wise to the first order leaves develops on these sprouts (brachyblasts) (fig. 10). In this case quasi «leave's rosette» (Kulkov, 1983) or "bunches of leaves" (Fedorov et al. 1956) are formed. There can be up to 7–8 leaves at the expense of formation of the several spurs in each of "rosette." It is noteworthy that the same principle works further during the formation of lateral branches, and also, for the formation of flower buds, when as a result of shortened internode's development not only single or paired flowers (fig. 11, page 68), but also inflorescences from 3–12 flowers could be formed (fig. 12, page 68).

In the course of growth on the youngest shoots (for a few days) reddish lines («wings") are formed, sweeping in parallel from one knot to another (fig. 13a). As in each knot the pair of decussate arranged leaves is cross-wise unfolded, 4 such lines are formed and the young stem gets 4-angled ("4-winged") form. When the stem thickens, the parallel lines start to incline, as a result of which it looks like a peculiar "grid" (fig. 13b). As it appears on the transverse section of

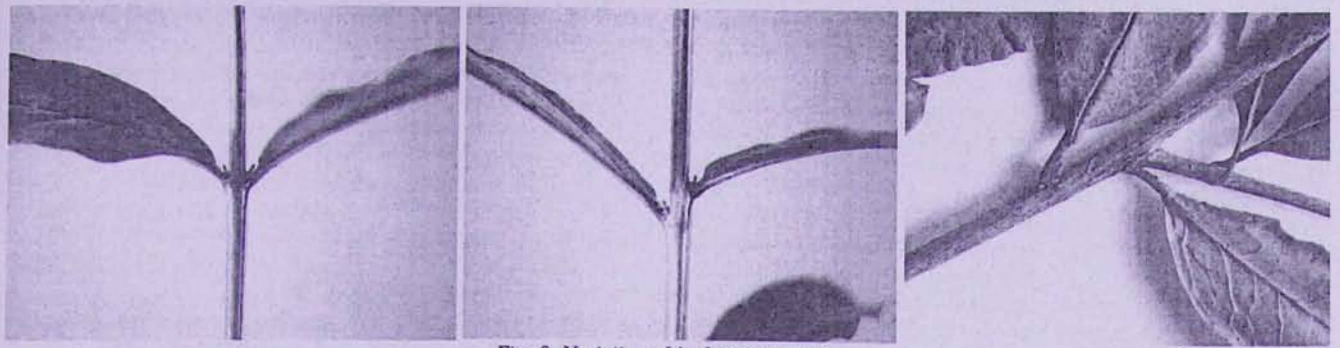

Fig. 8. Variation of leaf arrangement.

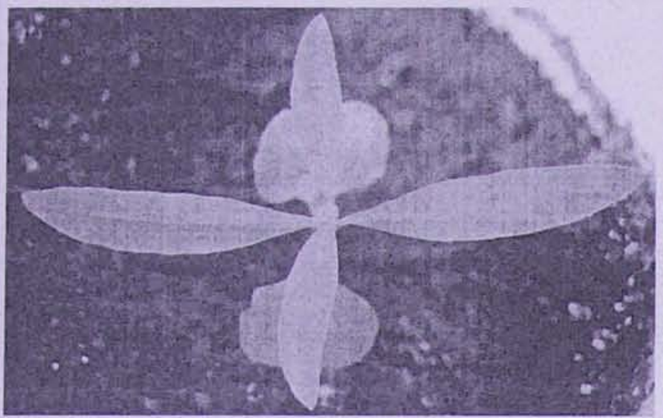

Fig. 9. Leaf arrangement (dorsal view)

Fig. 10. Formation of leaf rosettes

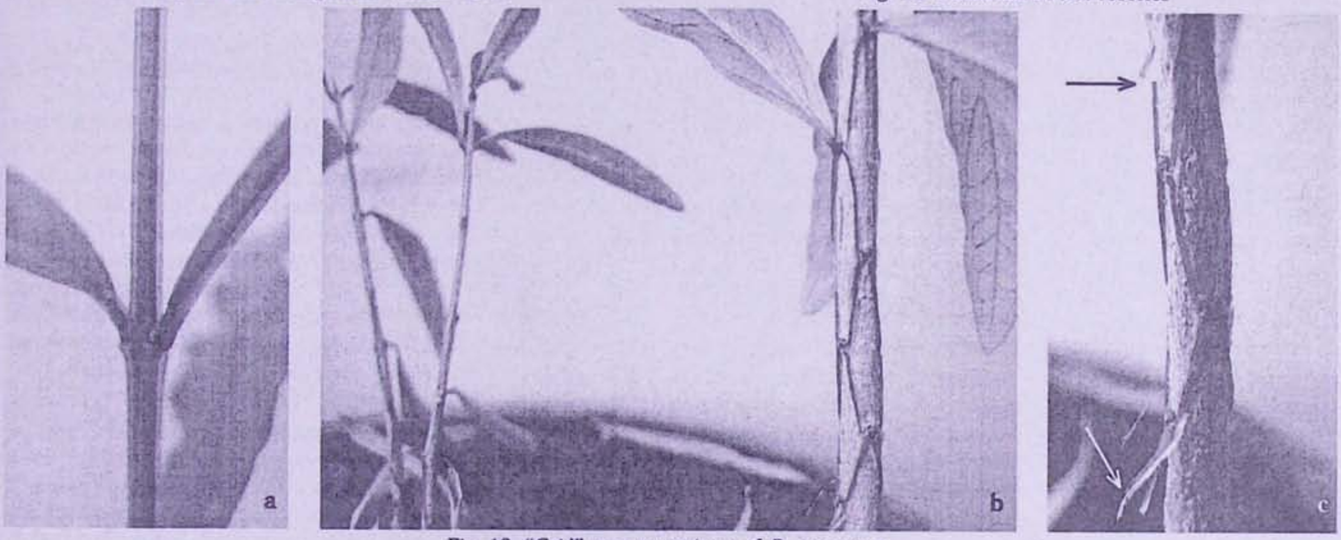

Fig. 13. "Grid" on young stems of *P. granatum*.

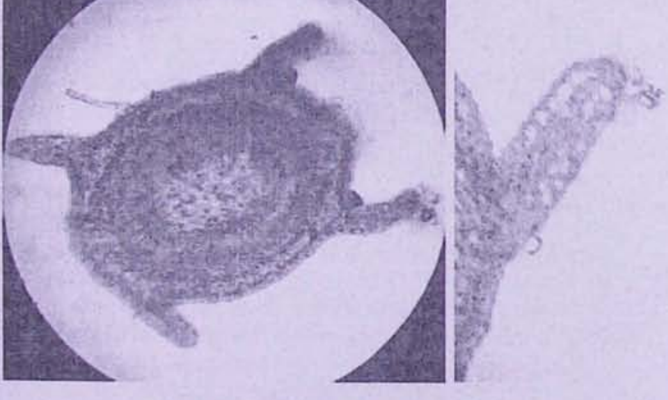

Fig. 14. Transverse section of a young stem

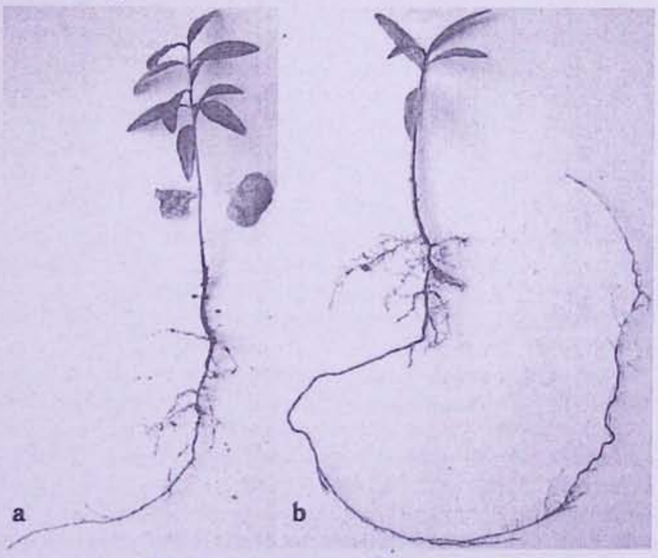

Fig. 15. Juvenile plants of pomegranate.

the young stem (fig. 14), specified "wings" are outgrowth of the bark covered with epidermis and cuticle. These lines which are thin and reddish at the beginning, get corky and become brownish bands on the stem's periderm. At the end of the vegetation period during the stem thickening, "grid" begins to rupture (fig. 13c), but separate threads remain for the second year.

From the axillary buds of the first order shoots on some plants already in the first year second order shoots develop, which, as well as leaves, have opposite arrangement. A big polymorphism is observed among the seedlings of pomegranate on branching degree: the greatest branching, as well as formation of leaves' rosettes, among the first year seedlings are with the plants of a pomegranate placed in a hothouse, that is – in more damp conditions.

On one of such a first year seedlings three buds were formed, one of which has revealed, the flower was physiologically mail – with rudimentary ovary. Another second year seedling had 13 buds, two of which have developed. Such an early flowering is rather often observed among the dwarf forms of a pomegranate – *P. granatum* var. *nana* Pers., but it is very unusual for seedlings sprouted of seeds of the high (up to 8 m) bush. Seedlings' flowering in the first years of vegetation is a phenomenon infrequent for fruits, however it is observed not only among seedlings from Armenian populations of *P. granatum*, but also in Azerbaijan, Uzbekistan, Tajikistan, Turkmenistan (Rosanov, 1961; Levin, 1978). Flowers of such a seedlings, as in our case, are always short-styled.

None of the first year seedlings had spines, although formation of spines (up to 3 cm in length) from the axillary buds is a very common phenomenon for adult plants of wild *P. granatum*, growing in xerophilous conditions. Spikes were also on a biennial seedling of pomegranate growing *in situ*.

The root system of a pomegranate develops both horizontally, in the surface layers of the soil, and vertically, getting deep into the soil – the so called, universal root system, that at various times could use a moisture of different horizons. The length of the main root of the one-year seedlings varies from 15 to 45 cm (fig. 15) and more.

In the autumn seedlings shed their leaves in an acropetal order. However juveniles, growing in the conditions of a north-western exposition and in the more protected from draughts place, remained leafy during all winter, and in the spring, as well as seedlings with sheded leaves, began to growth, though, because of their shaded position, the growth was much less intensive. It was noticed that the adult plants of *P. granatum* growing in greenhouse conditions with enough heat, and also in such areas with warm winters as, for example, in Florida, southern areas of China etc., are evergreen. This comes to prove that "the dormant period in a winter and leaf fall are not organically integral attributes of this species" (Rozanov, 1961: 51); that is to say, the leaf fall of the pomegranate, as well as of many other arborescents – is recent quality acquired in the course of an evolution.

It is noteworthy that in natural conditions the pomegranate has, basically, vegetative propagation, it forms dense coppice shoot and soboles – during observations *in situ* very rare seedlings of pomegranate have been noticed. More than half a century ago A. V. Ivanova argued (1950: 112) that: "seed renewal of the pomegranate occurs, but it is not abounding and is confined to the places protected from cattle bite.". Except pasture, at the present it is possible to specify two more causes of the seedlings young growth' death in natural populations: frequent frosting of the juvenile, not succeeding to form underground suckers (xylopodium), and oppression of seed reproduction owing to arid conditions of growth of Armenian populations of *P. granatum*.

Conclusion

The examination of the early stages of wild pomegranate's ontogenesis allowed to reveal a number of features:

existence of the dormant period at pomegranate's seeds;
heterospermy on germination's terms;
high germinating capacity;
strongly heliophilousness already at the early stages of ontogenesis;
crossed-opposite leaf arrangement with a length of internodes from shares of mm – to 7 cm;
formation of peculiar reddish "grid" on young stems;
increased branching in a damp conditions;
possibility of flowers formation at first year seedlings;
development of universal root system;
leaf fall is not integrally attributes of *P. granatum*.

Further examination of *P. granatum* in wild populations will allow to better understand processes of seed germination of a pomegranate and, probably, to elaborate measures for preserving seed reproduction *in situ*.

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