MORPHOGENESIS OF BUDS OF THE RARE AND DISAPPEARING PLANT ALNUS SUBCORDATA
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ABSTRACT
We investigated that the morphogenesis of the bud of Alnus subcordata C.A. Mey. which belongs to 3rd time and is one of the rare plants to determine the evolution for plant ontogenesis. Formation of meristematic bud of the plant required long time, so the study was divided into three stages that was totally duration 33-34 months. Although preformation of generative organs was occurred during winter development of the bud was continuously with exception a few short dormancy. It was revealed that the Alnus was evergreen in 3rd time and in very wide-area distributed over world in the present time. Besides it was determined that Alnus, was relict taxon, was capable of a very wide adaptation. In our results, flowering time of Alnus subcordata C.A. Mey. was on August.

Introduction
Many plant species which were widespread in the past time are disappeared by the times so distribution areas are the limited to current time. (1). The elements of Hirkan Region derived from Arcto-Tertiary that is dominant in Holoarctic. Today, several relict plants which survive between Black sea and Hazar sea coasts are the absence in Europe. The elements known as Euksin-Hirkan were distributed over large areas at Tertiary and Pleistosen. (2). Although Alnus subcordata C. A. Mey. is one of the relict elements, small populations were dispersed along side of little rivers in Hirkan region. The decreasing of population of the plants referred as rare plant is the very attractive topics for scientists to investigate the reasons of reduction. Determination of the genetic variation types in rare plants is significant to protect their habitats, to increase population and to determine potential translocation (3). Likewise, on rare plants, the investigations have denoted that the reasons of decreasing of any plant population may be originated from biological, genetic, ecological, anthropogenical and historical factors (4 -10). Due to anthropogenic constraints on populations, habitat destruction and fragmentation which genetical and ecological factors are responsible for is mainly caused for splitting of natural habitats and ecosystems into smaller and more isolated patches. The rare plants have small isolated populations in each habitat and contain small number of genetic variants in each population. One of the most important reason of this situation is that sexual isolation, increases the extinction probability in small populations, causes the stimulation a clonal population in the given area (11). Another reason may be augmentation of inbreeding. The investigations have indicated that reduction of population size could cause to decrease the genetic diversity and to increase the inbreeding. As a result, the number of genetic variants diminishes (it is referred as genetic erosion) in long period of time therefore it restricts possibilities of evolutionary variety generating to a novel environmental stress. Also, Inbreeding, have
very negative impacts on the survival opportunity of a species, results offsprings with innate characters which have not as strong as the character that is from their parent, furthermore it causes slow growth of plant with poor flower and seed quality, in short term (12). Due to destruction and fragmentation of habitat of *Alnus subcordata* C. A. Mey., is one of the monotypic species, inbreeding and decline in number of genetic variants was occurred hence it has led loss of subspecies or varieties. Historical reasons may be also important however while *Alnus subcordata* C. A. Mey. is widespread in Pleistosen, recently distribution area was restricted (13). Investigations related to bud structure are significant step to determine evolutionary relationships between morphogenesis and ontogenesis of the plant (14-17). It was reported that the average of seed yield of *Alnus subcordata* C. A. Mey. is as small as of 18 % hence it may be the reason that *A. subcordata* C. A. Mey. is a rare plant since it does not produce abundant fertile seeds (13).

In the present work, the bud morphogenesis was investigated in a rare and noteworthy plants, monotypic taxon *Alnus subcordata* C. A. Mey. pertaining to the 3rd glacial period from three geographically isolated populations by cytological studies.

**Materials and Methods**

Seeds, pollens, and buds of flowers and vegetative shoots of Alder plant that was 10 to 20 years old after the plant was introduced to a botanic garden from the wild type were used. Specimens were collected one month intervals in winter and one week intervals in the other seasons. Samples collected were put in plastic bags then as soon as they were moved to the laboratory, buds dissected were scanned by a MBS-9 microscope.

The methods described by Serebrakov (1952) and Hüseyinova (1988) were applied to study for morphogenesis of vegetative and generative buds (17-18). Cytological analysis was carried out by fixation solution (Kornuva liquid in the ratio of 6:3:1). After cross sections in 10-12 µm were prepared by microtome, they were stained by the Geydengayt method and ge-motosil. Photographs from diagrammatic figures which were drawn on cytological cards by a RA-4 machine were taken.

**Results and Discussion**

In the present work, it was revealed that the Alder has two types of buds, as vegetative and generative. While formation of male flowers were initiated at the beginning of summer, the emergence of female flowers was 2-3 weeks later. The formation of meristematic bud appeared after a long period of time thus processing of development with slow growth was the resembling to other woody (perennial) plants, accordingly, the development of the bud can be comprised of three phases.

First phase (required 13 months); formation of buds and its sections.

Second phase (required 9-11 months); development of buds, and formation of female and male flowers.

Third phase (required 10-11 months); pollination, fertilization and development of fruits and seeds (Fig. 1).

In our results, elapsed time to fruit maturation from bud initiation was 31 to 34 months. Accordingly, the process of the formation and development of the buds can be explained as below:

In the February, unopened buds was approximately 1 cm in length. The primordia of all foliage leaf and the buds of the second year onward from growth of first year’s bud (these parts which can observe only by optical tools are distinguished by two meristhematic layers) were the hidden within the parent buds (current year’s buds). While development of shoots and leaves emergence from parent buds were observed during spring, the length of leaves and the size of the buds the located on axes.
Fig. 1. The development of Alder's buds in different periods including the meristematic puffiness to shoot that produced fruit. I. The protection of bud's basis and formation of its parts. II. The development of male and female flowers from shoot found in the bud. III. Pollination, formation of fruit, development of seed and fruit.

Fig. 2. The cross section of heart-shaped bud of Alder in September (about 1 cm in length); leaflets (1), umbrella-shaped hairs (2), leaves scale (3), veins found in the leaves (4).

of leaves generally increased. Preformation of a bud initiated in the February and continued to February of second year and during this period the current year's bud produced 5-7 leaf primordia then the bud of the following year converted parent bud. The bud borne within the parent bud has been covered by two scales. These buds were named as daughter buds which initiated within parent buds the formed each year, then converted the parent bud in the second year (Fig. 2), thus it is provided the continuation of the bud. The exterior leaves within the parent bud are relatively thicker.
in comparison to the interior leaves, primordia of the interior leaves that is very thin has exuded sticky liquid which may protect the leaves from cold weather. Also, due to encompassed with umbrella-shaped hairs of all leaves within the parent buds, we consider that the major function of this liquid can be to maintain the leaves against extreme hot weather. Parent bud was surrounded with two large bud scales (exterior scale) protecting inner parts of the parent bud against external factors. When shoots growth initiated, a major section of exterior scales fell and resting part of interior scales survived to protect the base of shoot until the shoot elongation then it dropped after the bud opened completely. (Fig. 2). The phyllotaxy is the alternate in the bud and hairs present only on the leaflets, not on scales or other parts of the bud.

In the 3rd time, these scales did not have a significant role to protect the bud due to hot climate. After 3rd time climate altered depending on the cold conditions, the plants converted deciduous form as a result of the adaptation to new climate conditions furthermore overwintered parts such as outer scales of the plant become thickened. Following these variations, scales were not formed over the bud (then called as naked bud) and converted to buds the resembled to deciduous plants morphologically generating sections that provide for protection.

In present study, full formation and development of the bud has lasted for a period of short dormancy. The external factors that influence winter hardiness such as temperature, light, moisture did not prevent bud development therefore development was observed throughout the year. These results obtained in the experiment prove that Alder plant was an evergreen plant.

Thereupon the buds of Alder started to open with rising in weather temperature in March, shoot in the bud was ready to sprout and the length of actively faster growing buds reached to 30 cm by June. Male flowers occured earlier than female flowers from buds emerged from a few branches at the end of June and development of reproductive organs were observed during dry months of the summer to winter (June-February). Likewise, during this period, also second phase formation of the next year’s bud morphogenesis occured. Overwintering male catkins were 1 mm in length in the summer, elongating to 15 cm at flowering so continuously growth and development throughout the year was an evidence that Alnus plant were evergreen.

After the leaves, seeds and fruits dropped, there was a clear increase in bud dimensions of second year, in this period, five leaf primordiums and two scales (exterior scales) for each primordia already developed in these buds, when climate conditions were convenient, additional one or two leaves grew up during summer season. The male flowers of Alder released a great number of pollens to air in February. Fertilization occured in August and then fruits and seeds maturated in December. Percentage of seed germination was approximately 20 - 25 %. The development of reproductive organs in winter and many distinctive biological characteristics of the plant exhibited that Alder was a relict and evergreen plant.

When the pollens matured since February, female flowers and pedicel reached 0.7 cm and 8 to 9 mm in height, respectively, however female flowers was not ready to accept pollen grains for fertilization. Pollens were shed profusely in the air withering of anthers, then they land on female stigmas of the flower. Therefore third phase period consisted of fertilization, formation of fruits and seeds and shedding of seeds begun and were completed to 10-11 months. During of the formation of bud, extension of shoot, development of male and female catkins, pollination, fertilization, development of fruit and seed and shedding of seeds were more than 3 years (Figs. 3, 4 and 5). Thus, relatively long duration of bud morphogenesis may be the
Fig. 3. The ontogenesis of the shoot of heart-shaped Alder in different levels: development of shoot from bud that formed previous year (A), the length of buds that formed in present year and the development of male parts (B), the development of female parts and seed (C).

result of adaptation caused with cold climate conditions. The actively growing and development of the buds in Alder was observed from March to June and so shoot elongation was very fast reaching approximately to 30 cm in length. In this period, primordia of the male and female catkins at the June and July, respectively, was originated from a bud formed in axis of a formed leaves in the current growing season. However this processes in Alnus was much slower until about the end of summer, plant began for formation of generative organs in coming growing season, maturation of fruits and seeds, winter dormancy (leaf fall e.t.c.). Serebryakov (1952) reported that buds are distinguished in to two types depending on winter dormancy, whether generative organs in buds are occurred (18). Present data has suggested that *Alnus subcordata* C. A. Mey. belonged to the type which generative organs were not formed during the period of winter dormancy.

The shoot extension from bud of previous year begun in March and the length of shoot reached to 28 cm for three months (March-June), (Fig. 3A). The shoot extension and augmentation in length throughout the year are shown in Fig. 5, Phase-II.

The generative buds which begin to appear in June or July and will form male flower has developed for eight months and flowered in March (Fig. 3B). Therefore, in this time, the ovaries has not maturated yet. A few months need for maturation. The female flowers buds which begun to develop after a short time and flowered in March for eight months (Fig. 3C). Development periods of male and female buds
are shown in Fig. 5. Phase II. Development periods for both types of buds occurred in Autumn, winter and spring prove that Alder is an evergreen plant.

Leaf development evaluated for two years of development cycle (Fig. 4). Leaf development begun with shoot extension at the same time (Compare Fig. 3D and Fig. 4). Leaf development lasted from March to December. In this period, alteration of leaf number is as follows: March (0), April (1), May (1), June (2), July (3), August (4), September (5), October (5-6), November (6) and December (6-7) (Fig. 5-Phase I). Attending of leaf development in autumn and partly in winter proves that Alder is an evergreen plant.

Following fertilization, formation of fruits and seed spill lasted for ten or eleven months (Fig. 5, Phase-III).

In this study we observed that the initiation and development of buds lasted 3 years in Alder plant and the rhythm of development of inner parts of buds was permanent, and the formation of the flower occurred in winter. Although Alder was once considered cosmopolite species, it is considered an endemic species nowadays. All these facts proved that it was an evergreen plant. Furthermore, the flowering time of the plant was determined exactly. In addition, the normal development and formation of the Alder indicated that the main factor, which made it a rare plant, was not biological or ecological but rather anthropogenic. Some phenological investigations, which were made on the development of inner parts of bud, growing, flowering and the formation of fruit, showed that the Alder plant could be introduced to a botanic garden.

Because of being a rare plant belonging to the 3rd glacial period and the decrease in its number, heart-shaped Alder has been investigated by scientists for many years. According to related literature, formation of flowers of the Alder begins in March or April (1). However, in our study, the dispersion of pollens from male flowers, which were both natural and introduced specimens, was observed in February. At the same period, the formation of cones (female catkins), that included dark pink appendages were determined. Dimensions of these cones are 0.7 × 0.9 cm. Further investigations showed that female flowers were not ready to fertilize at this time; conversely, a few months were required from pollen dispersion to fertilization (19). In our study, fertilization occurred in August and maturing of fruit and seed occurred in December. Comparison with other study (1)
Fig. 5. The developmental cycle of buds of Alder from meristematic puffiness to mature shoot (between 1998 and 2000). 1st, 2nd and 3rd Phases:

- Formation of parts of bud
- Growing of shoot
- Development of male flowers
- Development of female flowers
- Maturing of fruit and seed
- Falling of seed

1st Phase (15 months)
2nd Phase (9-11 months)
3rd Phase (10-11 months)

The numbers, out of parenthesis, show the length (mm). The numbers, in parenthesis, show the increasing in leaf number by the time (Figure 5)
showed that fertilization, and formation of fruit and seed begun in later periods.

A study, which was made on Alder plant by Serebryakov (1952), showed that reproductive organs were not formed yet at the level of development of generative organs (18). In our study, formation of reproductive organs before winter dormancy supported the result of Serebryakov (1952). Another study that was made on Parrotia persica by Huseyinova (1988), showed that there were similarities between Alder and Parrotia persica in terms of shaping, formation and development of the buds (17).

Development of reproductive organs in winter and other biological features supported the theory that this species was one of the members of evergreen plant’s family.

REFERENCES