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Mechanism of seedlessness in Iranian seedless barberry (*Berberis vulgaris* L. var. asperma)

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ABSTRACT

Species of barberry (*Berberis vulgaris* L. var. asperma) is cultivated in arid and semi arid areas of Iran (South Khorasan province). It is widely used as a food additive. Fruits of this species are seedless, while wild type barberries produce seeds in the same area. In this study, we investigated the mechanism of seedlessness in seedless barberry by pollen viability test, field pollination experiments and microscopic observation of pollen tube growth in pistil and ovule development. For comparison, we also examined ovule development in wild type barberry (*B. crataegina* DC). In seedless barberry pollen germination was about 54%. Seedless barberry produced 20% seeded fruits when pollinated with pollen of wild type barberry. There was a sharp decrease in fruit set in emasculated unpollinated flowers of seedless barberry. In seedless barberry, a large number of pollen grains (about 370) were observed on stigma of each flower at 12 h after balloon stage (ABS). Most of them germinated and penetrated intracellular area of stigma surface, but no pollen tube reached ovary. In seedless barberry, many ovules did not have any embryo sac or had a very small incomplete embryo sac. In addition, unfused polar nuclei were clearly recognized in some cases at 14 days after full bloom (AFB). However, in wild type, double fertilization was accompanied by disappearance of polar nuclei. In seeded barberry, the cellularized endosperm became apparent at seven days AFB. At 21 days AFB, all ovules of seedless barberry were degenerated, while at the same time in wild type, one or two ovules of each flower were normal and were developing into complete seeds. Results showed that self-incompatibility has a main role in seedlessness of seedless barberry. However, the high frequency of abnormal ovules and single fertilization can be considered as two other reasons of seedlessness. Due to our results, fruits of seedless barberry were set by stimulative parthenocarpy.

1. Introduction

Seedless barberry (*Berberis vulgaris* L. var. asperma) is a particular crop which is cultivated in more than 6000 ha in South Khorasan, Iran. It is widely used as a food additive with cooked rice and rarely in jam and beverage. It has a high economical value for local farmers (Kafi et al., 2004).

The family of Berberidaceae is a well-defined and horticulturally important angiosperm family consisting of fifteen genera and about 650 species mainly found in the Northern Hemisphere and is native to Asia, Europe, North Africa, and to North, Central, and South America (Ahrendt, 1961). Barberry's hermaphrodite flowers are borne in racemes, usually solitary, located in the axial of fascicle of 2–4 leaves, on lateral shoots produced in the previous year. *Berberis* is self fertile and mainly autogamous. Self pollination is due to the seismonasty of stamen and to the behavior of honey bees when feeding with nectar. Pollen is sticky and produced in small amounts (Cadic, 1992; Sastri, 1969; Anderson et al., 2001). The ovule of these species is anatropus, bitegmic and crassinucellate. Development pattern of embryo sac is of the *Polygonum* type. The synergids show filiform apparatus and are persistent. Antipodals are big and ephemeral. Mature pollen grains are three-colpate and two-celled (Sastri, 1969).

Fruit set and seed set processes have important roles in garden management and fruit breeding. Seedless fruits are desirable in crops such as grape and citrus. However, seed set has an important role in breeding programs. In natural populations, seedlessness results from one of the following three causes: (i) lack of pollination, (ii) pollination occurring without fertilization (stimulative parthenocarpy), and (iii) fertilization followed by embryo (seed) abortion (stenospermocarpy) (Srivastava, 2002; Ebadi et al., 1996).

Plant reproductive process, especially seed set, may be affected by self-incompatibility or inbreeding depression (Franklin-Tong, 2008; Porcher and Lande, 2005). Self-incompatibility (SI) is a genetically controlled process in angiosperms that results in the recognition and rejection of self or self-related pollen and pollen

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tubes (De Nettancourt, 1997, 2001). Depending on the species, pollen is recognized and rejected in stigma and style by sporophytic or gametophytic SI (De Nettancourt, 1997, 2001). There are also many reports of pollen tubes entering the ovary, and even the ovule, before self-rejection occurs "late-acting SI" (Lersten, 2004). In one version of the late-acting SI, self-pollen tubes enter ovules but do not penetrate embryo sacs (Kenrick et al., 1986). In another case, self-pollen tubes deposit sperms into the embryo sac, but double fertilization does not take place (Cope, 1962). In some other instances, double fertilization occurs, but zygotes arising after self-pollination never divide (Sage and Sampson, 2003; Sage et al., 2006). Inbreeding depression is expected to cause embryo failure at a variety of developmental stages (Seavay and Bawa, 1986). SI plays an important role by reducing inbreeding depression and its harmful effects (De Nettancourt, 1997; Waser and Williams, 2001).

Fruits of seedless barberry do not have any seeds, while wild types growing in the area mentioned above produce seeded fruits. There are several species of seedless barberry in the world but there is only one report about existing SI in *Berberis corymbosa* (Anderson et al., 2001). Microscopic observation of pollen tube growth In *B. corymbosa* showed that pollen grains germinate on the stigmata, but pollen tubes do not grow beyond the stigmatic level (Anderson et al., 2001).

In the present work, we investigated several aspects related to seedlessness mechanism in barberry: pollen viability test, field pollination experiments, pollen tube growth on stigma and ovary, embryo sac development.

2. Materials and methods

2.1. Plant materials

Field observations and pollination experiments were done at a barberry germplasm in Mashhad Science and Technology Park, Khorasan, Iran (longitude 59°, latitude 36°). For histological study as well as pollen germination tube growth study, mature inflorescences of 10-year old seedless (*B. vulgaris* L. var. asperma) and seeded barberry (*B. crataegina* DC) (Browicz and Zielinski, 1975) were collected from a commercial farm in Birjand city (longitude 59°, latitude 32°) and the botanic garden of Agriculture college, University of Tehran (longitude 51°, latitude 35°), respectively.

To study the mechanism of seedlessness in seedless barberry, four experiments were conducted as follows:

2.2. Pollen viability

Pollens were collected from mature inflorescence with the *n*-pentan solution method (Cadic, 1992). Then, Pollens were cultured in Petri dishes in a solid medium containing sucrose 20%, agar 1%, 0.01% Boric acid, 0.01% KNO_3, 0.03% $Ca(NO_3)_2$ and 0.02% $Mg(SO_4)_2$ (Medium optimizing with primary experiment). They were incubated at 20 °C in light condition. Pollen germination percentage was calculated by dividing the number of germinated pollens to total observed pollens in three Petri dishes for any genotype 12 h after culture. Data were analyzed by SPSS 15.0 software and means were compared by *t*-test.

2.3. Pollen tube growth

In seedless barberry, 12 flowers were examined per each sampling time (one flower per inflorescence of each of the four main sides of three shrubs). Flowers were collected at 3, 6,12, 24, 36, 72, 144, and 288 h after balloon stage(ABS), fixed in FPA(Formalin: Propionic acid: 50% ethyl Alcohol, 5:5:90, v/v); the pistil of each flower was softened in 0.8 mol NaOH for 1 h at 60 °C, rinsed at least for 1 h in distilled water, stained with aniline blue (1%) in 0.1 mol K_3PO_4 for a minimum of 4 h, then squashed and pollen tube growth was observed using the fluorescence microscope (Nikon E1000) in excitation filter BP 395 to 425 nm and barrier filter of LP450. The following items were measured: (1) the average number of pollen grains on the stigma surface, (2) the number of germinated pollen grains on the stigma, (3) the number of pollen tubes penetrating among stigma papillate cells, (4) number of pollen tubes at the middle of stigma and (5) number of pollen tube at the base of ovary.

2.4. Field experiment

Fruit and seed set of seedless barberry were measured in the field on the basis of complete randomized block design with six treatments and three replications. Four inflorescences (13–20 flowers per inflorescence) on different sides of each shrub were selected for every replication. Pollens were collected from mature inflorescences with the *n*-pentan method (Cadic, 1992). To emasculate flowers, sepals, petals and stamens were removed at the balloon stage. Pollination was done at stigma receptivity time (one day after emasculation). Emasculated flowers were covered with waterproof pulpy bags. Pollen was placed on the stigma surface by a small brush and flowers were covered again.

There were six pollination treatments including:(1) Controlled pollination of emasculated flowers with seedless barberry pollens, (2) controlled pollination of emasculated flowers with mixed pollen of seeded barberries, (3) pollination of intact flowers (without emasculation) with mixed seeded barberry pollens, (4) covered emasculated flowers without any pollination, (5) covered intact flowers (without emasculation and pollination) and (6) open pollination of intact flowers.

After six months, the percentages of fruit set and seeded fruit set were calculated using following formula:

$$\text{Fruit set percentage} = \frac{\text{total fruit numbers}}{\text{total flower numbers}} \times 100$$

$$\text{Seeded fruit percentage} = \frac{\text{total seeded fruit numbers}}{\text{total fruit numbers}} \times 100$$

Statistical analysis was then performed by SAS 9.1 software to determine whether there were differences among pollination treatments using an ANOVA with LSD comparison procedures.

2.5. Embryo sac development and fertilization

This experiment was done in two years. In April 2008 and 2009, three similar shrubs of seedless barberry were selected. Flowers were sampled from synchronized flowers of each inflorescence of four main sides. All flowers were collected at full bloom, 7, 14, 21 days after full bloom (AFB). Harvested flowers were fixed in FPA, dehydrated in ethanol series, embedded in xylem and paraffin. Tissues were sectioned at 7 μm thickness with a rotary microtome and were stained with safranin and fast green. In seeded barberry, sampling was done only in April 2009 and flowers were collected at full bloom, 7, 14, 21, 32 days AFB and fixed in FPA. A transmission light microscope (Zeiss) was used to take images. The numbers and percentages of normal ovules (ovules containing normal size of embryo sacs), complete ovules (ovules containing embryo sacs with polar nuclei and filiform apparatus), degenerated ovules and fertilized ovules were measured.

3. Results

3.1. Pollen viability

Pollen of barberry started to germinate 2 h after culture and pollen germination rate was calculated after 12 h. The average of

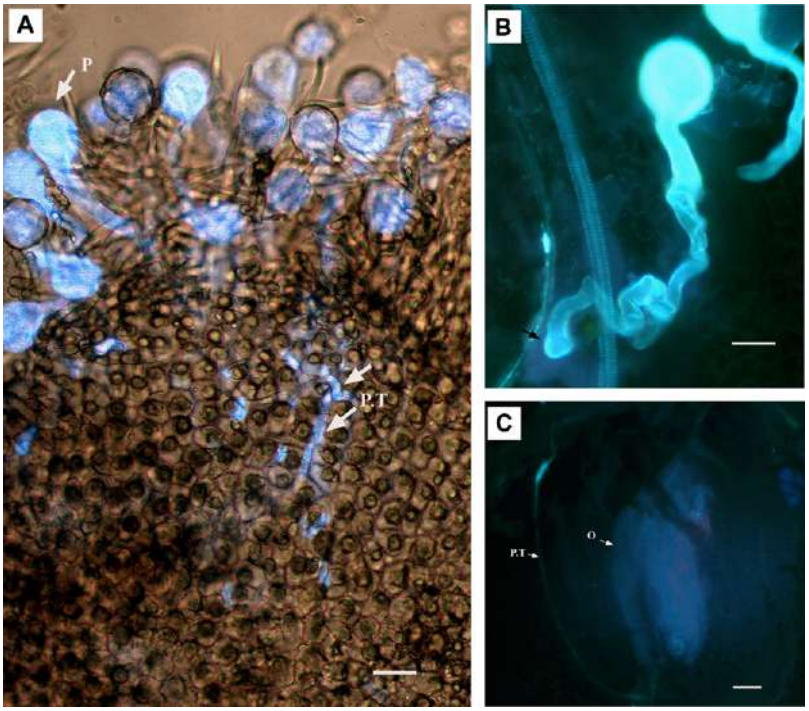

Fig. 1. Pollen germination and pollen tube growth in seedless barberry. (A) pollen germination and intercellular penetration of pollen tubes on stigma at 36 h ABS. Bar = 4 μm, (B) curvature and callose deposit in restricted pollen tubes in stigma. Bar = 4 μm, (C) pollen tube penetration into ovule at 72 h ABS. Bar = 100 μm, P: pollen, P.T: pollen tube. O: ovule. ABS: after balloon stage.

pollen germination was 57% and 69% in seedless and seeded barberry, respectively. However, percentages of pollen germination in seedless and seeded barberry were not significantly different ($p < 0.05$).

3.2. Pollen tube growth

Pollen tube growth (Table 1) was observed in seedless barberry without any pollination treatment. The surface of stigma was wet and covered by stigma secretion at receptive time (anthesis). Pollen grains inserted on papillate cells at the edge of stigma. In three to 12 h after the balloon stage (ABS), 10–32 pollens were placed on stigma. At 24 h ABS, the pollen number increased and reached to 285. The maximum number of pollen grains on stigma was 787 at 36 h ABS. The average number of pollen grains on stigma increased from 6 to 72 h (21.6–400.2) then decreased very slightly in 288 h ABS (Table 1). Pollen tubes initially grew intercellularly among the cells adjacent to stigmatic epidermal cells (Fig. 1A). Callose deposit was easily observed at the end of restricted pollen tubes among stigma cells (Fig. 1B). Only in two cases, two pollen tubes were observed at the base of ovary at 72 and 144 h ABS (Fig. 1C). There was no pollen tube at the base of ovary at 288 h ABS (Table 1), while the number and percentages increased on stigma from 3 to 288 h ABS. Most of pollen grains on stigma germinated and pollen tubes

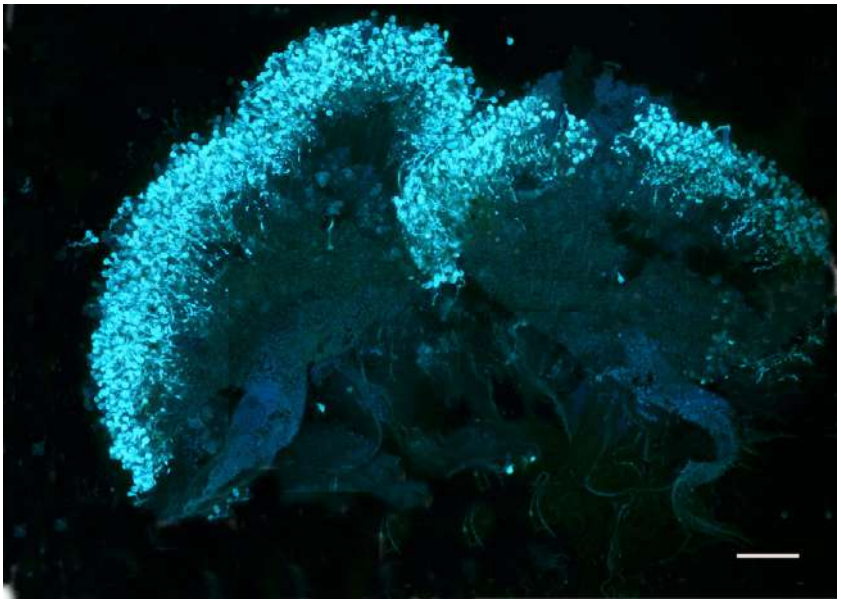

Fig. 2. Pollen germination and pollen tube growth on the stigma of seedless barberry and in upper ovary at 72 h ABS. Bar = 100 μm.

Table 1
Average number, range and percentage of pollen grains on stigma, germinated pollen grains and germinated pollen tubes at the base, mid and on stigma during hours of ABS[a].

Hours after ABS	Pollen grains (Average number[b], range and percentage)			Pollen tubes (Average number[b], range and percentage)				
	On stigma	Germinated		On stigma		At the mid-stigma		At the base of ovary
3	23 (10–32)	6 (3–9)	26%	3 (1–4)	13%	0	0	
6	21.6 (16–27)	9 (7–11)	41%	4 (2–5)	19%	1	5%	0
12	28 (17–30)	20 (18–22)	71%	12.5 (5–15)	45%	4 (3–5)	14%	0
24	138 (51–285)	88.2 (41–189)	64%	74 (30–140)	54%	13.8 (5–30)	10%	0
36	280.2 (241–787)	128.2 (80–140)	46%	119 (70–130)	42%	18 (9–24)	6%	0
72	400.2 (275–523)	156 (85–251)	40%	95.16 (60–100)	24%	45 (25–60)	11%	1
144	373.2 (316–455)	282 (264–270)	75%	231 (194–262)	62%	30 (20–45)	8%	1
288	359 (314–390)	330 (312–346)	92%	301.2 (285–315)	84%	40 (21–70)	11%	0

[a] ABS: Flower's Balloon Stage.
[b] Mean of12 flowers per sampling time.

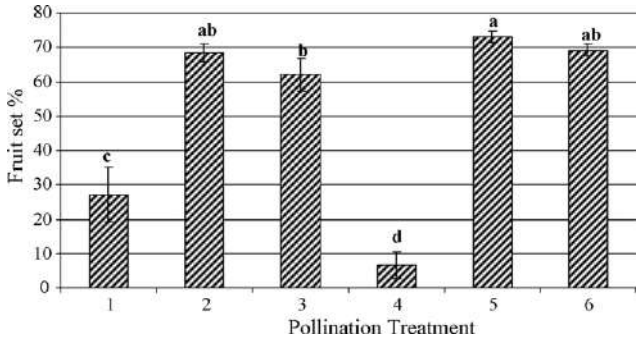

Fig. 3. Percentages of fruit set in six pollination treatments in seedless barberry.
Significant differences ($p = 0.01$) were observed by LSD test. Pollination treatments: 1: pollination of emasculated flower with self pollens; 2: cross pollination of emasculated flowers with mixed wild seeded types pollens; 3: cross pollination of intact flowers with mixed wild seeded types pollens (no emasculation); 4: covered emasculated flowers with no pollination; 5: covered intact flowers; 6: controlled open pollinated flowers.

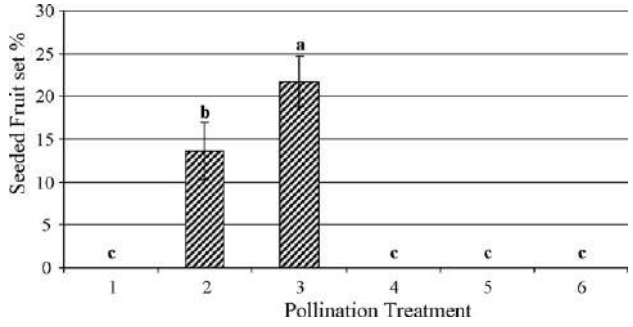

Fig. 4. Percentages of seeded Fruit set in six pollination treatments in seedless barberry.
Significant differences ($p = 0.01$) were observed by LSD test. Pollination treatments: 1: pollination of emasculated flower with self pollens; 2: cross pollination of emasculated flowers with mixed wild seeded types pollens; 3: cross pollination of intact flowers with mixed wild seeded types pollens (no emasculation); 4: covered emasculated flowers with no pollination; 5: covered intact flowers; 6: controlled open pollinated flowers.

penetrated the stigma cells, but all of them were rejected at the base of stigma (Fig. 2).

3.3. Field experiment

Pollination treatments had a significant effect ($p < 0.01$) on fruit set and seeded fruit percentages. Fruit set in normal flowers of seedless barberry was about 70% (Fig. 3). The minimum fruit set (6%^{d}) was observed in emasculated and unpollinated flowers (Treatment 4) (Fig. 3). Fruit set was also significantly decreased in treatment 1 (27.16%^{c}) (emasculated, self-pollinated and covered flowers) (Fig. 3). There were no significant differences in treatments 2 (68.46%^{ab}), 3 (61.99%^{b}), 5 (73%^{a}) and 6 (69.20%^{ab}) (Fig. 3). Only flowers pollinated with mixed pollen of some seeded barberry (Treatment 2 (13.67%^{b}) and 3 (21.72%^{a})) could produce seeded fruits (Fig. 4).

3.4. Embryo sac development and fertilization

In 2007, the embryo sacs in seedless barberry were mature at anthesis. In a normal embryo sac, two unfused polar nuclei were clearly recognized (Fig. 5A). An egg cell was visible in the middle of the two-synergid cells (Fig. 5B). Antipodals could not be observed at this time. The histological study of ovule development in seedless barberry showed that all four ovules in each ovary got black and degenerated at 21 days AFB (Table 2; Fig. 5C and D). Out of the 61 ovules at anthesis, only 72% of them had a normal embryo sac size and the rest were abnormal (either had no embryo sac (Fig. 5E) or had a very small embryo sac) (Table 2). About half (55%) of the ovules had an embryo sac which had its components (polar nuclei, filiform apparatus or synergids) (Table 2). At full bloom, none of the ovules had any signs of degeneration (Table 2). Seven days AFB, 57% of the ovules had a normal embryo sac size, 46% of them had a complete embryo sac and only 10% of them showed signs of degeneration (Fig. 5F). At 14 days AFB, 45% of the ovules were normal and the rest were degenerated. Unfused polar nuclei were easily observed at 14 days AFB (Fig. 5G). At seven days AFB, there was a zygote in an ovule (Fig. 5H). The nucellus tissue from the integument was separated at 14 days AFB in some cases (Fig. 5I).

In 2008, out of 20 ovules, 80% had a normal embryo sac size and the rest were abnormal at anthesis (Table 2). At 14 days AFB, 31.2% of the ovules looked normal and the rest were degenerated (Table 2).

In seeded barberry at anthesis, 66% of the ovules had a normal embryo sac size and 41% had a complete embryo sac (Table 3). Fertilization did not occur at anthesis. In seven days after anthesis, 31% of the ovules had signs of fertilization, such as existence of zygote (Fig. 6A). In fertilized ovules, the development of cellular endosperm was clear at 14 AFB (Fig. 6B). Aborted ovules included those which failed in fertilization (Fig. 6C, Table 3). At 14 days AFB, 37.5% of the ovules were fertilized and at 21 days AFB, the fertilized ovules were clearly recognizable from the unfertilized necrosed ovules (Fig. 6C and D).

4. Discussion

Seedless barberry is one of the major economical crops for local farmers of south Khorasan province in Iran. To our knowledge, there

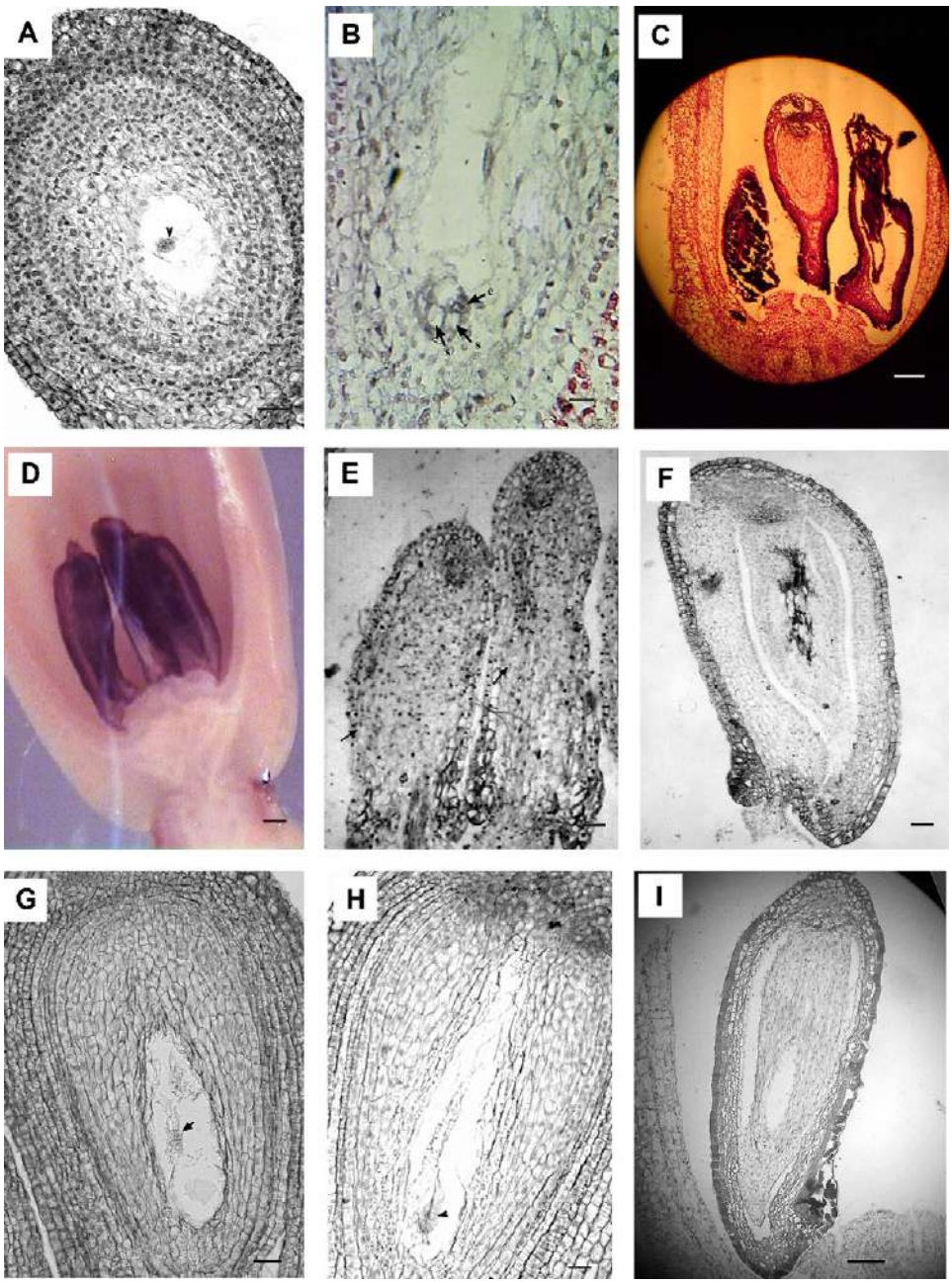

Fig. 5. Abnormal development of ovules in seedless barberry. (A) polar nuclei at full bloom stage. (B) Egg cell and synergid cells at full bloom stage., (C) and (D) degenerated ovules at 21 days AFB., (E) abnormal ovule without embryo sac at seven days AFB., (F) degeneration of embryo sac seven days AFB., (G) unfused polar nuclei at 14 days AFB., (H) a zygote at seven days AFB., (I) separation of embryo sac and nucleus from integuments at 14 days AFB. Bar in A, G, H: 20 μm; B: 10 μm; C, D, E, F, I: 40 μm. e: egg cell, S: synergids, AFB: after full bloom.

Table 2
Situation of ovules at 0, 7, 14 and 21 days after full bloom in seedless barberry in 2007 and 2008.

Year	Days after full bloom (AFB)	Number of studied ovules	Number of ovules with normal embryo sac size	Number of ovules with complete embryo sac	Number of degenerated ovules
2007	0	61	44 (72%)	34 (55%)	0
	7	64	37 (57%)	30 (46%)	7 (10%)
	14	44	20 (45%)	16 (36%)	24 (54%)
	21	32	–	–	32 (100%)
2008	0	20	16 (80%)	8 (40%)	0
	7	16	8 (50%)	3 (18%)	4 (25%)
	14	16	5 (31%)	4 (25%)	11 (68%)
	21	12	–	–	12 (100%)

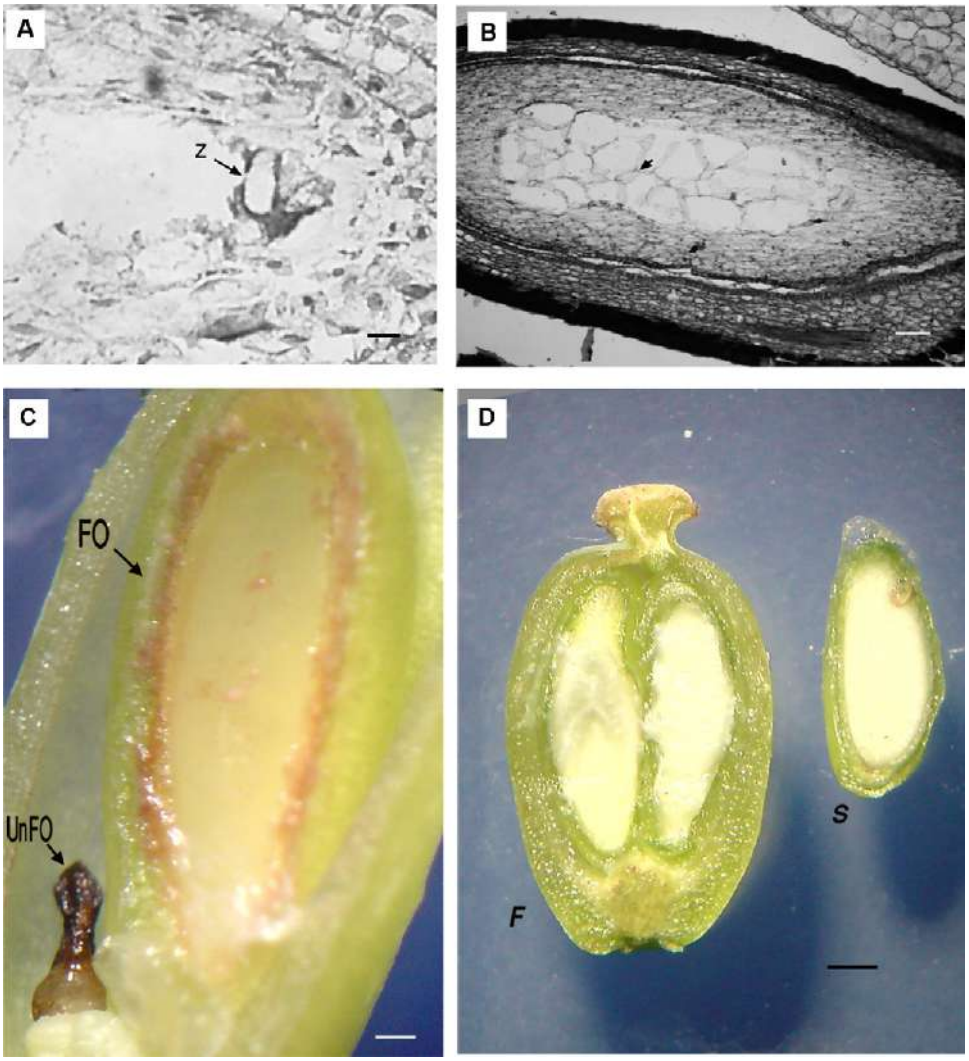

Fig. 6. Inner status of seed development in seeded barberry. (A) Zygote at 7 days AFB, Bar = 10 μm., (B) cellular endosperm at 14 days AFB, Bar = 20 μm., (C) fertilized and unfertilized ovule at 21 days AFB, Bar = 20 μm., (D) seed and fruit development in a normal fertilized seed at 40 days AFB, Bar = 40 μm. Z: zygote, F: fruit, S: seed, FO: fertilized ovule, UnFO: unfertized ovule, AFB: after full bloom.

is no report about the seedlessness mechanism of Iranian seedless barberry. In this study, we tried to clear some ambiguity of seedlessness mechanism and fruit set of seedless barberry that is important for designing a breeding program.

Pollen of seedless barberry showed high viability and pollen tube growth was normal in in vitro culture. Microscopic study of pollen tube growth and germination in natural conditions showed high pollen viability, too.

Male sterility, due to abortion in tetrad stage, has been reported in some barberry hybrids (*B. juliana* × *B. ottawensis*) (Cadic, 1992). Results of pollen germination in in vitro and in in situ conditions rejected the existence of male sterility in seedless barberry.

The numbers of pollen grains on stigma at the early stages after flower balloon stage, were much fewer than the later stages (Table 1). There is a mechanism through which the stamens automatically snap towards the stigma when touched or shook by strong wind gusts or insects. When stimulation has finished, stamens return to their normal position against the petal (Fleurat-Lessard and Millet, 1984). Barberry pollen grains are sticky and in several stages gradually placed on the stigma by seismonasty mechanism.

Results showed that several hundreds of pollens were landed on each flower stigma. Most of them germinated and penetrated in stigma intercellulary, but ceased after some penetration (Fig. 2). Each pollen tube showed curvature and callose deposit, especially at the end of the pollen tube (Fig. 3C). In genotypes with gametophytic self incompatibility, callose is deposited irregularly at the end of pollen tube (Heslop-Harrison, 1976). Pollen tube ceasing area

Table 3
Situation of ovules at 0, 7 and 14 days after full bloom in seeded barberry in 2008.

Days after full bloom (AFB)	Number of studied ovules	Number of ovules with normal embryo sac size	Number of ovules with complete embryo sac (unfertilized)	Number of fertilized ovules	Number of withered ovules
0	12	8 (66%)	5 (41%)	0	0
7	16	12 (75%)	7 (43%)	5 (31%)	0
14	16	–	–	6 (37%)	10 (63%)

depends on SI type and genetic characteristics. To date, all stylar SI is documented to be gametophytic (De Nettancourt, 1997, 2001) and stigmatic SI may be sporophytic or gametophytic (Sedgley and Griffin, 1989; De Nettancourt, 1997, 2001). Barberry flowers are styleless or with a very short style and all pollen tubes were rejected in stigma. Rejection of pollen tube in stigma has been reported in some cultivars of apple, pear, citrus and almond which were considered gametophytic SI (Sedgley and Griffin, 1989). On the other hand, barberry has regular flowers with moisturized stigma and two nuclei pollen grain with high viability (Sastri, 1969), which all are features of gametophytic SI (Sedgley and Griffin, 1989).

In controlled pollination with self pollens, fruit set was reduced about 40% in comparison to open pollination (Fig. 3). Reduced fruit set in controlled pollination may be the result of emasculation and isolation stress or the low number of pollens placed on stigma in controlled pollination (Nyeki and Soltesz, 1996). Dramatic reduction of fruit set in non-pollinated flowers indicated the pollen stimulus effect on fruit set. In stimulative parthenocarpy, pollination or other stimulus is required for fruit set (Saito et al., 2007). In this phenomena, fruit formation may be due to self incompatible pollens such as *Citrus reticulata* cv. Clemantin or due to killed pollens by temperature or irradiance e.g. in pear (Sedgley and Griffin, 1989).

Production of seeded fruits in cross pollination of seedless barberry with mixed pollens of wild types showed rejection of self pollens in seedless barberry. Self-incompatibility is a genetically controlled process in angiosperms that results in the recognition and rejection of self or self-related pollen and pollen tubes (De Nettancourt, 1997, 2001). Field experiments are a practical and acceptable method for compatibility and fertilization studies (Nyeki and Soltesz, 1996).

Histological sections have been used in many plants to study seed or fruit malformations (Ebadi et al., 1996; Miyajima, 2006). High percentages of abnormal ovules were observed in seedless and seeded barberry (26–54% in seedless and 25–33% in seeded barberry). There are two ovules in all prunes but only one of them is fertile and the second one is often undeveloped (Sedgley and Griffin, 1989). In *Pistacia vera*, up to 31% of the ovules lacked embryo sacs (Shuraki and Sedgley, 1996). In olive, some cultivars had more than 80% abnormal ovules (Rallo et al., 1981). Abnormal callose synthesis (Rosellini et al., 2003) or failure in meiosis (Siddiqi et al., 2000; Wilson and Owens, 2003) was reported to be the main cause of the failure in embryo sac formation.

Unfused polar nuclei were detected seven to 14 days AFB in normal embryo sacs of seedless barberry (Fig. 5G), while in seeded barberry, unfused polar nuclei were rarely recognized in seven days AFB. In *Berberis umbellata,* polar nuclei were fused before fertilization (Sastri, 1969). Joining of polar nuclei in cherry occurs a few minutes before or during the fertilization process (Nyeki and Soltesz, 1996). Successful fertilization can easily be recognized by polar nuclei disappearance several days after pollination (Miyajima, 2006). In zinnia, Polar nuclei can easily be observed in unfertilized ovules 10–15 days AFB (Miyajima, 2006).

In seeded barberry, cellularized endosperm was observed at 14 days AFB (Fig. 6B), while at this time, there was no sign of endosperm formation in seedless barberry. Existence of endosperm is a sign of fertilization that easily can be recognized (Lersten, 2004).

In few embryo sacs of seedless barberry, there was an undivided zygote without any sign of endosperm formation (Fig. 5H). The phenomenon in which the egg cell is fertilized while central cell fails to fertilize (single fertilization) has been reported in many plants, such as maize (Kato, 1997) and zinnia (Miyajima, 2006). In single fertilization, pollen tubes penetrated the embryo sac, but did not fuse with the polar nuclei. In some cases, sperms were discharged into the embryo sac but stopped later in synergid cell (Raghavan, 2003; Punwani and Drews, 2008). Single fertilization has been known as a type of SI (Sparrow and Pearson, 1948; Sage and William, 1991).

In seeded barberry, at 21 days AFB, fertilized ovules were completely distinguished (Fig. 6C), while at the same time, all seedless barberry ovules were blackened and degenerated (Fig. 5C and D). In self incompatibility, all ovules abort simultaneously (Lersten, 2004), while in inbreeding depression, abortion occurs at different stages of plant life cycle (from embryo development to juvenility stage of offspring or even at mature stages) (Kennington and James, 1997).

There is a close correlation between flower structure and breeding system in fruit trees (Nyeki and Soltesz, 1996). Special structure of barberry flowers cause to place many self-pollens on its large stigma. However, flowers of barberry with their attractive color and nectar have some visitors (pollen feeding insects and honey bees) that may facilitate some cross pollination. The selection pressure in domestication and breeding of the new cultivars enhanced self-fertilization. Self-fertile cultivars have more fruit set compared to cross fertile cultivars (Nyeki and Soltesz, 1996). Domestication has encouraged self fertilization in seedless barberry and self-incompatibility acts as a genetic mechanism developed for declining inbreeding depression effects.

In summary, with considering low seeded fruit set in crosspollination treatments with using seeded barberry pollen and rejection of self pollen tubes in stigma or style, self incompatibility is the main reason of seedlessness in seedless barberry, but high percent of malformed ovules and single fertilization, can be two other reasons that have some effects on seedlessness in this plant. Therefore, stimulative parthenocarpy is presumably the main mechanism of fruit set in seedless barberry.

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