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Original Article

Sexual Propagation of Persian Shallot (*Allium hirtifolium*) Through Manual Pollination and *In vitro* Ovary Germination

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Abstract

For sexual reproduction of *Allium hirtifolium*, collected bulbs were planted in the field. In order to develop ovary embryo and seed setting, control self-pollination and natural open pollination took placed using honey bees under cages. Ovary development and seed setting of *A. hirtifolium* extraordinary were affected by pollination and there was a significant difference between mean effect of self-pollination and natural open pollination using chi-Square test at α=0.05%. Open pollination using honey bees with average 23 numbers of seeds per flower stalk showed higher seed formation than control self- pollination. Out of 240 mature ovaries (25 and 35 day-old ovary), which have been isolated, 194 ovary were germinated (80.8%) from 25 and 35 days after pollination (DAP). Due to long embryo development in this species, all isolated embryo (less than 25 day-old) failed to develop direct plantlets on either MS hormone free or half-MS medium, for in vitro germination. Analysis of data originated from affect of culture media on embryo germination, indicated that there is no significant differences between culture media on embryo germination using Chi-square test at α=0.05 level. In spite of no differences, MS media showed higher ovary germination (average 54.33%) than half-MS medium (average 41.50%). Successful acclimatized plantlets transferred to green-house and then to the field.

Keywords: *Allium hirtifolium,* Mooseer, Ovary, Embryo, Sexual reproduction, Pollination

Introduction

The genus *Allium* includes more than 700 species, which grow in the temperate, semi arid and arid regions of the northern hemisphere. *Allium hirtifolium* Boiss, is one of this genus, which is produced through bulbs and called Persian shallot (Mooseer) [1]. Persian shallot (*Allium hirtifolium* Boiss.) is a wild, perennial, herbaceous and aromatic plant. The plant is native to some parts of west, south and central parts of Iran. It is widely used in the pharmaceutical and food industries in the country. Persian shallot is a nutritive plant with special taste and its dried bulb slices are used as an additive to yogurt and also pickling mixtures. Its powder is used as a tasty additive or spice for foods. Its fresh or dried bulbs are sold in small and medium quantities for domestic consumption and exported to Persian Gulf countries. Bulb proliferation is common natural propagation system of this species, which is intensively harvested. Due to such highly consumption of Mooseer bulb, attempt has to be done to develop reliable methods for asexual and sexual propagation. Little information on seed formation and germination is available on sexual proliferation of *A. hirtifolium* ecotypes. Seed dormancy is one of limitation factors in sexual proliferation of this species, and made it difficult. Many attempts have been developed to overcome seed dormancy such as cold stratification, chemical or mechanical scarification to reduce seed hardness and to improve germination, emergence rate and uniformity. In

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spite of highly consumption of Mooseer, successful method of breaking seed dormancy has not yet been reported. The aim of this study is to develop a reliable method for fully pollination and *in vitro* ovule embryo culture to increase seed germination and overcome seed dormancy barrier in sexual propagation of this species.

Material and Methods

Collected bulbs of *A. hirtifolium* from Agricultural Research Center of the Hamedan Province were vernalized in cold storage over the winter at 4 °C and then were planted in the field. Totally one hundred bulbs with 10 bulbs per row were planted in the experimental station with ten replicates. All recommended cultural practices like weeding; fertilizers and irrigation were done properly at the required time. Control self pollination took placed by putting cage on each individual plant. Natural open pollination was completed using honey bees for natural cross pollination. Flowers of all originated plants were examined for pollen production and pollination to encourage ovary development and see setting. For embryo germination, sixty isolated 25 and 35 day-old ovaries were transferred to half-MS and MS [2] hormone free agar medium containing 30 mg/sucrose. No growth regulators were added to the medium. The medium was autoclaved for 20 min at 120 °C and then dispensed in jars with 20 ml. Plantlets 1 to 2 cm in height were transferred to Jars containing the same medium and kept for two months before acclimatized. The numbers of germinated embryo have been counted and analyzed using Chi-square test. Acclimatized plantlets transferred to green-house and then to the field.

Results and discussion

Effect of Pollination on Number of Ovary Development

Different number of developed ovaries and seeds setting of *A. hirtifolium* extraordinary were affected by pollination and there was a significant differences between mean effect of self-pollination and natural open pollination using chi-Square test at α=0.05% (Table 1). Open pollination using honey been with average 23 seed per flower stalk showed higher seed formation than control self- pollination (Table 1). Different number of seed (1–3) per ovary capsule observed for both control self-pollination and open pollination. In contrast to the report of Jafari Mofidabadi [3] who were obtained higher seed per ovary in cross pollinated flower than selfing, we did not observe seed setting differences per ovary in cross and self-pollinated *A. hirtifolium* ovaries.

Effect of Ovaries Age on Embryo Germination

Out of 240 mature ovary (25, 35 days old ovary) which have been isolated, 194 ovary were germinated (80.8٪) from 25 and 35 days after pollination (DAP), While no plant have been obtained from capsule isolated less than 25 days old (Table 2). Ovary embryo culture have been used previously by Ragan [4] and Ramming [5] for different plant species. The analysis of collected data on *in vitro* embryo cultures using, 25 and 35 days old embryo, indicated that there is no significant differences among parent for mean number of developed embryo and its germination using Chi-square test at α=0.05 level (Table 2, Table 4). Fourteen days old embryo failed to develop embryo and germination. Due to long embryo development in this species, all isolated embryo (less than 25 day-old) failed to develop direct plantlets on either MS hormone free or half-MS medium, for in vitro germination. Age of embryo culture is a crucial factor which was reported by several researchers [6]. The same results for immature embryo isolation were reported for cotton [7-9] and other crop species such as poplar [6,14]. In contrast to the Moradi [1], who obtained hybrid plantlets from 3 days old embryos, using media supplemented with hormone growth regulators we were not able to develop embryos and germination in simple embryo culture. Successful results for diploid and tetraploid cotton hybridization using immature embryos (3 days old embryos) culture have been reported by Stewart and Hsu [10,11]. The same result has been reported by Moradi [1] for this species too. Due to long embryo development in cotton species, all isolated embryo (less than 45 days old) failed to develop direct plantlets on plant nutrition medium. Germination of embryos less than 45 days in poplar may be need to the media supplemented with different hormone concentration, hormone combination, sucrose and vitamins.

Table 1 Effective factors and it's significant on number of ovary development in *A. hirtifolium* t using Chi-square test

Factors	Average No. of ovary per stalk flower
Control self- pollination	16.3*
Open pollination	23

*= Significant different at α=0.05%

Table 2 Effect of embryo age on ovary germination

Age of Embryo (DAP*)	No of ovaries isolated	No of germinated ovaries	Mean no of germinated ovaries	Germinated ovaries
25	120	87	43.33	72.21
35	120	107	53.33	88.88
Total	240	194	-	-

*= Days after pollination

Table 3 Effect of culture media on ovary germination

Culture media	No of ovaries isolated	No of germinated ovaries	Mean no of germinated ovaries	Germinated ovaries (%)
MS1	120	109	54.33	90.83
MS2	120	85	41.50	70.83

MS1; = MS medium, MS2=half-MS medium

Table 4 Mean Effect of age of embryo and culture media on embryo germination and its significant using Chi-square test

Affective Factors	Embryo germination and its significant
Age of embryo	Germinated embryo (%)
25 DAP	43.33 ns
35	53.33
Culture media	Germinated embryo (%)
MS1	54.33 ns
MS2	41.5

ns; = no significant difference .
DAP= Days after pollination

Raghavan [12] reported culture of embryos with two developmental stage and stated pre-embryo development and germination is dependent on the endosperm and require a complex medium for germination such as amino acids, particularly glutamine, asparagine, various vitamins natural extracts, such as coconut milk and casein hydrolysate. Young embryos some time require a medium of high osmotic potential. Sucrose often serves both as a carbon source and osmoticum. High osmotic concentration in the medium prevents precocious germination and supports normal embryonic development. Ovaries with different development stage were used for embryo germination by Collins and Grosser [13] and Hu and Wang [14].

Effect of Culture Media on Embryo Germination

MS medium [2] is one of the most commonly basal media which were used for embryo rescue studies by several authors [15]. Media and its supplementation depend greatly on the stage of development of the embryo [16,17], Analysis of data indicated that there is no significant differences between culture media on embryo germination using Chi-square test at α=0.05 level. In spite of no significant differences, MS media showed higher ovary germination (average 54.33) than half- MS medium (average 41.50%) (Table 3.). MS hormone free medium was also used by Jafari Mofidabadi [3,18] for poplar mature embryo culture in interspecific hybridization. The same results were reported for poplar by Kalagari [15] and for Salix by Jafari Mofidabadi [3,18]. In contrast to the Raghavan [12,19] and Moradi [1] who were able to produce plant from immature embryo in MS medium and B-5 medium [20]

supplemented with different hormone growth regulators and vitamin, hybrid poplar was produced using half-MS and MS hormone free medium with 30 g/l sucrose [3,18] .

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