

Short Communication

Biological characteristics of pollens in some genotypes of *Rosa canina* as main factors affecting fruit set

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***Rosa canina* is a medicinal plant that its fruits have many important medicinal properties but unknown for many people's especially in Iran. However, this crop can be produced commercially and its orchards can be established such as other fruit trees. This research was carried out for studying pollen germination and tube growth as main factors affecting fruit set, in some Hashtroud and Maragheh indigenous genotypes of *R. canina*. Experiment was carried out based on completely randomized design (CRD) in ten genotypes (treatments) with four repeats. Pollens were cultured in an in-vitro medium containing 15% sucrose, 0.01% boric acid and 1.2% Agar and then maintained in 24°C in controlled condition. After 24 h pollens germination and growth were stopped with chlorophorm. Finally, pollen germination percentage (PGP) and pollen tube length (PTL) were measured by means of Light-Microscope at least in 7 randomized selected squares in each Petri Dish (repeats). Data analyzed with SAS software and results showed significant differences between studied characteristics of genotypes.**

Key words: *Rosa canina*, pollen germination, pollen tube growth, *in vitro*.

INTRODUCTION

The genus *Rosa* belongs to the Rosaceae family and contains approximately 100 species with mainly ornamental usages and which is widely distributed in Europe, Asia, Middle East, and North America (Nilsson, 1997). However, more than 50 rose species and cultivars have been reported in Iran and Iran can be regarded as a rose germplasm region. *Rosa canina* L. (dog rose, rose hip, briar rose) is one of the wild rose species and its fruits are considerably beneficial for human health based on containing organic and inorganic matters. Fruits of the rose species are rich in minerals, vitamins (A, B1, B2, B3, C and K), sugars, phenolic compounds, carotenoids, tokepherol, bioflavonoid, tannins, organic acids, amino acids, volatile oils, vanillin and other photochemical such as antioxidant and antimicrobials. Also; seeds of rose hip contain unsaturated and polyunsaturated fatty acids (Kazaz, 2009; Ozkan, 2004, 2002). Rose fruits has been used as medicinal and many other purposes. Fruit is eaten raw or cooked. It can be used in making delicious jams, syrups etc. The fruit can also be dried and used as

a tea. Moreover, Rose flowers are used for rose oil production, direct consumption or making various types of food products like tea, jam and candy (Nybom, 2009).

Medicinal properties and benefits of Rose are: Nutrient, mild laxative, mild diuretic, mild astringent, carminative, ophthalmic, tonic and vermifuge. Rose fruits tea has a mild diuretic and tonic effect, rich in vitamin C, traditionally made into conserves and purées, help the body's defenses against infections and especially the development of colds, help in cases of constipation and mild gall-bladder problems as well as conditions of the kidney and bladder, reduce thirst and alleviate gastric inflammation (Kaminski, 1994). Also, syrup made from the hips is used as a pleasant flavoring in medicines and is added to cough mixtures. Most of the rose species are diploid but there are several species with higher ploidy levels as well, especially tetraploids and also pentaploids, in section Caninae. Diploid species are usually self-sterile whereas the polyploids are self-compatible. In addition, these species are self-fertile, sometimes apomictic, and

Table 1. Analysis of variance for PGP and PTL based on micrometer in ten studied genotypes of *R. canina in vitro*.

Source of variation (SOV)	DF	PGP	PTL μm
Genotype	9	2338.7**	2284.1**
Experimental error	36	80.5	1.8
CV (%)		11.7	12.8

** : Significant at $P < 0.01\%$ level.

have reduced pollen viability but a high seed set (Sharafi et al., 2010).

However, fruits are the main part of Rose consumed by human. Fruits are results of pollination and fertilization of pollens with ovules (Cheung, 1996; Stosser et al., 1996). Pollination and fertilization are important in the plants which their fruit are main crop and based on these phenomenon studies of pollen traits such as longevity, germination and tube growth is necessary to be carried out in unknown plants such as rose species. *In vitro* pollen germination and tube growth is a speedy, simple and cheap method used by researchers in breeding programs for identifying favorable cultivars and genotypes which will be used as pollinizer in orchard establishment and plant breeding objectives. Some studies have been carried out on the relationships between viability and pollen germination. The rate of pollen germination of some plant species and cultivars varies depending on the medium or chemical concentration. For this reason, the suitable pollen germination medium should be obtained for each species and cultivar. Many stain tests have been used such as acetocarmine, propionyl carmine, aniline blue, Alexander's stain, IKI (iodine potassium iodide), FDA (fluorescence diacetate), NBT (p-nitro blue tetrazolium), MTT (2, 5-diphenyl tetrazolium bromide) and TTC (2, 2, 5-triphenyl tetrazolium chloride) to determine the pollen viability of fruit trees (Bolat and Pirlak 1996). In this study, pollen germination and tube growth of 10 rose genotypes investigated in the *in vitro* condition for determining suitable genotypes which will be used in the future breeding, orchard establishment and commercial production programs of rose hip in Iran.

MATERIALS AND METHODS

This study was designed to determine the qualitative characteristics of the pollen and to assess *in vitro* pollen germination of some rose genotypes that presently exist in Maragheh and Hashtrood towns. Ten genotypes of *R. canina* (M1, M2, M3, M4, M5, M6, H1, H2, H3 and H4), selected based on favorable flower, fruit and other main horticultural traits. In the spring of 2009, flower buds in balloon stage gathered and transmitted to laboratory. Petals and sepals were separated gently and anthers isolated from flower buds and placed in Petri dishes for releasing pollens. Pollens gathered in the jars and stored in refrigerator until using. Germination medium contained 1.2% agar, 15% Sucrose and 0.001% boric acid (Sharafi et al., 2010). Experimental design was Completely Randomized Design (CRD) with four replications. This research was carried out in department of Horticulture, College of Agriculture, University of Tabriz East Azerbaijan, Iran.

Table 2. Comparison of means of PGP and PTL in ten studied genotypes of *R. canina in vitro*.

Genotypes	PGP	PTL (μm)
M1	57.4 ^b	808.1 ^{ab}
M2	69.7 ^a	608.2 ^c
M3	66.8 ^{ab}	370.3 ^d
M4	12.5 ^d	320.4 ^d
M5	56.2 ^b	940.8 ^a
M6	11.9 ^d	381.7 ^d
H1	42.2 ^c	762.8 ^{cb}
H2	33.2 ^c	690.8 ^{cb}
H3	34.6 ^c	580.3 ^c
H4	57.4 ^b	885.7 ^{ab}

Pollens sowed with brush into the Petri dishes and maintained in 24°C in growth chamber for 24 h and then pollen tube growth was stopped with adding chlorophorm. Pollen germination percentage and tube growth were measured under light microscope in 7 randomized selected squares in each of the Petri dishes. A pollen grain was considered germinated when Pollen Tube Length (PTL) was at least longer than the grain diameter. Measurements of PTL were recorded directly by an ocular micrometer fitted on eyepiece of the microscope. Data were analyzed using SAS software and comparison of means was carried out with Duncan's multiple range tests.

RESULTS AND DISCUSSION

Analysis of variance for Pollen Germination Percentage (PGP) and PTL indicated significant differences among 10 studied rose genotypes (Table 1). PGP and PTL were ranged between 69.7 - 11.9% and 940.8 - 320.4 μm respectively. Maximum PGP and PTL observed in genotypes M2 (69.7%) and M5 (940.8 μm) respectively. Also, minimum PGP and PTL was observed in genotypes M6 (11.9%) and M4 (320.4 μm) respectively. Some of the genotypes have not shown significant differences in PGP and PTL (Table 2). Regarding data in Table 2 indicated that PGP has no relationship with PTL in studied genotypes. On the other hand, often genotypes with high PGP have no high PTL constantly although; genotype M2 showed high PGP had high PTL too. This phenomenon has been reported in some almond genotypes by Sharafi et al. (2010) and other genus and species such as hawthorn (Sharafi et al., 2010) loquat (Sharafi et al., 2010) and grapevine (Sharafi et al., 2010). *In vitro* germination of pollens has been used as a powerful tool for genetical, physiological, biochemical and cytochemical studies for a wide range of plant species belonging to different families. The possibilities of such an approach are experimentally shown for a number of plants (Heslop-Harrison, 1992). It is used to select favorable pollen grains to use in artificial pollination, breeding programs in biotechnological methods especially in tissue culture techniques and orchards establishment (Sharafi et al., 2010).

In general, there is a linear relation between pollen viability and germination capability in many fruit species and other plants (Ottavio, 1992). Germination capability of pollen depends on various factors, namely nutrition conditions of species and varieties used and environmental factors. To investigate pollination potential in breeding and growing programs, estimates should be made of pollen quantity and viability of cultivars and genotypes, as well as of pollen germination capability (Ercisli, 2007; Stosser et al., 1996). Pollen viability also has been tested according to Eti (1991) in 1% 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC), and Iodine Potassium Iodide (IKI) by some researchers (Bolat and Pirlak, 1999). Ercisli (2007) designed a research for *in vitro* pollen germination test of 2 rose species genotypes (*Rosa dumali* and *Rosa villosa*) with, 5, 10, 15, 20, 25, 30, 35 and 40% sucrose and 0.03, 0.01 and 0.1% boric acid in hanging drop method. In addition, 1% agar + 15% sucrose combinations in Petri dishes were also used. There were statistical differences ($p < 0.01$) among genotypes in pollen viability. Both the *R. dumalis* genotypes had higher pollen viability than *R. villosa* genotypes and he attributed this phenomenon to the effect of species. It was previously reported that pollen viability of *R. canina*, *R. dumalis*, *R. rubiginosa* and *R. villosa*, all belonging to section Caninae, varies considerably among species and pollen viability was recorded between 23 and 45%.

Pollen germination was increased with the increase of sucrose concentrations up to 35% in germination medium for *R. dumalis* and 30% for *R. villosa* genotypes. The highest pollen germination percentage (32.04-36.25) was observed in *R. dumalis* genotypes at 35% sucrose medium and 17.42 - 19.01% in *R. villosa* genotypes at 30% sucrose medium. Koncalova (1975) reported germination PGP of *R. hugonsis* at 30 and 35% sucrose concentrations which supports his findings. According to these results, Sharafi (2010) with the same study on some hawthorn genotypes, concluded that the best *in vitro* medium for hawthorn pollen germination and tube growth were composed of 15% sucrose with 0.005 - 0.01% boric acid and 1.2% agar and significant differences observed between different media in all of the studied pollen traits. Some favorable hawthorn genotypes by highest PGP and PTL were recognized for the future breeding and growing programs in Iran.

Conclusion

Findings of this study showed high difference among 10 studied genotypes in pollen PGP and PTL and genotype M2 recognized as the best one for the future breeding and commercial production in orchard establishment programs.

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REFERENCES

- Bolat I, Pirlak L (1999). An investigation on pollen viability, germination and tube growth in some stone fruits. *Turk. J. Agric. For.*, 23: 383-388.
- Cheung AY (1996). Pollen-pistil interactions during pollen tube growth. *Trends. Plant. Sci.*, 1: 45-51.
- Ercisli S (2007). Determination of pollen viability and *in vitro* pollen germination of *Rosa Dumalis* and *Rosa Vilosa*. *Bangladesh J. Bot.*, 36(2): 185-187.
- Heslop-Harrison y, Heslop-Harrison J (1992). Evolution of the actins cytoskeleton and wall during hydrating, activation and tube emergence. *Ann Bot.*, 69: 385-394.
- Kaminski P, Katz R (1994). The complete holistic herbal encyclopedia of medicinal plants flower. *Essence Repertory. The Flower Essence Society*; ISBN: 0-9631306-1-7.
- Kazaz S, Baydar H, Erbas S (2009). Variations in chemical compositions of *Rosa damascena* Mill and *Rosa canina* L. fruits. *Czech. J. Food Sci.*, 27: 178-184.
- Koncalova MN (1975). Studies on *Rosa* pollen. I. *In vitro* germination of pollen grains of *Rosa hugonsis*. *Preslia.*, 47(1): 22-25.
- Koncalova MN, Jicinska D, Sykorova O (1976). Effect of calcium and sucrose concentrations on pollen germination *in vitro* of *Sis Rosa* species. *Biol. Plantarum.*, 18(1): 26-30.
- Ma LG, Fan QS, Yu ZQ, Zhou HL, Zhang FZ, Sun DY. (2000). Does aluminum inhibit pollen germination via extracellular calmodulin? *Plant Cell Physiol.*, 41(3): 372-376.
- Nilsson O (1997). *Rosa*. In: Davis PH (ed.): *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, 4: 106-128.
- Nybohm H (2009). Genetics and genomics of Rosaceae, *Plant Genetics and Genomics*. Swedish University of Agricultural Sciences, Kristianstad, Sweden.
- Ottavio E, Mulahey D, Sari Gorla M, Mulahey GB (1992). *Angiosperm pollen and ovules*, Springer-Verlag.
- Ozkan G, Sagdic O, Baydar NG, Baydar H (2004). Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol.*, 10: 277-281.
- Sharafi Y, Bahmani A (2010). Study of pollen germination and tube growth in some Iranian Loquat cultivars and genotypes. 3th International Symposium on Loquat May. Antakya. Turkey, pp. 03-06.
- Sharafi Y, Karimi M, Ghorbanifar M (2010). Pollen germination, Cross compatibility and Fruit set in some of the Iranian Almond genotypes. *Afr. J. Plant. Sci.*, 4(5): 135-137.
- Sharafi Y, Hajilou J, Mohammadi SA, Dadpour MR, Skandari S (2009). Pollen tube growth and fruit set of some almond genotypes obtained from a breeding program for selecting suitable pollinizer. 5th International Symposium on Pistachios and Almonds. 6-10 October. Sanliurfa, Turkey.
- Sharafi Y, Babash pour M, Karimi M (2010). *In vitro* pollen germination and pollen tube growth in some hawthorn genotypes. *International medicinal and aromatic plants*. Shiraz. Iran, pp. 29-31.
- Sharafi Y, Karimi M (2010). Genetic diversity in Maragheh grapevines pollen traits. 10th International Conference on Grapevine Breeding and Genetics. USA. Geneva New yourk, pp. 1-5.
- Stosser R, Hartman W, Anvari SF (1996). General aspects of pollination and fertilization of pomes and stone fruits. *Acta Hort.*, 423: 15-21.