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Full Length Research Paper

Suitable *in vitro* medium for studying pollen viability in some of the Iranian hawthorn genotypes

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Hawthorn is one of the most important medicinal plants with more nutrients, medical, ornamental and sanitary properties and used as a main dry tolerant rootstock recently. Commercial part of the hawthorn is its fruits and the most important factors involved in fruit set are pollination, pollen tube growth and fertilization. This study was carried out to investigate pollen germination and tube growth of some East-Azerbaijan, Iran indigenous hawthorn genotypes in different *in-vitro* media for receiving the suitable medium for pollen germination and identifying favourable genotypes for using in the future breeding and orchard establishment programs. Experiment accomplished based on completely randomized design (CRD) in four repeats. Pollens gathered from anthers and maintained at refrigerator until usage. *In-vitro* medium has different concentrations of sucrose (5, 10, 15 and 20%), boric acid (0.005, 0.01 and 0.02%) with (1.2%) agar. Pollens were cultured in different media and maintained about 24 h in 22˚C and then, their growth was stopped with raising cholorophorm. Finally, pollen germination percentage and tube growth were measured under light-microscope in 7 randomized selected squares. Results showed that the best *in-vitro* medium for hawthorn pollen germination and tube growth were composed of 15% sucrose, 0.005 - 0.01% boric acid and 1.2% agar and significant differences observed between various media in the studied pollen traits

Key words: Hawthorn, *Crataegus*, pollen germination, pollen tube growth, *in vitro* medium.

INTRODUCTION

Currently, reduction of precipitations and snow in most of the agricultural regions of the world made to growers and breeders to regard those crops need to little water (especially, horticultural crops for instance fruit trees which need much water). However, fruit trees such as apples and pears need much water and show graft-compatibility on hawthorn rootstocks. Therefore, breeders and growers protect the stated problems with dry tolerant rootstocks, for example *Crataegus*, although, they have some difficulties on such rootstocks. Moreover, the genus Crataegus has some species, which could be cultivated commercially as a fruit tree, medicinal and ornamental plant and have much other usefulness (Sharafi et al., 2010). Crataegus is a complex group of trees and shrubs native to northern temperate zone with about 250 currently recognized species. *Crataegus* belongs to the subfamily Maloideae in the Rosaceae with the ability to interbreed freely by 17 basal chromosome numbers.

Around the world, hawthorns are used for a wide range of purposes.

Many hawthorn species are grown for their edible fruits in Asia, Central America, and various Mediterranean countries. Some cultivars have been selected for improved fruit size, yield, and ease of harvest, and these are grown for production of jellies, juices, preserves, and wine. However, until propagation, production, and harvest techniques are improved, limited supplies of fruits derived from orchard-grown plants will necessitate further collection of fruit from native. Hawthorns are recommended commonly by professionals as land-scaping and shelterbelt plants that can attract wildlife. Most developing countries have viewed traditional medical practice as an integral part of their culture. In Iran, a country where agricultural and livestock industries have an important role, the need for such fruit trees regarded as medicinal plants has been increasing gradually (Sharafi et al., 2010; Lasseigne and Frank, 2009).

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Moreover, Hawthorn has actinomorphic-perfect flowers and possesses a hypanthium on which perianth and androecium are inserted. The corollas comprise five free white to pinkish petals. Flowers grow in small clusters of 9 -18 and are entomophilous of the 'generalist' bowl form (Chacoff et al., 2008). Hawthorns contain mixture of flavonoids mainly oligomeric procyanidins, responsible of the plant medicinal benefits, congestive heart failure, migraine, high and low blood pressure. Hawthorn perhaps is one of the most widely plant used for treatment of mild heart disorders (Gabriel, 2006; Long et al., 2006; Mahrik et al., 2009; Committee, 1999).

Based on statements, fruits are the main part of hawthorn consumed by human. Fruits set after favorable pollination and fertilization of pollens with ovules in all of the plants is important in the plants, especially with their edible fruits. However, pollen germination and pollen tubes growth are, in principle, necessary for fertilization and seed formation in flowering plants. Study of pollen traits such as pollen longevity, germination and tube growth is necessary to be carried out in unknown plants such as hawthorn species. *In vitro* pollen germination and tube growth is a speedy, simple and cheap method used by researchers in breeding programs for identifying favourable cultivars and genotypes which will be used as pollinizer in orchard establishment and breeding objectives (Sharafi et al., 2010; Stosser et al., 1996). Pollen of most species germinates and grows like a tube when placed in a solution of calcium, boron, and an osmotic in tissue culture technique. Even though in species, germination and tube growth are robust under experimentally defined conditions, rendering in *vitro*-based studies of relevance to the *in vivo* situation (Cheung, 1996; Taylor, 1997). 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 stint tests have been used such as acetocarmin, propione carmine, aniline blue, Alexander's stain, IKI (iodine potassium iodide), FDA (fluorescein diactate), 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, 1999).

In this study, pollen germination and tube growth of some Iranian hawthorn genotypes were investigated in different *in vitro* condition for determining suitable medium and genotypes which will be used in the future breeding, orchard establishment and commercial production programs of hawthorn in Iran.

MATERIALS AND METHODS

This study was designed to determine the favourable *in vitro* medium for pollen germination and tube growth of some hawthorn genotypes that presently are exist in East-Azerbaijan Province, Iran. Ten genotypes of hawthorn [5 genotypes from *Crataegus douglasii* (black hawthorn) and 5 genotypes from *Crataegus Oxyacantha* (red hawthorn)] were selected. In the spring of 2010, 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 usage. Experimental design was completely randomized design (CRD) with four replications. This research was carried out in Department of Horticulture, Callage of Agriculture, University of Tabriz, East Azerbaijan, Iran. 7 germination media (treatments) were involved: (i) Sucrose solutions: 5, 10, 15 and 20%. (ii)Sucrose and boric acid combinations: sucrose 10 + 0.005% H3BO3; sucrose 10 + 0.01% H3BO3 and Sucrose 10 + 0.02% H3BO3.

Pollens sowed to the different media and maintained about 24 h in 22°C and then tube growth was stopped with adding chlorophorm. Pollen germination percentage (PGP) and pollen tube length (PTL) were measured under light- microscope in 7 randomized selected squares. A pollen grain was considered germinated when pollen tube length (PTL) was at least equal to or greater than the grain diameter. Measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece on 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 effects of sucrose solutions and sucrose-boric acid combination on pollen germination percentage (PGP) and pollen tube length are shown in Table 1. Results showed significant differences among treatments (different sucrose, boric acid and their combination) on pollen germination percentage (PGP) and pollen tube length (PTL) at $p < 1\%$ level (Table 1). PGP and PTL were measured for 10 genotypes and mean of them was analyzed because significant difference was not observed between 2 species (data not shown). PGP was ranged between 16.9 to 50.8% in different studied media in all of the genotypes. Different sucrose solutions were compared to each other. Maximum percentage for pollen germination was achieved in Sucrose 10 + 0.005% H3BO3 with a mean of 50.56% in all of the studied hawthorn genotypes (Table 2). Effect of boric acid on germination percentage was significant too. With comparing means of pollen germination percentage in medium with boric acid and without boric acid, they concluded that pollen grains of *Crataegus* had a maximum germination percentage mean in medium without (or very low concentration) boric acid (data not shown). Therefore, this cheap medium can be used as a suitable medium for investigation of the hawthorn pollen germination, viability and longevity in the future breeding (tissue culture), orchard establishment (pollination studies such as artificial pollination, pollen-pistil compatibility relationships of cultivars and genotypes, selecting suitable pollinizers, finding effective pollination period in fruit trees and etc) and commercial production programs (Sanzol and Herrero, 2001; Sharafi et al., 2009, 2010).

Analysis of variance for pollen tube length (PTL) showed

Table 1. Analysis of variance for pollen germination percentage (PGP) and pollen tube length (PTL based on micrometer) in ten studied genotypes of hawthorn in different studied *in vitro* media.

Source of variation (SOV)	DF	PGP%	PTL µm
Media	6	784.7**	827.1**
Experimental error	21	7.5	45
CV (%)		12.4	13.8

**: Significant at p < 0.01% level.

Table 2. Comparison of means for PGP and PTL in studied genotypes of hawthorn in different *in vitro* media.

Media%	PGP	PTL (µm)
Sucrose 5	35.5[b]	95.1[d]
Sucrose 10	32[b]	141. 7[b]
Sucrose 15	31.7[b]	133.6[bc]
Sucrose 20	16.9[d]	116. 8[cd]
Sucrose 10 + 0.005H3BO3	50.8[a]	100.5[cd]
Sucrose 10 + 0.01 H3BO3	35.9[b]	231.7[a]
Sucrose 10 + 0.02 H3BO3	25.2[c]	147.6[b]

Same letters show not difference among genotypes of each column.

significant differences in media with sucrose and media with sucrose + boric acid (Table 1). PTL was ranged between 95.1 - 231.7 µm in different studied media in all of the genotypes. Different sucrose solutions were compared to each other and maximum pollen tube length (PTL) was achieved in 10 - 15% sucrose. Moreover, maximum pollen tube length was achieved in 0.01% boric acid with a mean of 231.7µm (Table 2). It was distinguished that only low concentration of boric acid stimulated pollen germination and pollen tube growth, whereas boric acid with high concentration inhibited pollen grain germination and pollen tube elongation, which is in agreement with the finding reported for grapevine, loquat, almond, rose, apple, quince and cherries (Sharafi et al., 2009; Sharafi et al., 2010). The common condition for successful germination of other plants pollen *in vitro* seems to be a relatively requirement of different sucrose concentration in the cultivation media based on their genotype characteristics (Sharafi, 2010). Pollen viability also has been tested in 1% TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) and IKI (Iodine Potassium Iodide) by some researchers (Bolat and Pirlak, 1999).

In general, there is a direct relationship between pollen viability and germination capability in many fruit species. Germination capability of pollen depends on various factors, namely nutrition conditions (Calcium, Boron, Hydrocarbons, Osmotic, etc), genotype (various genusand species) and environmental factors (temperature, light, humidity and etc). To investigate pollination potential, estimates should be made of pollen quantity and viability, as well as of pollen germination capability for breeding and growing programs (Ercisli, 2007).

The dependence of pollen germination on sucrose concentration in germination media was proved in several Rosaceae species too, (Du et al., 2006; Sharafi, 2010). Ercisli (2007) designed a research for *in vitro* pollen germination test of 2 rose species (*Rosa dumali* and *Rosa vilosa*) 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.

Conclusion

Findings of this study 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 favourable genotypes by highest PGP and PTL were recognized for the future breeding and growing programs in Iran which will be introduced for these objectives after some other studies on them.

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