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Evaluating Micropropagation of Kashan Damask Rose, Yasooj Aromatic Rose and Their Hybrid

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Abstract**—***According to the value of damask rose and aromatic rose, crossing of Kashan damask rose × Yasooj aromatic rose has high importance in terms of its essential oil performance, rose water quality and its usage as a medicinal plant. With considering propagation problems of this plant, mass reproduction with tissue culture can help in a short time. The samples of hybrid plants and their parents were collected from Research Institute of Forests and Rangelands. After pre-sterilization and sterilization, explants were established on the MS medium. When length of explants became around 2 cm, were transferred to MS medium with 3 different treatments for shooting and it was indicated that best shooting treatment for all 3 genotypes was MS medium with IBA (0.1 mg/l), 2ip (0.5 mg/l) and BA (0.5 mg/l). G22 genotype produced better shoots, so it was established on 8 different rooting treatments and finally MS media with IBA (2 mg/l) showed best result (100%). Then explants of (G22×G4) and G4 genotype also were established on the best rooting media and produced 28% and 0% roots, respectively. All the rooted plantlets were transferred to plastic pots containing cocopeat+ perlite+ vermicompost with the ratio of 2:1:0.5 and placed in the laboratory for compatibility. Totally it was concluded that between 3 genotypes (crossed (G22×G4), damask rose as the paternal line (G4) and (G22) aromatic rose as the maternal line), G22 had the highest results of micropropagation, G4 had lowest results and crossed plant (G22×G4) was in the interstitial situation.*

Keywords**—Aromatic rose, Crossing, Damask rose, In vitro.**

I. INTRODUCTION

Damask rose is one of the most important roses and famous plants in the horticultural history of the world (Heidari *et al.*, 2005). Damask rose with the scientific name of *Rosa damascena* Mill. and English name of Persian rose, is from Rosacea family. This species has leaves consisted of seven and often nine serrate leaflets and it is a hybrid of *R. gallica* and *R. cunina* (Tabaei Aghdaei *et al*., 2009). Flowers are pink, somewhat big, mostly compacted in an inflorescence with few flowers, short pedicle and large petals (Mirheidar, 1996). Damask rose is one of the most important medicinal and aromatic species, which is cultivated in some parts of the world. Iran has been known as the source of this valuable plant (Chevallier, 1996). In the past, Persia was the main center of rose and other medicinal plants. Most of workshops for producing rose water were placed in Fars province (Meimand and Firuzabad cities). Today, Isfahan province, especially Kashan city, is one of the most important centers of cultivating damask rose, producing rosewater and different odors. In addition to Kashan, in other provinces such as Fars, Kerman and Azarbayejan sharghi also it has been cultivated (Tabaei Aghdaei & Rezaei, 2004). Damask rose has been placed in the category of roses (Bealis, 1990). Most important essence source of rose it is in their petals, which had different uses from last centuries. Petals of damask rose and it is products, reduce the amount of blood cholesterol. The plant is resistant to salt and drought, even it can be grown in the poor soils. In addition, it has been considered as an oily rose species (Gunes, 2005). Present day's damask rose is being used in the preparation of herbal medicines (Nikbakht, 2003). Volatile oil compounds or an essence, which is used for making odors, also it is used as tranquilizer, anti-depressant and anti-inflammatory (Haji-akhondi and Baligh, 2002). Iran is the most important producer and

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exporter of rosewater and damask rose essence (Guenther, 1952). Products of damask rose are dry flower, rosewater and essence (Tabaei Aghdaei *et al*., 2009). Debergh and Maene (1981) and Soomro *et al*., (2003) reported that, some of rose species hardly propagate by classic methods, because rooting is weak in some cuttings. In the other hand, in vitro culture of explants is a suitable and fast propagation method for rose varieties. Nikbakht *et al*., (2004) reported that usual method of propagating damask rose is cutting. However, in this method, the risk of viral and bacterial disease diffusion is high and it is not a safe method for supporting high recommendation of healthy shrubs and trees. Vander Salm *et al*., (1994) reported that propagating seedlings by tissue culture is a suitable way for achieving plant material, which are free of disease and having new characteristics. According to high requirements for re-cultivation of damask rose lands, breeding them with native plants and propagating them by tissue culture is the best way to achieve hybrids with excellent characteristics. Therefore, this research work was carried out to investigate the micropropagation of Kashan damask rose × Yasooj aromatic rose (G4×G22), Kashan damask rose (G4) and Yasooj aromatic rose (G22).

II. MATERIAL AND METHODS

2.1 Pre sterilization and sterilization

Shoot tips of Kashan damask rose, Yasooj aromatic rose and their hybrids, was used for microporopagation process (Ghamari Zare, *et al.*, 2001). Pre sterilization was done with water and washing liquid, by brushing the samples and then sterilization was done with two different treatments, according to Table. 1.

Table 1. Sterilization treatments

Code	No.	$HgCl_2$ (0.1%)		NaOCl (1.5%)	
		D. (min)	H. (%)	D. (min)	H. (%)
G4	20	5	80	10	55
G22×G4	22	5	90	10	82
G22	20	5	75	10	75

*G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid, H: Healthiness, D: Duration

Three genotypes of G4, G22 and G22×G4 were sterilized with two different treatments, $HgCl_2$ (0.1%) for 5 min and NaOCl (1.5%) for 10 min. The number of sterilized samples of G4 and G22 was 20 and hybrid G22×G4 was 22. After one month, treatments were evaluated and the best sterilization treatment was recognized.

2.2 Establishment in the shooting media

In this stage, healthy sterilized explants, which had a bud with length of 1- 1.5 cm were selected. Explants of all the samples (G4, G22 and G22×G4) were exposed to 3 different treatments (Table. 2) to recognize the best shooting treatment. Each treatment contained 5 replications and each replication consisted of 5 explants. Complete Randomized Design was used to analyze the shoot proliferation treatments.

Table 2. Shoot proliferation treatments

Media	BA (mg/L)	2ip (mg/L)	IBA (mg/L)
GM3	0.5	0.5	0.1
GM5	3	-	-
GM6	1.5	1.5	0.1

*Base media: MS media (Murishig & Skooj, 1962) + 100 mg/L Ascorbic acid

2.3 Establishment in the rooting media

In this stage, the best genotype in the case of shooting was recognized and transferred to modified MS media (half macro elements, twice phosphate and 100 mg/L Ascorbic acid) with 8 different treatments, 5 replications and 5 explants in each replication. After analyzing data by Complete Randomized Design, the best rooting treatment was recognized and used for all genotypes.

Table 3. Rooting treatments

Treatments	Hormones	Hormones (mg/L)
GMr1	NAA	0.2
GMr2	IBA	0.2
GMr3	NAA	1
GMr4	IBA	1
GMr5	NAA	2
GMr6	IBA	2
GMr7	IBA، GA	1 & 2
GMr8	-	-

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2.4 Establishment of the seedlings in the soil

In order to access compatibility of the seedlings, all rooted seedlings were transferred to plastic pots, which contained soil (cocopeat + perleat + vermicompost with the ratio of 2:1:0.5 respectively). Pots were covered with a transparent plastic and some pores were created on the plastic, gradually. After 20 days, all the plants were compatible.

2.5 Analysis of data

Experiments data entered in the Excel software and analyzed by SAS software, in the form of Complete Randomized Design.

III. RESULTS

2.6 Sterilization

Between all the three genotypes, highest percentage of healthy samples (G22×G4 (90%), G4 (80%) and G22 (75%) was obtained from sterilizing in $HgCl_2$ (0.1 %) solution. Percentage of healthy seeds which sterilized with NaOCl (1.5%) was G22×G4 (82%), G22 (75%) and G4 (55%). In both of the treatments, G22 showed 75% of healthiness. Totally, G22×G4 showed highest percentage of healthiness in compare with other genotypes, in both of the treatments.

2.7 Shoot proliferation

Between all the three genotypes, highest shoot length was obtained from G22 in GM3 media. Comparison of GM5 and GM6 media showed that, G22 and G4 produced better and longer shoots in GM6 media and G22×G4 in GM5 media (Fig.1).

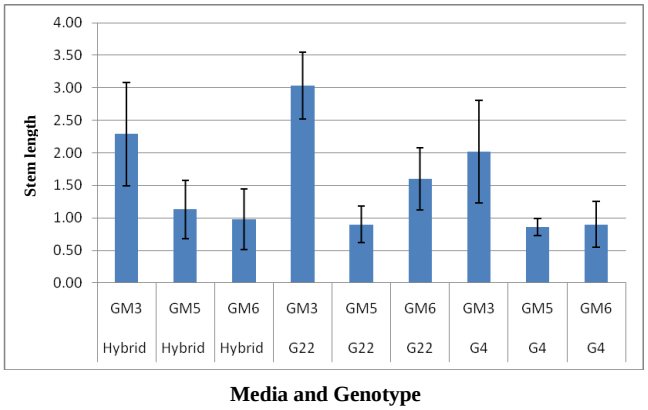

Fig. 1. Effect of different media on shoot length (cm) in G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid rose.

Analysis of variance showed that G4 and G22×G4 had higher shoot proliferation in GM5 and GM6 media, respectively. However, G22 produced more shoots in GM6 and GM5, respectively. All the genotypes produced lowest shoot proliferation in GM3 media (Fig. 2).

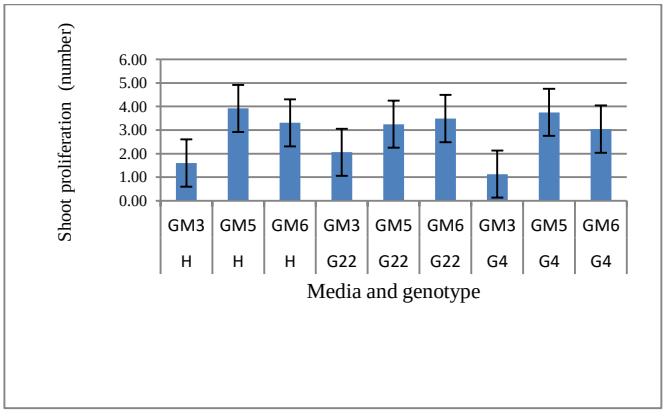

Fig. 2. Effect of different media on shoot proliferation (number.) in G4: Damask rose, G22: Aromatic rose, G22×G4: Hybrid rose.

2.8 Establishment of plant samples in the media

Totally, all the genotypes were established in three different media (Fig.3, 4, 5 & 6) and there was no significant difference between them. G22 showed 100% establishment in all media. G22×G4 showed 100% establishment in GM3 and GM5 and 90% in GM6. G4 showed 100% establishment in GM5, 80% in GM6 and 68% in GM3.

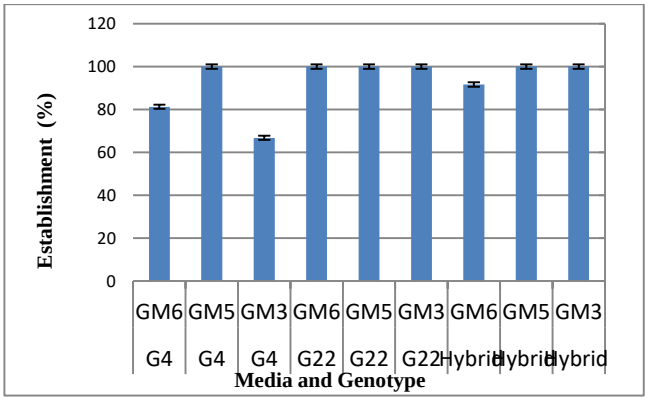

Fig. 3. Effect of three different media as main factor and three genotypes of roses as secondary factor on soil establishment

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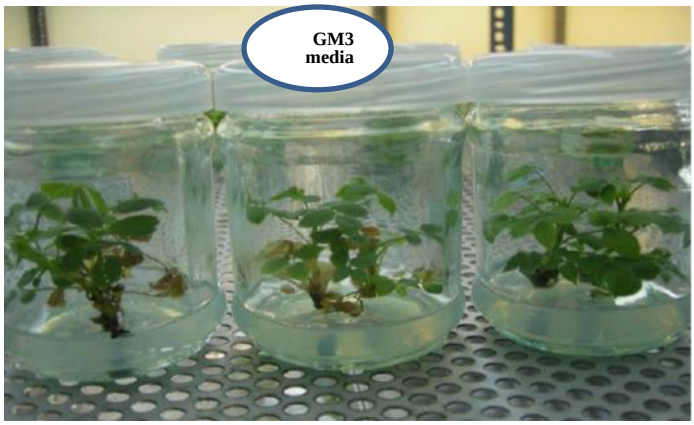

Fig. 4. Establishment of G22, G4 and G22×G4 on GM3 media for shoot proliferation

Fig. 5. Establishment of G22, G4 and G22×G4 on GM5 media for shoot proliferation

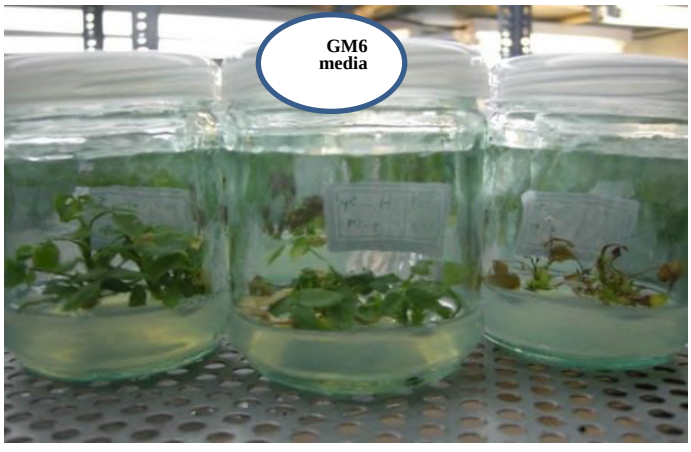

Fig. 6. Establishment of G22, G4 and G22×G4 on GM6 media for shoot proliferation

2.9 Rooting of plant samples in the media

According to the results of shoot proliferation, it was shown that aromatic rose (G22) had the best shooting. So, it was cultured in 8 different media to recognize the best rooting media. G22 produced 100% roots in GMr4, GMr5 and GMr6 rooting media, in GMr1 83%, in GMr2, GMr3 and GMr8 50%. There was no rooting in GMr7 media (Fig.7). Speed of rooting was faster and its density was higher in GMr6 in compare with GMr4 and GMr5 (Fig.8). On the base of results, GMr6 showed best rooting. So, rooting of G22, G4 and G22×G4 were compared in GMr6 rooting media. G22 and G22×G4 were rooted 100% and 28%, respectively. However, G4 did not produce any root (Fig.9).

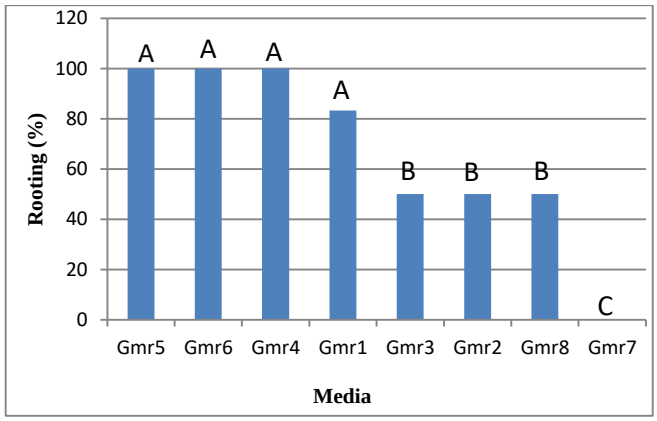

Fig. 7. Effect of 8 rooting media on G22 genotype

G22 G4 G22×G4

Fig. 8. Establishment of G22, G4 and G22×G4 on GMr6 rooting media

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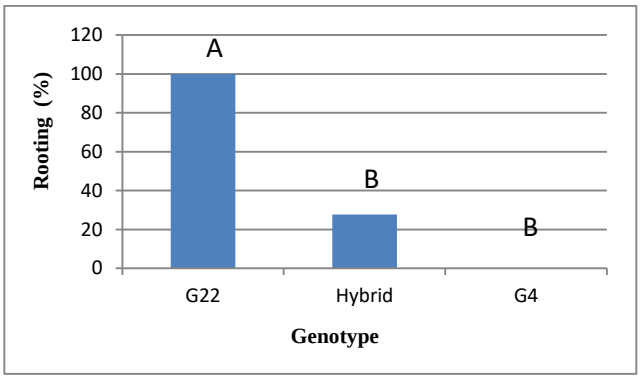

Fig. 9. Rooting of G22, G4 and G22×G4 on GMr6 rooting media

2.10 Establishment of plant samples in the soil

In this stage, rooted seedlings were transferred to plastic pots, which contained soil (cocopeat + perleat + vermicompost with the ratio of 2:1:0.5 respectively). Pots were covered with transparent plastic and some pores created on the plastic, gradually. After 20 days, all the plants were compatible. G22 showed highest incompatibility in the soil (Fig. 10).

Fig. 10. Establishment of G22 in the soil

IV. DISCUSSION

In some research works, success of micropropagation in rose cultivars has been reported (Pati *et al*., 2001 & 2010, Rout *et al*., 1999). Success of these methods for rose cultivars, it is dependent on their species and genetically field. Some cultivars can not grow through in vitro condition and their propagation happens slowly (Kornova & Michailova, 1994). Also, micropropagation of damask rose was done by Khosh – khui and Sink (1982), and because of its problems in shoot proliferation still it is continuing (Kumar *et al*., 1999). Most of the studies are on phyto-chemical aspects, such as methods of extracting the essence and analyzing the essential oils. Therefore, in order to extend the cultivation of damask rose flowers, solving problems of growers and introducing new varieties, which are superior in all aspects of qualitative and quantitative characteristics of flowers, essence and flowering period, this research work was laid out in the Research Institute of Forests and Rangelands. For this purpose, 80 genotypes of damask rose, were collected from different parts of the country and established in the institute. Jabbarzade and Khosh-Khui (2005) reported that explants of *Rosa damasena* Mill. was sterilized with chlorox (10%) for 15 min, NaOCl (1.5%) for 10 min and $HgCl_2$ (0.1%) for 5 min. results showed that in all the studied genotypes, $HgCl_2$ (0.1%) showed better results. Allahverdi *et al.*, (2010) also found that sterilization of Damask rose explants with $HgCl_2$ (0.1%) for 5 min was best treatment, which are in agreement with our findings. Our results, in case of using growth regulators was in agreement with findings of Rout *et al*., (1999), who showed that cytokinins are effective on blossoming buds and BA has significant effect on shoot proliferation in the in vitro condition. Nak-Udom *et al*., (2009) also studied micropropagation of *R. hybrida* L., they cultured the explants on MS media containing 3 mg/l BA and 0.003 mg/l NAA, which produced plantlets with best shoot proliferation and complete rooting. These results are same as our results for G4 and G22×G4, but G22 got its best results in GM6 media with 1.5 mg/l BA, 1.5 mg/l 2ip and 0.1 mg/l IBA. Nikbakht *et al*., (2005) studied the in vitro culture of *Rosa damasena* Mill., species of Azaran and Ghamsar, with 32 treatment levels contained BA (0, 1, 2 & 3 mg/l), GA_3 (0, 0.1, 0.25 & 0.5 mg/l) and NAA (0 & 1 mg/l). Best shoot proliferation was achieved in Azaran species with BA (1 & 2 mg/l), GA_3 (0.1 mg/l) and NAA (0 & 1 mg/l) and in Ghamsar with BA (1 & 2 mg/l), GA_3 (0.1 mg/l) and NAA (0 mg/l). Their results were in agreement with our results in case of G4 genotype, which showed the best results of shoot proliferation with BA treatment. BA has been used for many ornamental plants in laboratory condition. Wang *et al*., (2002), Carelli and Echeverrigary (2002) and Alsemaan (2013), studies micropropagation of *Rosa damasena* Mill., they studied effect of different concentrations of BA (0, 0.5, 1 & 2 mg/l) and GA_3 (0, 1 & 2 mg/l) on shoot proliferation and concluded that BA (2 mg/l) and GA_3 (2 mg/l) had highest effect for best shoot proliferation. Alseman (2013) used half strength of MS media, 3 gr/l activated charcoal and IBA (0, 1, 2 & 3 mg/l) for rooting and found that IBA (2 mg/l) had highest effect on rooting of plantlets. These results are in agreement with our findings, which showed that in all

three genotypes, BA (3 mg/l) in shoot proliferation and IBA (2 mg/l) in rooting had the best effect. Tabesh *et al*., (2013), used activated charcoal (3 mg/l) for removing phenol compounds of *Rosa damasena*, but we used ascorbic acid (100 mg) instead of activated charcoal. Also, Jabbarzadeh and Khosh-Khui (2005), showed that IBA (0.1 mg/l) and BA (2.5 & 3 mg/l) were best treatment for shoot proliferation of *Rosa damasena* Mill. In our research also, BA (3 mg/l) was best for shoot proliferation, but IBA (2 mg/l) had highest effect for rooting.

V. CONCLUSION

Totally our results showed that, between all three genotype, G22 (mother parent) had the best micropropagation results, G4 (father parent) was the worse one and G22×G4 hybrid was between them. However, in some characters such as stem length, shoot proliferation and rooting percentage, G4 was better than G22. Best rooting was recorded for G22 and G4 did not produce any root.

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