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Seed germination in rose (Rosa spp.) as influenced by pre-sowing treatments and genotypes

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Abstract
Low seed germination is a major problem in commercial rose breeding and in this study we have attempted to address this problem by various pre-sowing treatments across multiple seed parents. The study confirmed the seed germination to be dependent on genotypes. Among the five rose genotypes selected for the present work, maximum seed germination (11.46%) was observed in the genotype 'IIHRR-3-7-12'. Minimum germination (1.06%) was observed in genotype 'IIHRR-4-15-12'. Among the different treatments, maximum seed germination (13.33%) was observed in the treatment where mechanical scarification was attempted.

Keywords: genotypes, rose, germination, seed, dormancy

Introduction
Rose is one of the most important commercial crops worldwide because of its value as an ornamental plant, as a medicinal plant for flower production besides its adaptability to a wild range of habitats (Hornero-Mendez and Minguez- Mosquera, 2000; Uggla, 2004; Winther, *et al*., 2005) [5, 12, 13]. Conventionally, rose is propagated mainly by vegetative methods as stem cutting, layering, budding and grafting (Pati *et al*., 2006) [8]. However seed germination is a pre-requisite for hybridization in a breeding program. Seed germination is essentially required in rose hybridization program to develop new varieties and rootstocks. Most of the commercial rose varieties do not set seed. Seed setting ability varies among genotypes and attempts to enhance seed set starts from selection of parents to method of crossing.
The structure of the rose seed is botanically defined as an achene (Pipino *et al*., 2013) [9]. Fertilized flowers will develop in to fruits, which are called hips (Reddy and Nagaraju, 2004) [11] and every rose hip will have multiple achenes. Rose seeds do not germinate easily due to inherent seed dormancy problem. Reasons responsible for seed dormancy ranges between endogenous and exogenous dormancy (Bosco *et al*., 2015) [4], which requires more time and expensive treatments to overcome these problems (Zlesak 2006; Pipino *et al*., 2011) [16, 10]. Since seed dormancy is incompletely understood, significant experimentation is necessary to determine treatments that can give maximum germination. In this study we mainly addressed seed germination problem from its genetic background. Removal of hard seed coat that is an impediment for seed germination along with possible hormonal support was evaluated for their ability to resolve seed germination problem.

Materials and Methods
The present investigation entitled effect of pre-sowing treatments and genotypes response on seed germination was carried out at ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta Lake Post, Bengaluru, situated at an altitude of 890 meter above mean sea level and latitude 12^0 58´ north latitude, 78^0 45´ east longitudes respectively. Removal of hard seed coat either by mechanical clipping or softening the seed coat by mechanical scarification were attempted in combination with GA_3.
The experiment was laid out in Factorial Completely Randomized Design (FCRD) with 25 treatments and three replications per-treatment. Each replication comprised of 25 seeds. Genotypes were considered as one factor and the second factor was different seed treatments. Treatment of mechanical scarification of seeds was attempted by stirring the seeds in mixer grinder, these seeds were further clipped by secature to expose the embryos by removing one

side of the seed coat. Prior to sowing, seeds were treated with 1 per cent sodium hypochloride for five min + 3 per cent H_20_2 for two days as a precaution to avoid any fungal infection. In order to understand the need of GA_3, the seeds were set for germination with or without 1000 ppm of GA_3 in combination with clipping.

To compare the different seed treatments, a control was used that consisted of regular practice being followed at ICAR-IIHR rose laboratory where the experiment was conducted. This control involves pre sowing treatment steps of keeping the seeds in hot water for two days then subsequently shifting of seeds to water with one per cent charcoal followed by GA_3 1000 ppm for two days.

Table 1: Genotypes and pre-sowing treatments used in rose seed germination studies

Factor A Genotypes	G_1: IIHRR-3-7-12 G_2: Crifty Duty G_3: Care Free Beauty G_4: Berry N' Cream G_5: IIHRR-4-15-12
Factor B Pre-sowing treatments	T_1: Mechanical scarification T_2: Mechanical scarification + Clipping T_3: Mechanical scarification + 1000 ppm GA_3 T_4: Mechanical scarification + Clipping + 1000 ppm GA_3 T_5: Control

Open pollinated seeds of five rose genotypes 'IIHRR-3-7-12', 'Crifty Duty', 'IIHRR-4-15-12', 'Berry N' cream' and 'Care Free Beauty', were used for seed germination studies. After harvesting of hips, the seeds were extracted by cutting open the hips into two halves and seeds were scooped out by forceps. For mechanical scarification, seeds were soaked in water for two days and subjected to mild stirring using mixer grinder. Clipping was done by cut opening of individual seeds with secature. Depending upon the treatment choice, seeds were soaked in 1000 ppm GA_3 either soon after mechanical scarification or followed by clipping after mechanical scarification. After the pre-sowing treatments, seeds were sown in polythene cover filled with mixture of cocopeat and perlite in the ratio of 1:1. Covers were sealed and kept at constant temperature of 15^OC. Seed germination was recorded till four months after sowing.

Table 2: Description of the rose genotypes utilized for seed germination studies

Name of the cultivar	Plant growth habit	Flower type	Number of petals	Number of colours in Petal	Colour of the majority portion of the petal	Flower diameter (cm)	Flower fragrance	Size of Hip	Hip shape of longitudinal section	Hip colour
Crifty Duty	Intermediate	Semi-double	Medium (20-30)	One (single)	Red purple group 68-A	Large (8.1-10.0)	Absent	Large	Pitcher shaped	Yellow
Berry N' Cream	Intermediate	Semi-double	Few (<20)	One (single)	Red purple group 73-B	Medium (6.1-8.0)	Absent	Medium	Pitcher shaped	Yellow
Care Free Beauty	Semi-upright	Semi-double	Few (<20)	Two (double)	Red purple group 68-B	Medium (6.1-8.0)	Absent	Large	Pitcher shaped	Orange
IIHRR-3-7-12	Bed	Single	Few (<20)	One (single)	White group 55-B	Large (8.1-10.0)	Absent	Medium	Pitcher shaped	Brown
IIHRR-4-15-12	Bed	Semi-double	Few (<20)	One (single)	Red purple group N 57-C	Medium (6.1-8.0)	Absent	Large	Pitcher shaped	Brown

Result and Discussion

The results with respect to seed germination percentage in five rose genotypes with different treatments and their interaction effects are presented in Table 3. There was no seed germination observed in control. Significant variation was noticed among genotypes and seed treatments, as well as among their interaction effects between genotypes and seed treatments. One of the factors contributing to poor germination in rose is the genetic composition of the seed (Anderson and Byrne, 2007). Our study clearly indicated the genotypes having differential performances. Among the five genotypes, maximum seed germination (11.46%) was observed in the genotype IIHRR-3-7-12 and a minimum germination (1.06%) was observed in genotype of IIHRR-4-15-12. Pre-sowing treatments were found to have significant influence on seed germination percentage. Among the pre-sowing treatments, maximum seed germination (13.33%) was observed in T_1 (Mechanical scarification).

Table 3: Effect of various seed treatments on germination percentage of five rose genotypes

Treatments	G_1	G_2	G_3	G_4	G_5	Mean
T_1	41.33 (39.77)	6.66 (12.42)	0.00 (0.57)	16.00 (23.57)	2.66 (7.88)	13.33 (16.76)
T_2	0.00 (0.57)	0.00 (0.57)	2.66 (7.88)	0.00 (0.57)	0.00 (0.57)	0.53 (2.03)
T_3	0.00 (0.57)	0.00 (0.57)	24.00 (29.15)	16.00 (23.57)	0.00 (0.57)	8.00 (10.89)
T_4	16.00 (23.47)	2.66 (7.88)	12.00 (20.02)	2.667 (7.88)	2.66 (7.88)	7.20 (13.44)
T_5	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)	0.00 (0.57)
Mean	11.46 (12.99)	1.86 (4.40)	7.73 (11.65)	6.93 (11.15)	1.06 (3.49)	
	S. Em ±		C.D. @ 5%		C.D. @1%	
Genotypes (G)	1.52		3.27		4.36	
Treatments (T)	1.52		3.27		4.36	
G xT	3.40		7.31		9.76	

Note: Analysis is based on values of arc sine transformation. Values in parenthesis represent arc sine transformation values.
T_1: Mechanical scarification, T_2: Mechanical scarification + Clipping, T_3: Mechanical scarification + 1000 ppm GA_3, T_4: Mechanical scarification + Clipping + 1000 ppm GA_3, T_5: Control.

Germination might be prevented by inhibitors like ABA in the seed coat, as well as by mechanical hindrance by the pericarp **(**Nadeem *et al*., 2013) [7]. Gibberellic acid is known to break seed dormancy and to enhance germination (Hosafci *et al*., 2005; Pipino *et al.,* 2011 and Zhang *et al.,* 2016) [6, 10, 15]. We experimented by mechanically removing the seed coat and in combination with pre-treatment of seed with gibberellic acid. With respect to various seed treatments, T_1 (Mechanical scarification) gave highest seed germination in all genotypes except in 'Care Free Beauty'. In 'Care Free Beauty', seed did not germinate in T_2 (Mechanical scarification + clipping), rather germination was highest in T_2 (Mechanical scarification + clipping). Attempts were made by different workers to remove the seed coat by chemical (Bhanuprakash *et al.,* 2004 and Younis *et al.,* 2007) [3, 14] and also by mechanical means. Our study also indicated the essentiality of mechanical scarification. We failed to obtain germination in control where no mechanical scarification was attempted. For majority of the genotypes, mere mechanical scarification to remove the impediment caused by hard seed coat could result in better germination. In *Rosa spp*. the mature embryo is protected by a seed coat called the testa, which forms the actual seed coat and is enclosed by the woody and hard pericarp (Zlesak, 2006) [16]. This woody pericarp covering the rose seeds prevents water absorption and air diffusion of the seed and at same time is a physical barrier to embryo expansion.

In addition to mechanical scarification, genotype specific pre-sowing treatments could further enhance seed germination as evidenced in our study. By adopting genotype specific pre-sowing treatments, seed germination could be enhanced. With respect to individual genotypes, IIHRR-3-7-12 genotype recorded 41.33% seed germination in T_1 (Mechanical scarification), Crifty Duty genotype recorded 6.66% seed germination in T_1 (Mechanical scarification), Care Free Beauty genotype recorded 24.00% seed germination in T_2 (Mechanical scarification + clipping), Berry N Cream genotype recorded 16.00% seed germination in T_1 (Mechanical scarification) and IIHRR-4-15-12 genotype recorded 2.66% seed germination in T_1 (Mechanical scarification).

Germination of rose achene is a challenging task due to the presence of endogenous and exogenous dormancy (Hosafaci *et al*., 2005 and Alp *et al.,* 2010) [6, 1]. Poor seed germination is a problem for rose hybridizers. Results indicated the hardness of seed coat as the major impediment that could be addressed by mechanical scarification and clipping of the seed coat. We could establish the necessity of preferential genotype and genotype specific pre-sowing treatments as essential pre-requisites before initiating rose hybridisation program.

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