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Improved technique for treating seed dormancy to enhance germination in Rosa × hybrida

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Abstract: Rose breeders need reliable and efficient germination protocols to exploit all viable embryos in their breeding programs. In this study, different treatment combinations were assessed to overcome the mechanical resistance of the pericarp and enhance germination of rose seeds obtained from hybridisation among hybrids roses. In the 3 treatments, 30 days of a warm temperature followed by 60 days of cold stratification and 30% sulphuric acid treatment for 10 min proved more effective in getting higher seed germination (18.54%) and seed vigour index (261.18) of the progenies. This led to lower germination (18.20 days) and imbibition (23.64) periods with respect to the other 3 treatments including the control. This treatment also helped increase the total length of progeny (14.1 cm) and number of leaves per progeny (6.44), whereas the response of other growth parameters of progeny was variable to this treatment. Correlation (r) between pericarp thickness and seed germination percentage, germination period, and imbibition period proved highly significant (r = 0.347, P < 0.05).

Key words: Rosa × hybrida, dormancy, achene, germination, stratification

1. Introduction

Roses (Rosa spp.) are one of the most important flower crops in the world and have an economic value in ornamental, pharmaceutical, and cosmetic trade. Growing roses from seeds is the oldest form of propagation, but it is necessary to germinate seeds through cross-pollination, or the "mating" between 2 rose species. Seed germination is a much more important issue for getting brand new, never before seen roses that are produced from seeds after successful crosses. In spite of performing many precautions and treatments, rose seed germination is achieved less often, as several factors contribute to successful germination. In order to get good percentages of seed germination, it is necessary to break the seed dormancy, which is a complicated process and results in changes in the pericarp, testa, and embryo. The extent of dormancy and level of dormancy control differs among species, varieties, and seed lots, and even among hips within a single bush (Meyer, 2008). Rose seeds normally show dormancy at maturity due to hard pericarp, inhibitors in pericarp and testa (Zhou et al., 2009), and physiological hindrances in the embryo (Jackson & Blundell, 1963; Densmore & Zasada, 1977; Bo et al., 1995). Even high concentrations of abscisic acid (ABA) are present in the pericarp and testa of rose seed, which may block germination (Bo et al., 1995). The germination

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of seeds in the genus Rosa is not easy due to the presence of endogenous and exogenous dormancy (Baskin & Baskin, 1998; Hoşafaçı et al., 2005; Alp et al., 2008; Werlemark et al., 2009). De Vries and Dubois (1987) and Gudin et al. (1990) revealed that limited germination of rose achenes, due to pericarp and endocarp thickness, can be controlled by factors such as temperature during maturation of achenes and genetic factors. Zhou et al. (2009) suggested that every treatment for breaking dormancy is less effective alone than in combination, i.e. scarification treatment with sulphuric acid and cold stratification (Densmore & Zasada, 1977; Bhanuprakash, 2004; Zlesak, 2005). Many studies have been conducted on other crops for seed germination responses including Orchis galilaea (Houri et al., 2012), Mediterranean woody species (Çatav et al., 2012), mustard (Sarkar et al., 2012), safflower (Namdjoyan et al., 2012), dahlia (Tariq et al., 2012), Tuscan populations of Fritillaria montana (Mancuso et al., 2012), and Ludwigia species (Oziegbe & Faluyi, 2012), but less attention is paid to the seed germination studies in roses. There is thus a need to further investigate the effect of different treatments in combinations to enhance the germination and ultimately for the rescue of the hybridisation product. The purpose of this study was to develop methods to increase germination percentage, shorten germination time, provide more

synchronous germination, and result in more efficient seed propagation techniques for rose seeds.

2. Materials and methods

Nine hybrid rose cultivars collected from different nurseries in Pattoki, Pakistan, namely Autumn Sunset (V1), Iceberg (V2), Paradise (V3), Angel Face (V4), Casino (V5), Louise Odier (V6), Grand Margina (V7), Handel (V8), and Gruss an Teplitz (V9), were used for this study. The included cultivars were selected because they have good hip-bearing and seed-producing ability in this geographical region. In 2007, all the cultivars were planted in the field area of Rose Project, University of Agriculture, Faisalabad, and they were established in the field for 2 years. In 2009, a hybridisation program was performed with a diallel cross to obtain seeds for possible hybrids with new contrasting characters. Afterwards, obtained hips from new successful crosses were harvested when their colour was turning to brown followed by yellow (Meyer, 2008). Harvested hips were weighed and stored at room temperature to dry (Figure 1a). Twenty days later seeds were extracted manually from hips (Figure 1b), and the

length and width of achenes and pericarp were measured by Vernier calliper (Table 1).

Three different treatment combinations were assessed to overcome the mechanical resistance of the pericarp and enhance germination including control (T_0), following the procedures described by Svejda (1968). For the control (T_0), seeds were kept at room temperature (24 ± 2 °C) for 90 days. The following 3 treatments were applied on the obtained seeds to enhance seed germination:

 T_1 - 30 days at room temperature, then chilling temperature (4 °C) for 60 days.

 $\rm T_2$ - 90 days at room temperature + 30% $\rm H_2SO_4$ for 10 min.

 $\rm T_3$ - 30 days at room temperature, then 60 days at chilling temperature (4 °C), and finally treated with 30% $\rm H_2SO_4$ for 10 min.

All treated seeds were washed with distilled water. The seeds were also dipped into water; floating seeds were considered dead and were discarded, leaving only viable sunken seeds. Seeds from all treatments were sown 3 times in the germination media consisting of peat moss and sand (3:1), by using the randomised complete block design



Figure 1. A- Hips of V3 × V1, B- achenes of progenies, C- progenies in pots, D- blooming offspring.

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Genotypes	Hip fresh wt.	Seed dry wt.	Length of seed	Width of seed	Pericarp thick.
$\frac{+\times 0}{V_3 \times V_7}$	4 17 + 0 67	20 57 + 2 27	4 87 + 0 25	2.67 ± 0.10	0.54 ± 0.05
$V_3 \times V_7$ $V_3 \times V_8$	4.17 ± 0.07 4.37 ± 0.15	20.37 ± 2.27 19 30 ± 0.85	4.67 ± 0.23 4.53 ± 0.58	2.07 ± 0.10 2.71 ± 0.06	0.54 ± 0.03
$V_{0} \times V_{0}$	4.37 ± 0.13	17.50 ± 0.85	4.35 ± 0.35	2.71 ± 0.00 2.55 ± 0.03	0.50 ± 0.05
$V_{0} \times V_{0}$ $V_{0} \times V_{3}$	4.53 ± 0.01	17.50 ± 0.00 19.67 ± 0.47	4.77 ± 0.13	2.33 ± 0.03	0.58 ± 0.03
$V0 \times V3$ $V1 \times V9$	4.55 ± 0.15	19.07 ± 0.47	4.87 ± 0.25	2.71 ± 0.00	0.58 ± 0.05
VIX VO	3.93 ± 0.13	18.10 ± 0.30	4.43 ± 0.23	2.33 ± 0.04	0.38 ± 0.03
$V_{0} \times V_{1}$	4.13 ± 0.13	18.70 ± 0.36	4.00 ± 0.30	2.60 ± 0.08	0.59 ± 0.12
V9 × V8	3.43 ± 0.06	17.67 ± 1.27	3.90 ± 0.20	2.49 ± 0.04	0.52 ± 0.06
$V4 \times V8$	4.40 ± 0.10	18.90 ± 2.02	4.57 ± 0.31	2.54 ± 0.02	0.62 ± 0.07
$V4 \times V1$	4.50 ± 0.10	18.27 ± 1.59	4.73 ± 0.32	2.54 ± 0.01	0.63 ± 0.06
$V8 \times V9$	4.63 ± 0.06	21.07 ± 1.57	4.93 ± 0.15	2.65 ± 0.08	0.66 ± 0.11
$V4 \times V9$	4.27 ± 0.06	18.33 ± 3.36	4.10 ± 0.44	2.58 ± 0.03	0.55 ± 0.01
$V8 \times V6$	4.60 ± 0.10	21.27 ± 2.85	4.83 ± 0.25	2.70 ± 0.02	0.62 ± 0.02
$V1 \times V5$	3.33 ± 0.15	18.33 ± 1.15	3.60 ± 0.20	2.42 ± 0.01	0.50 ± 0.05
$V5 \times V8$	3.63 ± 0.23	19.57 ± 1.18	4.63 ± 0.23	2.72 ± 0.05	0.52 ± 0.06
$V9 \times V7$	3.53 ± 0.15	17.70 ± 0.79	3.80 ± 0.10	2.44 ± 0.10	0.56 ± 0.04
$V6 \times V1$	3.87 ± 0.35	18.17 ± 1.33	4.60 ± 0.36	2.66 ± 0.10	0.59 ± 0.05
$V3 \times V1$	4.13 ± 0.06	18.30 ± 1.15	4.23 ± 0.31	2.59 ± 0.17	0.62 ± 0.06
$V7 \times V8$	4.50 ± 0.10	19.77 ± 1.46	4.00 ± 0.17	2.65 ± 0.09	0.52 ± 0.06
$V7 \times V9$	4.50 ± 0.26	20.57 ± 2.00	3.83 ± 0.15	2.67 ± 0.12	0.60 ± 0.05
$V4 \times V5$	4.40 ± 0.10	21.27 ± 2.65	4.53 ± 0.21	2.48 ± 0.12	0.52 ± 0.06
$V6 \times V7$	4.50 ± 0.20	20.93 ± 1.56	4.67 ± 0.15	2.58 ± 0.13	0.54 ± 0.09
$V1 \times V3$	3.40 ± 0.10	17.97 ± 1.33	3.73 ± 0.21	2.48 ± 0.06	0.53 ± 0.09
$V9 \times V1$	3.57 ± 0.12	19.53 ± 0.49	3.50 ± 0.10	2.52 ± 0.04	0.55 ± 0.01
$V1 \times V6$	3.40 ± 0.10	17.67 ± 0.64	3.60 ± 0.26	2.47 ± 0.05	0.48 ± 0.07
$V7 \times V1$	4.33 ± 0.06	20.57 ± 2.90	4.70 ± 0.20	2.55 ± 0.10	0.61 ± 0.02
$V1 \times V4$	3.30 ± 0.10	17.53 ± 0.85	3.57 ± 0.15	2.54 ± 0.11	0.54 ± 0.02
$V8 \times V9$	4.60 ± 0.10	18.70 ± 1.13	4.27 ± 0.06	2.63 ± 0.06	0.57 ± 0.01
$V6 \times V3$	4.37 ± 0.12	19.47 ± 1.06	4.67 ± 0.15	2.65 ± 0.09	0.61 ± 0.05
V6 x V8	4.20 ± 0.00	18.77 ± 1.35	4.27 ± 0.06	2.65 ± 0.02	0.58 ± 0.03
$V8 \times V1$	4.53 ± 0.25	16.63 ± 4.86	5.10 ± 0.10	2.73 ± 0.02	0.59 ± 0.02

Table 1. Morphological parameters of hips and achenes measured before applying treatment.

LSD (5%) = 0.425.

statistical scheme. Seeds were finally transplanted in pots with similar media (Figure 1c) and started blooming after 2 months (Figure 1d). Germination of seeds was monitored regularly and pots were irrigated with distilled water. Seedlings were considered germinated when the cotyledon and hypocotyl had grown to 0.5 cm above the media surface. Germination percentage and germination period were determined along with imbibition period, which was recorded as the number of days from sowing to start of the germination of the achenes. Seed vigour index of the progenies was estimated according to Abdul-Baki and Anderson (1973) as germination percentage × seedling total length, i.e. total shoot and root length. Performance of genotypes/progenies (seedlings) against each treatment was also estimated by measuring morphological parameters including shoot length (cm), root length (cm), seedling total length (cm), root to shoot ratio for length, internodal distance, and number of leaves per seedling.

2.1. Statistical analysis

Data were analysed using statistical software Minitab 15. Analysis of variance was performed to estimate the significance of treatments for different parameters. Means were compared by a least significance difference (LSD) test at a probability level of 0.05. The Pearson correlation coefficient (r) was also computed to check the dependency of variables.

3. Results

Results related to physiological parameters revealed that the germination period (F = 89.95, P < 0.05), germination percentage (F = 67.16, P < 0.05), imbibition period (F = 26.13, P < 0.05), and seed vigour index (F = 112.2, P < 0.05) were affected significantly by all treatments. Growth parameters including internodal distance (F = 4.42, P < 0.05), shoot length (F = 32.99, P < 0.05), root length (F = 34.79, P < 0.05), and seedling total length were also significantly affected. However, treatments had no significant effect on the number of seedling leaves (F = 2.26, P > 0.05) and root/shoot ratio (F = 0.38, P > 0.05). Comparison of the means related to the effect of different treatments to enhance seed germination showed that germination and imbibition periods were reduced to 18.20 and 23.64 days, respectively, in seeds treated with 30 days of warm (24 ± 2 °C) stratification + 60 days cold stratification + 30% H_2SO_4 treatment for 10 min (T₂), as compared to the seeds kept at room temperature (24 \pm 2 °C) for 90 days (T₀) (23.82 and 27.32, respectively). However, treatment of 30 days at room temperature + 4 °C for 60 days (T_1) and 90 days at room temperature +

30 % H_2SO_4 for 10 min (T₂) had quite a similar effect on these parameters (Figure 2a). T₃ was again found to be better to accelerate the seed vigour index to 261.18 (Figure 2b) and germination percentage to 18.54% (Figure 2c), as compared to control (T₀) at room temperature at 24 ± 2 °C for 90 days. Growth attributes like shoot length, root length, total seedling length, and internodal distance were greater among seeds that germinated earlier, as they started early growth and accumulated more biomass compared to seeds that germinated later, where maximum shoot length (7.40 cm), root length (6.74 cm), seedling total length (14.10 cm), and internodal distance (1.49 cm) were observed at T_{2} (30 days warm (24 ± 2 °C) stratification + 60 days cold (4 °C) stratification + 30% H_2SO_4 treatment for 10 min) as compared to all other treatments (Figure 3a). Treatments T_1 and T_2 showed the same response to generate the number of leaves in progenies (Figure 3b). Root/shoot ratio for length was higher under the influence of treatment T₂, producing a root/shoot ratio of 0.931, followed by treatment T_3 (Figure 3c).

Similar to germination percentage, genotypes (progeny) were significantly affected for seed vigour index, germination period, and imbibition period. A minimum germination period consisting of 18.53 days and imbibition period of 24.08 days were observed in the progenies of V1 × V5 and V9 × V1, in contrast to V6 × V1 and V8 × V1 with the maximum germination and imbibition periods of 22.08 and 29 days, respectively (Figure 4). However, a maximum seed vigour index was exhibited by the progeny of V1 × V6 (286), followed by V7 × V9 (256) and V3 × V4 (247), whereas minimum seed vigour index was possessed



Figure 2. Performance of genotypes against all treatments for: a- seed germination and imbibition period, b- seed vigour index, and c- seed germination percentage.

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Figure 3. Contribution of all treatments for: a- internodal distance, shoot, root, and total length; b- number of leaves; and c- root/ shoot ratio for seedling length.

by the genotype of V4 × V5 (98) (Figure 5a). Maximum germination percentage (18.83%) was observed in the progeny of V6 × V8, followed by the progeny of V9 × V1 with a germination percentage of 17.22%. Minimum germination percentage (12.92%) was observed in progeny of V4 × V5 against all treatments (Figure 5b).

Results regarding morphological parameters showed that treatments had significant effects (P < 0.05) for seedling total length, length of shoot, and length of root (Figure 6). Maximum total length (18.558 cm) of seedlings was yielded by the progenies of V1 × V6 and minimum by V4 × V5 against all treatments. Overall, seeds treated with 30 days of warm and 60 days of cool temperature and 30% sulphuric acid (T₃) showed maximum total length (14.107 cm). Interaction between genotypes (progenies) and treatments was also significant in the case of germination percentage (F = 1.61, P < 0.05), germination period (F = 2.27, P < 0.05), and imbibition period (F = 1.91, P < 0.05), where interaction between T₃ and V9 × V7 resulted in maximum germination percentage (24.3%), followed by V1 × V8 yielding 22% germination (Figure 7). For

germination period, interaction between treatments and genotypes revealed that maximum germination period (28.333 days) was observed in progenies of V6 \times V1 at T_o , while V1 × V5 took fewer days (16) for germination at T_3 (Figure 8). For imbibition period, T_3 and progenies of V6 \times V4 took 33 days to imbibe, which is maximum as compared to the interaction effect of T_4 and $V9 \times V3$, in which seeds imbibed only for 18 days, which is the minimum for imbibition period (Figure 9). However, for seed vigour index, number of leaves, shoot length, root length, total seedling length, internodal distance, and root/ shoot ratio for length, interactions were not significant. Pericarp thickness was significantly correlated (r = 0.347, P < 0.05) with seed germination percentage. However, length of seed, width of seed, and hip fresh weight did not show significant correlation with germination percentage, as presented in Table 2.

4. Discussion

Germination of rose achene is a bit of a challenging task due to the presence of endogenous and exogenous dormancy



Figure 4. Response of genotypes for imbibition and germination period as affected by treatments.



Figure 5. Response of genotypes for: a- seed vigour index and b- germination percentage as affected by treatments.



Figure 6. Growth parameters of seedlings as affected by various treatments.



Figure 7. Interaction plot for germination percentage against all treatments.

(Baskin & Baskin, 1998; Hoşafaçı et al., 2005; Alp et al., 2008; Werlemark, 2009). Proper storage conditions and any treatment given to the achene to regulate the physiological and morphological conditions of seeds are important to consider. Storage life of rose seeds is much less as the

viability of seed is affected by duration of storage and dry period (Crocker & Barton, 1931). After applying several amendments to the storage temperature and pericarp loosening, it was clear that there is variable response of progenies to germination potential of seeds as the extent



Figure 8. Interaction plot for germination period for all treatments.

of dormancy and level of dormancy control differs among species, varieties, seed lots, and even among hips within a single bush (Meyer, 2008).

In this study, germination might be prevented by inhibitors like ABA in the seed coat, as well as by mechanical hindrance by the pericarp. The improvement in germination percentage from 12.7% to 18.5% in the achene seemed to be due to the activation of embryo by chilling temperature (4 °C), as mentioned by Hartmann and Kester (1975) after storing at room temperature, and to loosening of the pericarp and testa by acid treatment as argued by Younis et al. (2007) for a period 10 min in 30% H₂SO₄. The results of the present study support the findings of Rober and Shardlow (1979) and Zlesak (2005), who found similar results. Results related to effect of pericarp thickness on seed germination percentage also showed that there was a positive correlation (r = 0.247, P < 0.05) between these 2 variables. These results are similar to the findings of Vasil'eva (2009), who found that germination of the genus Rosa can be enhanced by scarification and stratification to a better value. In the case of Rosa canina,

it was 50% because cracking the pericarp alone does not remove dormancy (Tincker & Wisely, 1935). Moreover, duration of scarification and stratification treatments also affects germination rates.

There is another hypothesis that seed set is the indication of successful fertilisation, but seeds may or may not reach maturity alive (Bo et al., 1995) and the embryo often deteriorates before the seeds have matured (Fagerlind, 1954), which may cause a loss in germination, i.e. about 81.5%-87.3% in the current study. In that case, achenes appear normal from external morphology, but are actually either empty or contain shrivelled remains (Erlanson, 1931; MacPhail, 2007), and they ultimately fail to germinate or the seedlings might die soon after germination (Svejda, 1974). Some seedlings grow well, but are unable to initiate flowers. Even if one of the F1 seedlings is able to set seeds, the seeds might not germinate or the second generation of seedlings might be sterile. In the present study, warm stratification followed by cool stratification and sulphuric acid was provided to the seeds, and it showed significant results with respect to



Figure 9. Interaction plot for imbibition period of the progenies against all treatments.

	Achene length	Achene width	Pericarp thickness	Seed dry weight	Hip fresh weight
Cormination norcontage	0.053	0.153	0.347	0.032	0.112
Germination percentage	(0.00) **	(0.03) *	(0.00) **	(0.001) **	(0.01) **
Commination noniod	-0.276	-0.331	0.213	0.210	0.021
Germination period	(0.00) **	(0.00) **	(0.00) **	(0.00) **	(0.00) **
Turk : h : h : h : h : h : h : h : h : h :	-0.205	-0.266	0.366	0.331	0.044
inibidition period	(0.004) **	(0.00) **	(0.00) **	(0.002) **	(0.003) **

Table 2. Correlations among various physical and germination attributes. *P < 0.05, **P < 0.01.

seeds that were not treated with a warm period of storage. This appears to confirm the hypothesis of Werlemark et al. (1995) and Zhou et al. (2009), who revealed that warm plus cold stratification alone appeared to be an effective remedy to get rid of dormancy of rose achenes. Seedlings with the maximum total length, number of leaves, and number of branches may be a result of the early germination by the seedlings induced by the method of dormancy breakage. Seed germination of Terminalia ivorensis, as observed by Oni (1991), also had significant relationships between seed morphological characteristics and seedling vigour. These growth parameters are attributed to the promotion of rapid production of vigorous seedlings for nursery establishment or species for plantation establishment (Okunlola, 2011). Galston and Davies (1969) and van Overbeck (1966) stated that seed treatment improves the growth of seedlings due to the interactions between promoters and inhibitors. ABA in the inhibitor interacts with gibberellic acid, which is increased after the inhibitor is removed, to increase growth (Lipe & Crane, 1960). Furthermore, the nutrient level in the growth medium may have also contributed to this increased growth (Okeyo & Ouma, 2008).

By adopting seed treatments to break dormancy, we can rescue valuable germplasm achieved after many years

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of struggle. Among the 3 treatments of the present study, 30 days of warm temperature followed by 60 days of cold stratification and 30% sulphuric acid treatment for 10 min was the best in getting higher seed germination (18.54%) and seed vigour index (261.18) of the progenies, leading to lower germination (18.20 days) and imbibition (23.64) periods compared to the other 3 treatments including the control. This treatment also helped obtain increased total length of progeny (14.1 cm) and number of leaves per progeny (6.44). Correlation (r) between pericarp thickness and seed germination percentage, germination period, and imbibition period proved highly significant (r = 0.347, P < 0.05). Thus, to get rid of seed dormancy, warm stratification followed by cool temperature storage and sulphuric acid treatment is proven to be a good tool for seed germination.

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