Indian J. Hort. 69(3), September 2012: 374-378

Evaluation of rose varieties for pollen efficiency

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ABSTRACT

An experiment was conducted to study the pollen viability, *in vitro* pollen germination, absolute pollen viability, pollen diameter and hip set in ten varieties of rose namely; Africa Star, Century Two, Dr Bharat Ram, Eiffel Tower, Folklore, Gold Medal, Kiss of Fire, Pinata, Pusa Ajay and Sadabahar. Five treatments were used for *in vitro* pollen germination, *viz*., control (distilled water) (T1), 15% sucrose (T2), 20% sucrose (T3), 15% sucrose + 100 mg/l boric acid (T4), 20% sucrose + 100 mg/l boric acid (T5). Ten varieties were crossed with three female parents, *viz*., Queen Elizabeth, Pink Parfait and Raktagandha in the field and hip set was noted. Pollen viability varied from 6.45 (Folklore) to 78.07% (Africa Star) among the varieties. The percentage pollen germination was highest in Dr Bharat Ram followed by Africa Star. The pollen of the varieties Folklore and Pusa Ajay failed to germinate in all the five treatments, neither did they set hip. Higher hip set was recorded with pollination of varieties Africa Star and Dr Bharat Ram. The treatment T2 (15% sucrose) recorded the highest percent of pollen germination compared to other treatments used. Pollen diameter was highest (51.22 µm) in variety Africa Star followed by Dr Bharat Ram (50.21 µm), while it was lowest in Pusa Ajay (25.25 µm). Pollen viability, *in vitro* pollen germination and hip set were positively correlated with pollen diameter. The varieties Africa Star and Dr Bharat Ram were found to be the best pollen donor among the varieties used for screening. Pollen viability percent and pollen diameter were found to be the best parameters for ascertaining the pollen fertility.

Key words: Pollen viability, *in vitro* pollen germination, pollen diameter, hip set, rose.

INTRODUCTION

Rose is one of the most economically important ornamental species used as landscape and cut flower plant in the world. The ability to consistently produce new rose cultivars is essential for the breeder to maintain a market share. Roses are complex hybrids involving inter-specific hybridization, polyploidy (triploid, tetraploid and aneuploid) with high female and male sterility, cytological abnormalities and heterozygous genotypes perpetuated by vegetative propagation. Thus, in an ongoing breeding programme for roses the viability and germinability of the pollen grains of the cultivars, which are to be used as parents should first be checked. Rose breeders often complain about low seed set in the crosses between rose cultivars. This can be caused by, e.g. inadequate pollen germination (De Vries and Dubois, 1) or sub-optimal (too low) temperatures during pollination (Visser *et al*., 6). To investigate pollination potential, estimates should be made of pollen quantity and viability, as well as of pollen germination capability. Pollen tube growth after a certain stage is dependent on nutrients available in the pistil tissue. The most important substance is the sucrose, which has a two-fold effect; as it regulates the osmotic pressure of the pollen grain and is used as a nutrient for the pollen tube growth. For *in vitro* experiments, addition of external minerals and growth regulators such as boric acid, calcium nitrate, potassium nitrate, magnesium sulphate and gibberellic acid can stimulate pollen germination and pollen tube growth (Westwood, 8). Screening of male donor parents for pollen efficiency will provide information on different cultivars of rose for their suitability in breeding programme. It may allow progress towards a better management of hybridization and consequently a means to optimise rose breeding programme. This study was designed to determine the pollen efficiency among different cultivars of rose based on pollen stainability, *in vitro* pollen germination, pollen diameter and hip set.

MATERIALS AND METHODS

Experiments were carried out at the Research Farm, Division of Floriculture and Landscaping, IARI, New Delhi. Ten rose varieties, namely, Africa Star, Century Two, Dr Bharat Ram, Eiffel Tower, Folklore, Gold Medal, Kiss of Fire, Pinata, Pusa Ajay and Sadabahar were used for present study. The experiment was laid out in Randomized Block Design with three replications. Observations were recorded on pollen viability, *in vitro* pollen germination, pollen diameter and hip set.

Pollen grains from each of the varieties under study were collected separately from fully developed

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unopened flower buds and percentage viability was found out by acetocarmine test (Johansen, 2). The germination capacity of fresh pollen grains was assessed with the hanging drop technique (Voyiatzi, 7). Five treatments were used for *in vitro* pollen germination, *viz*., control (distilled water) (T1), 15% sucrose (T2), 20% sucrose (T3), 15% sucrose + 100 mg/l boric acid (T4), 20% sucrose + 100 mg/l boric acid (T5). A drop of germination medium was placed on a cover slip and the pollen was dusted onto the drop. The cover slip then was inverted and placed over a concave depression (cavity) slide, using liquid paraffin to seal the cover slip and prevent desiccation. Three slides per treatment were prepared, each slide with pollen of a different flower. Unless stated otherwise the pH of the medium was between 5.5 and 6.5, and the pollen grain preparations were incubated at 25°C. After 18-24 h, counts for germination were made on each slide under a compound microscope at 20x magnification (Nikon, Japan).

The pollen diameter was measured according to Pipino *et al*. (5). Dry pollen was dusted onto a glass slide without a cover slip. No hydration or embedding was performed, because imbibed pollen swells, resulting in significant changes in pollen diameter and a globular shape. Microscopic observations (80x) were made using a stereo zoom microscope (Carl Zeiss, Germany). To measure the pollen diameter, the major axis (transverse diameter) of the ellipsoidal dry pollen grain was considered. To study the hip set the ten varieties were crossed to three female parents, *viz*., Queen Elizabeth, Pink Parfait and Raktagandha. For emasculation the fully developed flower buds which were likely to open the next day were selected. Pollination was done in the next day morning between 10 AM to 11 AM.

RESULTS AND DISCUSSION

Pollen viability varied from 6.45 to 78.07 per cent in the ten male donor parents (Table 1). Africa Star with 78.07 per cent showed the highest pollen viability followed by Dr Bharat Ram (65.82%) and Pinata (47.06%). The lowest pollen viability was observed in variety Folklore (6.45%) followed by Century Two (12.75%) and Pusa Ajay (13.31%). Kumar (3) reported similar pollen sterility ranging from 78.52 to 92.95% in rose cv. Folklore and its 12 induced mutants. Although various stains have been used in studies of pollen viability, staining may not indicate true viability. One of the drawbacks of pollen viability is due to a certain variation in colour among the pollen after staining, making the decision to what degree of colour to accept as viable is somewhat subjective.

The results of *in vitro* pollen germination are presented in Table 2. In the hanging drop method, none of the cultivars germinated in distilled water (control). In general, all the varieties showed low pollen germination and it did not exceed 12%. These results were in line with findings of Voyiatzi (7) in roses who observed that generally there is a low germination capacity and it didn't exceed 18% with any of the treatments. The percentage pollen germination in Dr. Bharat Ram (11.31%) was significantly higher than all other varieties. The highest percentage of pollen germination (17.56%) in Africa Star was observed in case of treatment T4 (15% sucrose + 100 mg/l boric acid) followed by treatment T2 (15% sucrose). The varieties Folklore and Pusa Ajay failed to germinate in all the five treatments. The treatment T2 (15% sucrose) varied significantly from all other treatments. The treatment T2 (15% sucrose) recorded the highest percent of pollen germination (5.75%) compared to

Table 1. Pollen viability of different male donor parents in rose.

Variety	No. of viable pollen	No. of non-viable pollen	Pollen viability (%)
Africa Star	255.33	72.00	78.07
Century Two	26.33	182.67	12.75
Dr. Bharat Ram	110.00	50.67	65. 82
Eiffel Tower	37.00	117.67	23.90
Folklore	16.00	218.00	6.45
Gold Medal	21.67	56.67	27.01
Kiss of Fire	49.33	122.33	28.36
Pinata	55.67	55.33	47.06
Pusa Ajay	32.00	200.67	13.31
Sadabahar	50.67	91.00	37.15
CD at 5%	43.21	84.22	14.88

Table 2. *In vitro* pollen germination (%) in different male donor parents in rose.

Variety	T1	T2	T3	T4	T5	Mean
Africa Star	0.00	14.76	3.46	17.56	3.18	7.79
Century Two	0.00	0.00	2.33	0.00	0.00	0.47
Dr Bharat Ram	0.00	28.37	3.77	18.42	6.01	11.31
Eiffel Tower	0.00	0.00	2.50	0.00	0.00	0.50
Folklore	0.00	0.00	0.00	0.00	0.00	0.00
Gold Medal	0.00	0.00	2.17	0.00	0.00	0.43
Kiss of Fire	0.00	0.00	0.00	0.00	3.54	0.71
Pinata	0.00	4.33	0.00	0.00	0.00	0.87
Pusa Ajay	0.00	0.00	0.00	0.00	0.00	0.00
Sadabahar	0.00	10.00	0.00	0.00	0.00	2.00
Mean	0.00	5.75	1.42	3.60	1.27	
	Variety (V)		Treatment (T)		V × T	
CD at 5%	0.96		0.68		2.14	

T_1 = Control (distilled water), T_2 = 15% sucrose, T_3 = 20% sucrose, T_4 = 15% sucrose + 100 mg/l boric acid, T_5 = 20% sucrose + 100 mg/l boric acid

all other treatments. This result is in line with findings of Visser *et al*. (6). Treatment T2 (15% sucrose) was followed by treatment T4 (15% sucrose + 100 mg/l boric acid) which recorded 3.56% pollen germination. Positive influence of boron on *in vitro* germination was observed. The efficiency of germination, staining is best assessed *in vitro* by the percentage of normal pollen grains that are found viable. In the present research, the percentage of rose pollen grains that germinated was lower than the percentage of viable pollen assessed by acetocarmine technique. Similar results were obtained by Pearson and Harney (4), and Visser *et al.* (6) in roses. There is influence of medium on *in vitro* germination, but the present study indicates dominating influence of the variety for this parameter. Pollen diameter varied significantly among the ten male donor parents (Fig.1). Effective pollen donors were having the highest pollen diameter. Highest pollen diameter was observed in the variety Africa Star (51.22 µm) followed by Dr Bharat Ram (50.21 µm). Lowest pollen diameter was observed in the variety Folklore (23.90 µm) followed by Pusa Ajay (25.25 µm).

Results of crosses made between varieties varied for hip set. The varieties Africa Star and Dr Bharat Ram were able to produce best hip set (Table 3). This was due to the sound pollen quality and efficiency exhibited

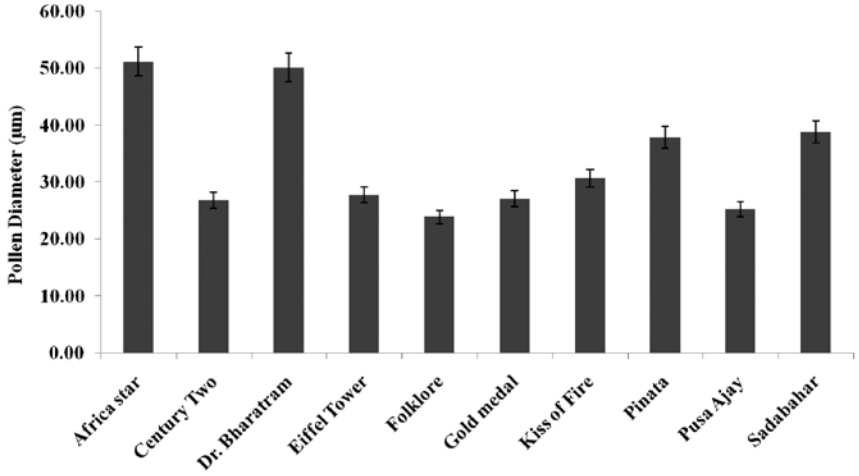

Fig. 1. Pollen diameter of different male donor parents in rose.

Table 3. Hip set (%) with the different male donor parents in rose.

Female (F) / Male parent (M)	Raktagandha	Queen Elizabeth	Pink Parfait	Mean
Africa Star	66.67	86.67	66.67	73.33
Century Two	13.33	26.67	20.00	20.00
Dr Bharat Ram	60.00	86.67	66.67	71.11
Eiffel Tower	20.00	33.33	26.67	26.67
Folklore	0.00	0.00	0.00	0.00
Gold Medal	26.67	40.00	33.33	33.33
Kiss of Fire	26.67	46.67	33.33	35.56
Pinata	40.00	53.33	46.67	46.67
Pusa Ajay	0.00	0.00	0.00	0.00
Sadabahar	33.33	46.67	40.00	40.00
Mean	28.67	42.00	33.33	
	Male (M)	Female (F)	M × F	
CD at 5%	8.42	4.61	NS	

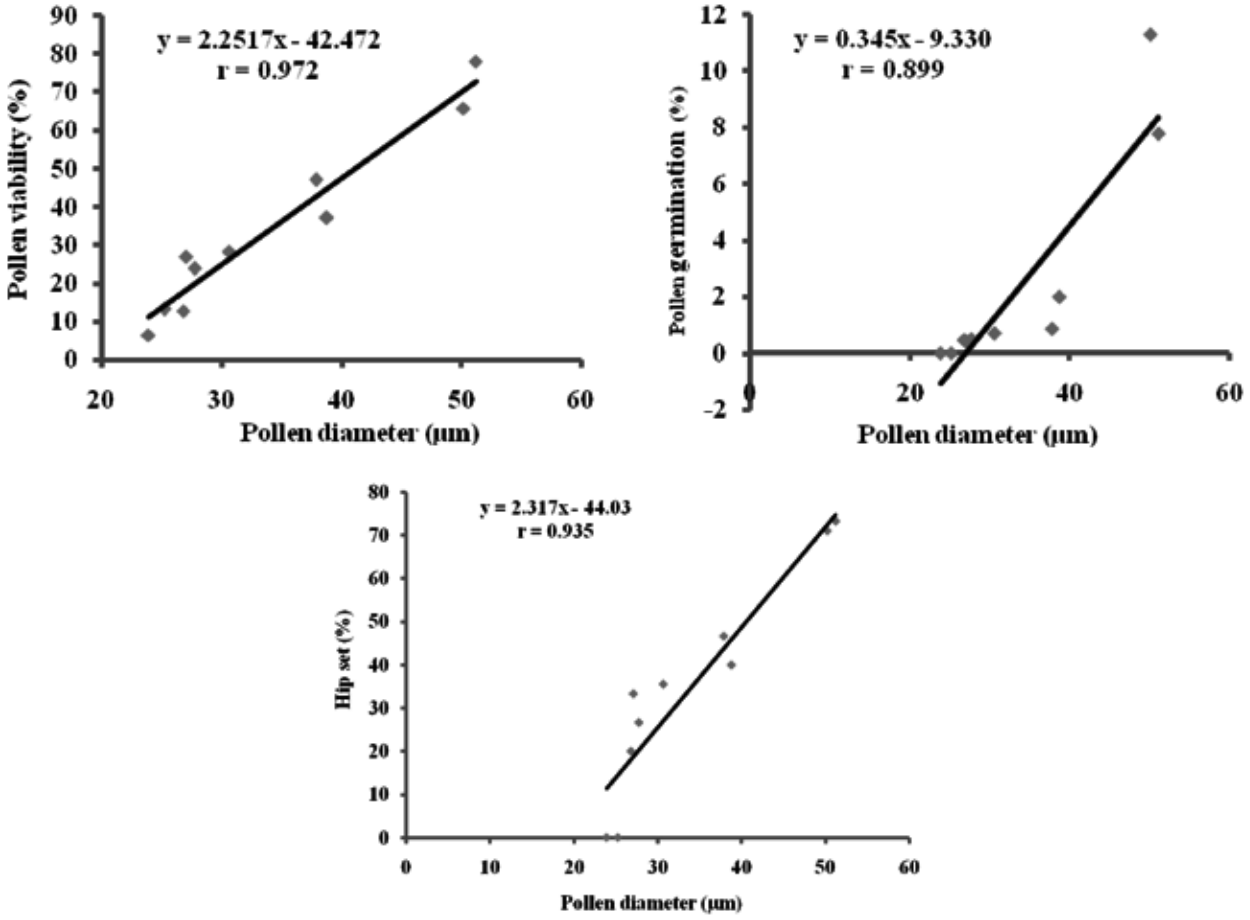

Fig. 2. Correlation between pollen diameter and (a) pollen viability, (b) pollen germination and (c) hip set in rose.

by these two varieties with respect to higher pollen stainability and pollen germination. These results are in line with the earlier findings of Visser *et al*. (6). No hip set was observed with pollen of Folklore and Pusa Ajay with all the three female parents. Mean pollen diameter was observed to be an effective parameter for the evaluation of male fertility. Varieties having pollen size less than 25 µm were not effective in producing a satisfactory hip set. Therefore, in some cases *in vitro* germination does not completely reflect germination capacity *in vivo*. It seems quite typical of Hybrid Tea roses. These observations are in line with the findings of Pipino *et al*. (5) in low fertile rose genotype 2695. The varieties Pusa Ajay and Folklore showed very low pollen stainability, nil *in vitro* germination and consequently no hip set. Visser *et al*. (6) reported that this variation was due to variation in pollen viability rather than incompatibility.

Correlation between pollen diameter and (a) pollen viability, (b) *in vitro* pollen germination and (c) hip set was studied (Fig. 2). High positive correlation (r = 0.972) was observed between pollen diameter and pollen viability. Also there was significant positive correlation (r = 0.899) between pollen diameter and *in vitro* pollen germination. There was a positive correlation (r = 0.935) between pollen diameter and hip set. Zlesak (9) used pollen size to define ploidy levels in roses. However, pollen size is not informative for ascertaining the ploidy level. The present study relates pollen diameter to pollen fertility in roses. Based on the pollen diameter the varieties Africa Star and Dr Bharat Ram can be grouped as cultivars with high pollen fertility. The varieties Century Two, Eiffel Tower, Gold Medal, Kiss of Fire, Pinata and Sadabahar can be grouped as genotypes with medium pollen fertility, while Folklore and Pusa Ajay can be grouped as low pollen fertility types. The present study shows that the evaluation of pollen size can help breeders to select good candidate pollen donor parent. This can potentially increase the number of genotypes used as pollen donor in rose breeding and also help in finding the most valuable progenitors, thus enhancing the efficiency in rose hybridisation.

REFERENCES

1. De Vries, D.P. and Dubois, L.A.M. 1983. Pollen and pollination experiments. X. The effect of repeated pollination on fruit and seed set in crosses between the Hybrid Tea- rose cvs. Sonia and Ilona. *Euphytica*, **32**: 685-89.
2. Johansen, D.A. 1940. *Plant Microtechnique*. McGraw Hill, New York.
3. Kumar, S. 1990. Morphological and cytological studies on some of the induced mutants of cv. 'Folklore'. M.Sc. thesis submitted to IARI, New Delhi.
4. Pearson, H.M. and Harney, P.M. 1984. Pollen viability in Rosa. *HortSci.* **19**: 710-11.
5. Pipino, L., Leus, L., Scariot, V., Giovannini, A. and Van Labake, M.C. 2010. Pollen diameter relates to seed production in cut roses. *Acta Hort.* **870**: 143-46.
6. Visser, T., De Vries, D.P., Welles, G.W.H. and Scheurink, J.A.M. 1977. Hybrid Tea- rose pollen. I. Germination and storage. *Euphytica*, **26**: 721-728.
7. Voyiatzi, C.I. 1995. An assessment of the *in vitro* germination capacity of pollen grains of five tea hybrid rose cultivars. *Euphytica*, **83**: 199-204.
8. Westwood, M.N. 1978. *Temperate - Zone Pomology*. W.H. Freeman Company, New York, pp. 5.
9. Zlesak, D.C. 2009. Pollen diameter and guard cell length as predictors of ploidy in diverse rose cultivars, species, and breeding lines. **In**: *Floriculture and Ornamental Biotechnology*, Vol. 3, Special Issue, D.C. Zlesak (Ed.), Global Science Books Ltd., UK. pp. 53-70.

Received : October, 2011; Revised : December, 2011; Accepted : February, 2012