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DETERMINATION OF POLLEN FERTILITY AND HYBRIDIZATION SUCCESS AMONG *ROSA HYBRIDA*

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Abstract

Breeding among *Rosa* species is a tedious process with low success rate due to presence of variability in pollen fertility and environmental conditions. Introduction of varieties and development of new hybrid lines is direly needed for rose industry in Pakistan, particularly in Pothwar area. Present study was designed to introduce existing varieties to this area and to check their adaptability and pollen fertility for initiating conventional breeding. Experiment was laid out at University Research Farm Koont by using factorial RCBD. Twenty-one varieties were scrutinized for pollen traits to get effective pollen donor parents for improvement of desired traits in locally-adapted cultivars. The number of anthers per flower, pollen viability, germination percentage and pollen diameter were observed for selection of seed setting and pollen donor parents. 30 cross-combinations were formed by use of 3 seed setting and 10 pollen-donor parents. Therefore 10 crosses were performed for each cross combination in this study. Successful hip setting was recorded after doing the recommended pollination practices. Variations in results was found regarding pollen behavior and its success rate in open field. Helen naude and Angel face contain significant number of anthers. Highest pollen viability percentage was observed in "Gruss-an-teplitz" while maximum hip set percentage and seeds were produced by Bora bora (V14) crosses when both were compared to "Gruss-an-teplitz (V21)" and "Midas touch (V6)" crosses. Hip fresh weight positively correlated with number of seeds in each cross combination. Overall Gruss-an-teplitz proved better as female parent and yielded significant hip set percentage after hybridization.

Key words: *In vitro*; Pollen behavior; Conventional breeding; *Rosa* species; Pothwar Pakistan.

Introduction

The history of roses (*Rosa hybrida*) cultivation started about 5000 year ago from ancient China civilization, Western Asia and North Africa due to its aesthetic value (Gudin, 2000). Plantation of millions of roses in garden or pots for production of cut flowers, increases its popularity as an ornamental plant across the globe (Khosh-Khui & Teixeira, 2006). Newly adapted hybrids were evolved in current genome from the old ancestors, having unique characters through historic evolution (Scariot *et al.*, 2006; Cock *et al.*, 2007). For breeding, successful seed production is pre-requisite for evolving new species and rootstocks (Pipino *et al.*, 2013, Bosco *et al.*, 2015). Natural hybridization occurs among polyploid species in Mediterranean region due to favorable geological and climatic conditions, which popularizes it as biodiversity hotspot region (Marques *et al.*, 2018). Evolution and adaptation of new hybrids in modern breeding programs includes controlled pollination, viable pollen parent, effective time of pollination, hip set for seed formation and maturation of viable seed to acquire new hybrid population. So, the selection of male parent with their known fertility status (number of seed produced per cross) is critical to avoid the risk of low seed yield (Zlesak, 2009). Modern rose cultivars (tetraploids) are usually associated with varying level of fertility, which could be due to their primordial interspecific origin (European tetraploids and Asian diploids) as well as intensive inbreeding (Zlesak, 2009; Pipino *et al.*, 2011). Interploidy or interspecific hybridization is a new breeding technique to develop novel fertile progenies with desirable traits, in order to enrich genetic diversity (Chen *et al.*, 2003). Rose (*Rosa hybrida*) remains the main breeding focus in *Rosa*ceae family for achieving desirable characters, post pollination flower performance, problem of seed production and germination (Pipino *et al.*, 2013; Caser *et al.*, 2014; Bosco *et al.*, 2015). Pollen traits including pollen viability, pollen germination and tube growth are important aspect for rose breeding process (Nadeem *et al.*, 2014). Pollen viability percentage just after anthesis is critical phenomenon for successful crossbreeding (Macovei *et al.*, 2016). Pollen morphology of *Rosa* L. genus is critically and systematically most important because of polymorphism (hybridization and polyploidy) which has been evolutionary carried out in 19th century by numerous researchers (Zielinski, 1985; Popek, 1996; Wronska-Pilarek & Jagodzinski, 2009). Selection of potential cultivar with desired traits takes years to be completed with success in breeding programmes. The seed production mainly correlates with pollen fertility and effective pollination (Pipino *et al.*, 2009). The round pollen considered as viable and it increase the rate of self-pollination (Entomophilous in hybrid tea and floribundas). The slow opening of petals (25-35) is the main cause of self-pollination as it does not give access to insects or wind to shed or spread pollens, but remain in unopened bloom and increase its seed setting. Thus, it can be used for breeding programme to get desired traits (Bell, 1988). Studies regarding improvement in scented variety (Farooq *et al.*, 2016) and heterosis breeding for qualitative and quantitative traits among seed bearing parents (Nadeem *et al.*,

2015) were conducted in Faisalabad region of Pakistan, but the rain-fed Pothwar Plateau in Punjab has yet to be explored. The agro-climatic conditions of the Pothwar region like elevation, temperature and precipitation are entirely different from Faisalabad (Farooq *et al*., 2011). The survival rate of rose depends on growing conditions, photosynthetic activity (Wojtania & Matysiak, 2018) and environmental conditions i.e ample sunlight, moderate temperature and precipitation. All of them for this region are different from rest of rose growing area in Pakistan. Seed setting is desirable features of rose for artificial pollination for evolving new hybrid varieties (Wagner *et al*., 2000). The primary objective was to introduce rose cultivars in this region to evaluate seed setting success (Khan *et al*., 2019; Khan *et al*., 2020). So, keeping in view the given scenario, a research trial was planned with prime objective of evaluating pollen performance for selecting desirable varieties and consequently enhancing success rate of hybridization among existing gene pool of *Rosa hybrida* species.

Materials and Methods

The research work regarding conventional breeding was conducted in year 2016 at PMAS Arid Agriculture University Research Farm Koont (Latitude 33° 11' N, Longitude 73° 01' E) (Khan *et al*., 2020) and pollen examination of twenty-one varieties was performed in the Cytogenetics Laboratory, Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.

Experimental procedure and data collection

Number of anthers per flower: Number of anthers per flower was counted in the month of April, with three replications for each variety.

Pollen viability percentage: Three flowers at colored bud stage/ open stage from each variety were harvested from selected plants. Required quantity of anthers were sliced on a new microscope slide to shed pollens and 2-3 drops of aceto-carmine (2%) were used as staining solution for pollen viability test (Nadeem *et al*., 2014). Later, the samples were covered with a cover slip and pollen viability was observed under using stereomicroscope (Nikon SMZ 1500). The pollen grains having red stain were considered as viable pollen (Eti, 1990; Ercisli, 2007; Crespel *et al*., 2015).

Pollen diameter: Diameter of viable pollens was also measured by using micrometer at 10X magnification in microscope for each sample and value was expressed in micrometer (µm).

Pollen germination percentage: Pollen germination percentage was observed to check the feasibility of pollens donor parents by using three prepared growing medias having 10, 15 and 20% sucrose in 2% agar media, respectively for each sample. Pollen after dehiscence were poured at growing media and placed in incubation for 24 hours by maintaining temperature at 24 ± 2°C. Germination percentage was observed under stereomicroscope (Nikon SMZ 1500). When pollen growth attained a size 1.5 times greater than diameter, it was considered as germinated pollen (Leus, 2005).

Hybridization study: The selective varieties already evaluated on the basis of morphological performance (Khan *et al*., 2019) were used for hybridization. Conventional approach was used to develop a new hybrid lines, by using cross combination among the three seed bearing and ten viable pollens parents (Abdolmohammadi *et al*., 2014). Both emasculation and pollination were performed in open field (Wagner *et al*., 2000). Varieties used in this study are polyploid (Khan *et al*., 2019) and genetically their self-compatibility varies according suitable environment (Chimonidou *et al*., 2007). Female flowers at reflex stage (when sepal starts bending and two-three petals opened) were selected. Anthers were smoothly clipped by using Forcep (to avoid self-pollination) without disturbing stigma of female flower (Dhyani *et al*., 2004). The list of selected parents and their cross combinations are presented in Table 1.

Table 1. Cross combinations made between selected varieties used in breeding program.

Varieties	Midas touch (V6)	Bora bora (V14)	Gruss-an-teplitz (V21)
Eye paint (V4)	V6 x V4	V14 x V4	V21 x V4
Fragrant plum (V5)	V6 x V5	V14 x V5	V21 x V5
Elina (V7)	V6 x V7	V14 x V7	V21 x V7
Anee Marie Trechslin (V9)	V6 x V9	V14 x V9	V21 x V9
Bridal pink (V12)	V6 x V12	V14 x V12	V21 x V12
Morstylo (V13)	V6 x V13	V14 x V13	V21 x V13
Pat austin (V15)	V6 x V15	V14 x V15	V21 x V15
Hot cocoa (V17)	V6 x V17	V14 x V17	V21 x V17
Broceliande (V19)	V6 x V19	V14 x V19	V21 x V19
Scentimental (V20)	V6 x V20	V14 x V20	V21 x V20

*Cross combinations used in hybridization study. *Seed setting parents are shown in vertical and pollen donor are represented in horizontal line. Varieties Midas touch, Bora bora and Gruss an teplitz good seed-bearing parents. Pollen donor parents were selected on the basis of pollen viability and pollen germination percentage

Data collection

The successful hip formation for each combination divided by the number of total crosses multiplied with one hundred to get combination success percentage. The hips maturation data was recorded in days from day of crossing (April), till maturity of hips (August). The harvested hips fresh weight (g) and diameter (cm) were measured by using electric balance and Vernier caliper, respectively. Seeds were extracted manually from hips and counted in three replications for each combination. Then seeds were stored at 4°C for treatment of germination improvement.

Meteorological data of experimental site: The meteorological data including temperature (maximum, minimum and average) and relative humidity was given in Fig. 1.

Statistical analysis

Ten crosses were performed for each cross combination and their results were described in percentage. In field, experiment was laid out under factorial Completed Randomized Complete Block Design (RCBD) with three replications. Data regarding parameters was compared by using LSD test with 5% significance level and Statistix 8.1 software was used for analysis of variance (ANOVA). In laboratory analysis, Completely Randomized Design (CRD) was used with 1% significance level. Correlation among field data was done by using PAST software (Steel & Torrie, 1980; Farooq *et al*., 2016).

Results

Number of anthers per flower: On statistical analysis of data, significant variation was observed regarding number of anthers per flower in all varieties as shown in Fig. 2. Higher numbers of anthers per flower (136.30) were observed in var. Helen naude followed by var. Angel face (producing 129.90 anthers per flower). Lowest numbers of anthers per flower (25.57) were exhibited in var. Gruss-an-teplitz. The results regarding number of anthers per flower varied significantly among other varieties used in this study. The number of anthers in var. Bora bora (103.10), Elina (102.80), Fragrant plum (70.00) and Broceliande (68.87) were at par with each other and not significantly different.

Pollen viability percentage (%): For the selection of desired pollen parents, observation of pollen viability percentage is considered important. Statistical analysis depicted that viability percentage varied significantly among all varieties (Fig. 3). Gruss-an-teplitz showed highest pollen viability percentage (67.40 %) where, the lowest pollen viability was recorded in var. Candy stripe (28.60 %) followed by Jude-the-obscure (30.20 %). The pollen viability in varieties Helen naude (56.80 %), Midas touch (56.80 %), Magic lantern (55.60 %), Pat austin (54.80 %), Morstylo (54.60 %) and Mr Waqar (53.60 %) were noted and found at par with each other, having non-significant difference. Nevertheless, differences were significant among other varieties.

***In vitro* pollen germination percentage:** After pollen viability test, their germination percentage was also recorded for each pollen donor parents as selected for hybridization by conventional breeding method. A contrasting behavior regarding pollen germination was observed for estimation of pollen vigor to get high success rate after pollination. Rose pollens were placed in three different media of sucrose concentration (10%, 15% and 20% with 2% agar) under controlled condition. After incubation for 24 hours, variation in pollen germination was significantly different in all tested varieties. Statistical analysis showed that highest pollen germination percentage (54.23%) was observed var. Gruss-an-teplitz at 15% sucrose media followed by var. Midas touch (42.60%) and var. Fragrant plum (42.43%) at same *in-vitro* germination media (Fig. 4). Lowest germination percentage was shown by var. Jude-the-obscure (6.90%), followed by var. Elina (7.70%), var. First prize (8.30%) and var. Candy stripe (8.66 %), respectively. Overall 15 % sucrose with 2% agar germination media proved best in this experiment as compared to other tested mediums (Table 2).

Pollen diameter (µm): The pollen diameter of all varieties varied significantly. The pollen diameter was significantly differed in var. Angel face (32.33 µm), var. Gruss-an-teplitz (32.26 µm) and var. Bora bora (31.86 µm) that are shown in Fig. 5.

Hybridization success results: The result of ANOVA showed that all parameters varied significantly and their mean values are shown in Table 3. The maximum hip set percentage (67%) was observed in Gruss-an-teplitz x Pat austin (V21 x V15). Overall maximum hip set percentage (67-44%) was observed in crosses with var. Gruss-an-teplitz (V21) followed by crosses with var. Bora bora (V14= 56-44%) and var. Midas touch (V6= 56-33%). The maximum hip size (2.16 cm) was observed in Midas touch x Eye paint (V6 x V4). The minimum hips size (1.44 cm) was observed in Gruss-an-teplitz x Morstylo (V21 x V13). The hip size in crosses of var. Bora bora (V14) remained intermediate (1.86-1.84 cm), diameter was recorded less (2.16-2.09 cm) than crosses of var. Midas touch (V6) and greater (1.49-1.44 cm) than crosses of var. Gruss-an-teplitz (V21). The maximum hip fresh weight (5.63 g) was observed in cross of Midas touch x Anee Marie Trechslin (V6 x V9). The fresh weight of crosses Bora bora (V14) was remained second (4.48-4.31 g) among other female parents. The lowest value of hip fresh weight (2.01 g) was observed in cross of Gruss-an-teplitz x Bridal pink (V21 x V12). Days to hip maturity all values showed non-significant interval (90.77-53.66 days) among all crosses. The maximum number of days taken to maturity (90.77 days) was observed in crosses of Gruss-an-teplitz x Eye paint (V21 x V4) and minimum (52.66 days) in cross of Gruss-an-teplitz x Anee Marie Trechslin (V21 x V9). The maximum number of seeds per hip (15.00) were counted in cross of Bora bora x Bridal pink (V14 x V12), followed by with slight margin Midas touch X Bridal pink cross (V6 x V12= 14.33). The minimum number of seeds (7.33) were observed in cross of Gruss-an-teplitz x Elina (V21 x V7), followed by (8.00) the cross of Gruss-an-teplitz x Fragrant plum (V21 x V5).

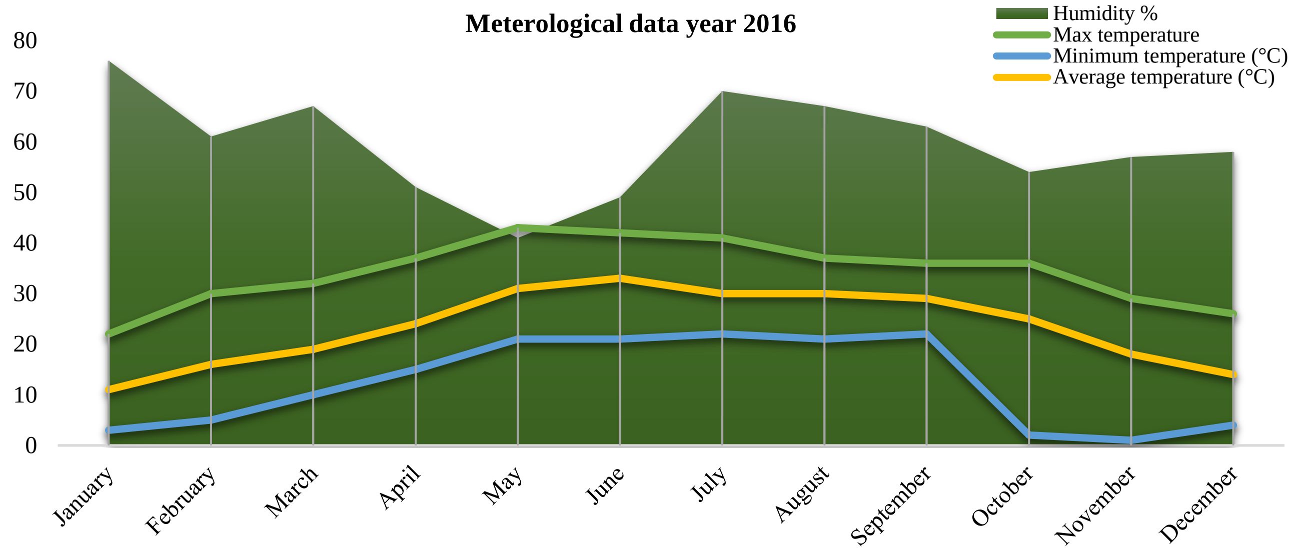

Fig. 1. Meteorological data for the year 2016.

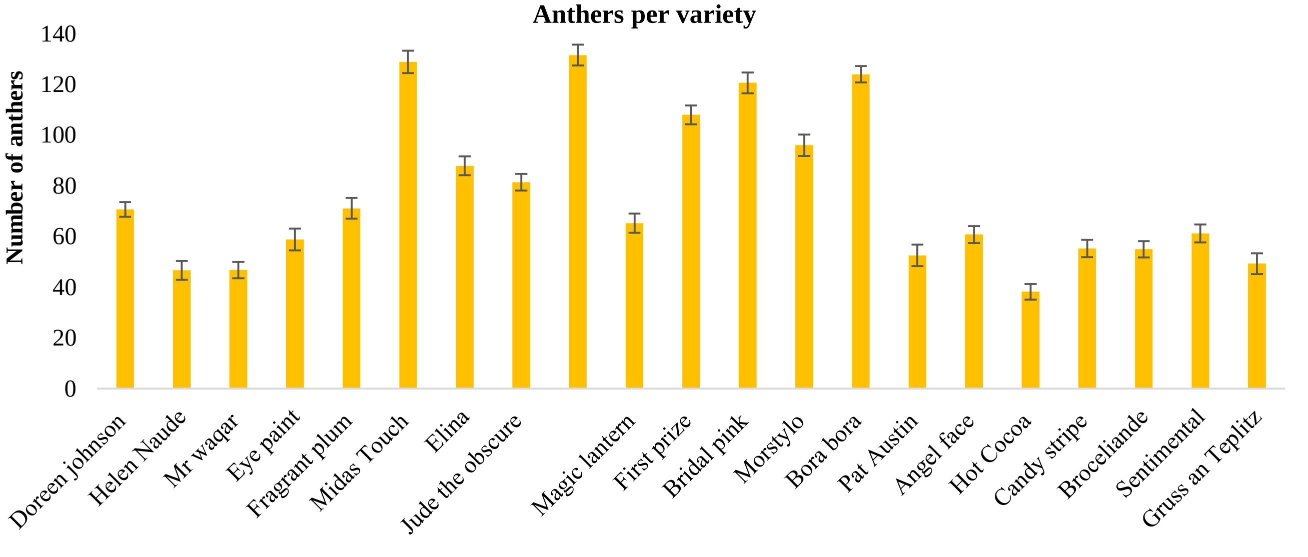

Fig. 2. Average number of anthers for each *Rosa* hybrida varieties.

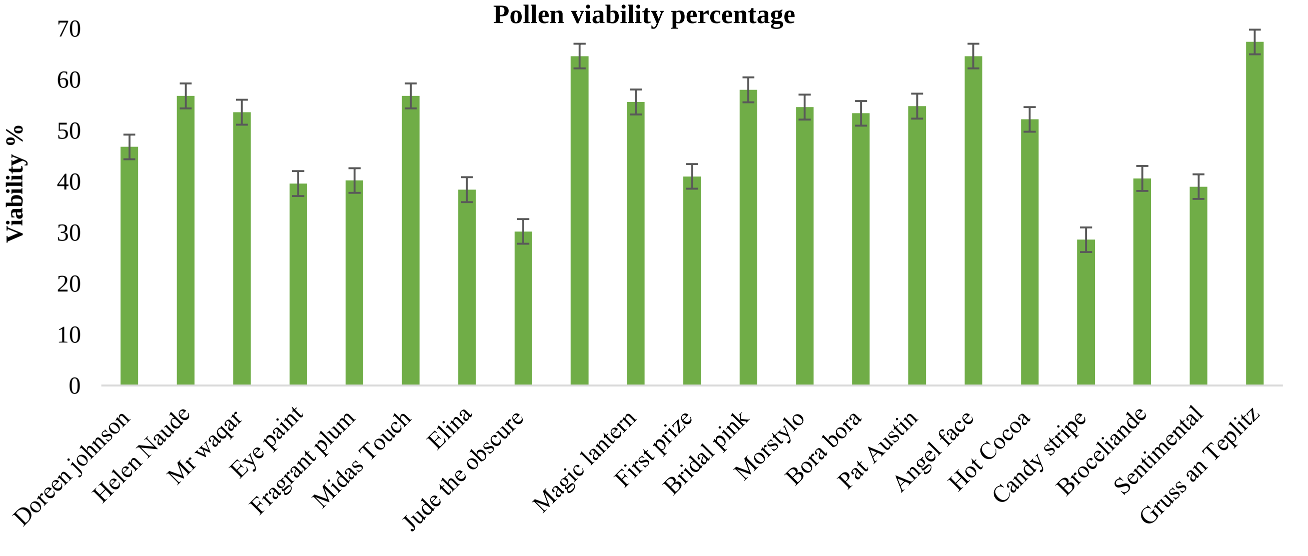

Fig. 3. Average pollen viability percentage of each variety.

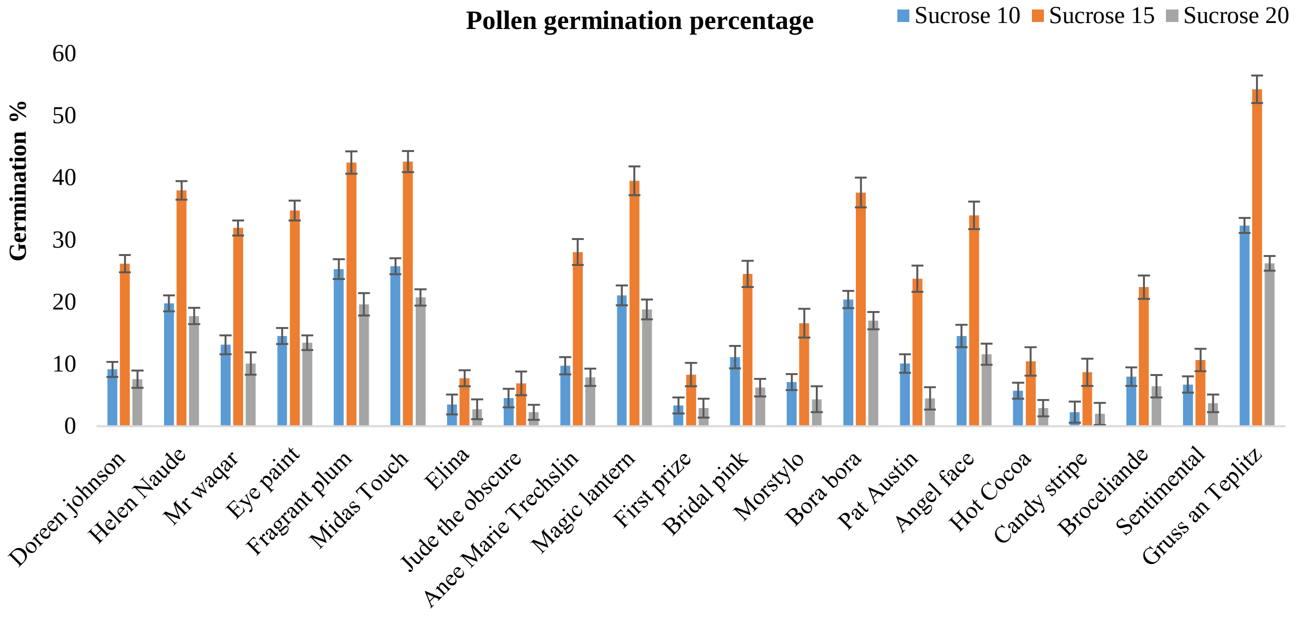

Fig. 4. Average pollen germination of each variety in different growing media. 2% agar in each sucrose media.

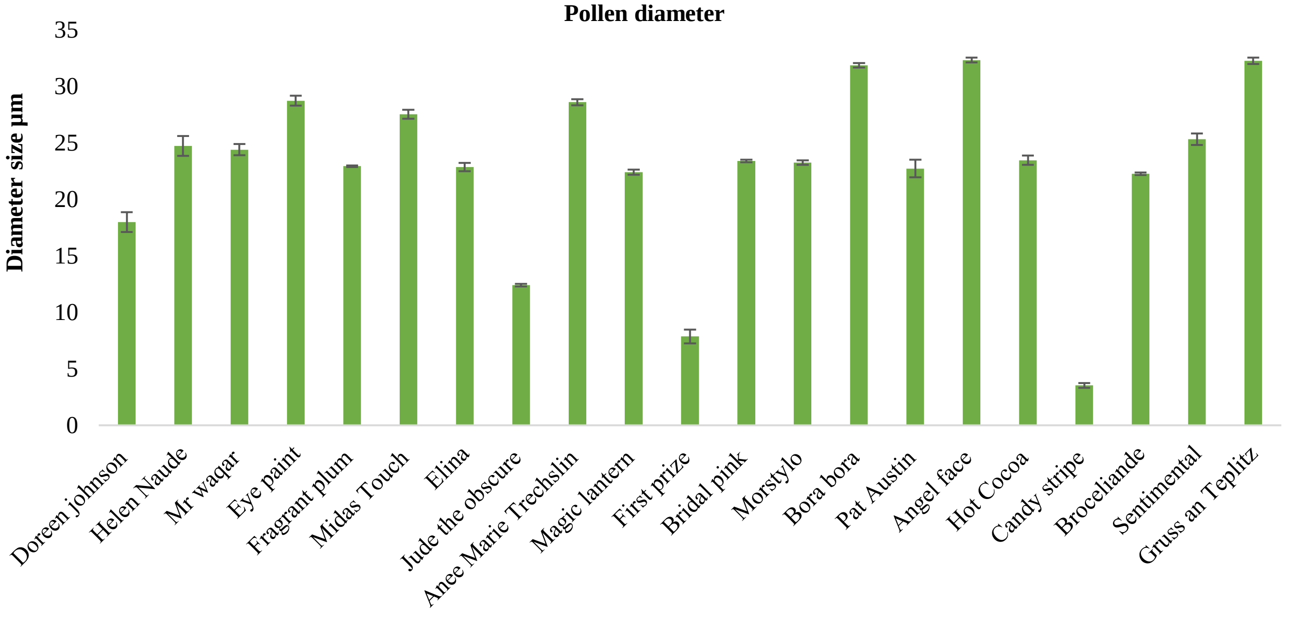

Fig. 5. Average pollen diameter of *Rosa hybrida* varieties.

In hybridization, successful crosses relationship was observed by grouping in cluster and represented in Fig. 6. The cluster 1 was comprised of (V21 X V13, V21 X V5 and V21 X V12), cluster 2 (V14 X V7, V6 X V12 and V14 X V12), cluster 3 (V21 x V15), cluster 4 (V21 x V4), cluster 5 (V21 x V9), cluster 6 (V6 x V20 and V6 x V4), cluster 7 (V6 x V15), cluster 8 (V6 x V9 and V14 x V9), cluster 9 (V14 x V5 and V6 x V5), cluster 10 (V21 x V7) and cluster 11 (V14 x V15 and V14 x V13). By clustering, all pollen parents show significance results and combining ability was positively assessed by their success percentage. All parameters were statistically analyzed to check their degree of relationship with each other. The first two components of PCA analysis showed 79.05 % and 13.43 % variance while grouping of crosses of *Rosa hybrida* specie were shown in Fig. 7. The grouping of the data confirmed that results of cluster analysis at linkage distance equal to 10. Hip set percentage has no significant correlation with all studied parameters as shown in Table 4. The maximum value (r =*0.68203) show positive correlation among hip fresh weight and seeds per hip which showed positive strong correlation, while days to hip maturity showed positive correlation with hip fresh weight and seeds per hip. Diameter of hip showed positive correlation (r =0.12358 & r =0.030417) with hip fresh weight and seeds per hip. These findings also correlated with the Pipino *et al*., (2012) as significant positive correlation was observed regarding number of seeds after successful hybridization and it confirmed pollen quality seems to be most promising factor.

Table 2. *Rosa hybrida* varieties results for examined traits of pollen and parentage characters.

Varieties	No of anther	Pollen diameter (µm)	Character	Seed setting
Doreen johnson (V1)	95.00 ± 0.89gh	18 ± 0.88h	Father	No
Helen naude (V2)	136.00 ± 2.31a	24.73 ± 0.87def	Mother	Yes
Mr waqar (V3)	104.00 ± 0.11ef	24.4 ± 0.50d-g	Mother, Father	Yes
Eye paint (V4)	72.00 ± 0.62kl	28.73 ± 0.43b	Mother, Father	Yes
Fragrant plum (V5)	70.00 ± 1.35 l	22.93 ± 0.08fg	Father	No
Midas touch (V6)	130.00 ± 1.46b	27.53 ± 0.40bc	Mother	Yes
Elina (V7)	103.00 ± 3.30f	22.86 ± 0.37fg	Father	No
Jude-the-obscure (V8)	78.67 ± 1.45j	13.2 ± 0.12i	Father	No
Anee Marie Trechslin (V9)	112.33 ± 0.91d	25.8 ± 0.27cd	Father	No
Magic lantern (V10)	81.67 ± 0.29ij	22.4 ± 0.23g	Mother, Father	Yes
First prize (V11)	107.67 ± 0.59e	7.86 ± 0.60j	Mother	No
Bridal pink (V12)	83.33 ± 0.88i	23.4 ± 0.12efg	Father	No
Morstylo (V13)	98.00 ± 2.06g	23.26 ± 0.20efg	Mother, Father	No
Bora bora (V14)	103.00 ± 0.59f	31.86 ± 0.19a	Mother	Yes
Pat Austin (V15)	53.33 ± 0.99n	22.73 ± 0.77fg	Mother, Father	No
Angel face (V16)	117.00 ± 2.06c	32.33 ± 0.20a	Mother	Yes
Hot cocoa (V17)	93.00 ± 0.59h	23.46 ± 0.40efg	Father	No
Candy stripe (V18)	64.33 ± 0.22m	3.53 ± 0.21k	Father	No
Broceliande (V19)	68.67 ± 1.24l	22.26 ± 0.10g	Father	No
Scentimental (V20)	74.33 ± 2.33k	25.33 ± 0.50de	Father	No
Gruss-an-teplitz (V21)	25.67 ± 1.06o	32.26 ± 0.28a	Mother, Father	Yes

Table 3. Comparison results for hip set (%), days to hip maturity, number of seeds per hip, diameter of hip and hip fresh weight after harvesting.

Cross	Hip set (%)	Days to hip maturity	No. of seeds/hip	Diameter of hip (cm)	Hip fresh weight (g)
v6 x v4	33%	90.77 ± 0.6a	14.00 ± 0.2 ab	2.16 ± 0.0 a	5.57 ± 0.1ab
v6 x v5	44%	78.33 ± 1.0ab	11.33 ± 0.2 a-c	2.12 ± 0.1ab	5.54 ± 0.2ab
v6 x v7	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0f
v6 x v9	44%	77.22 ± 1.8ab	9.667 ± 0.2 a-c	2.12 ± 0.2 ab	5.63 ± 0.1a
v6 x v12	56%	80.11 ± 1.2ab	14.33 ± 0.3 ab	2.10 ± 0.1 b	5.39 ± 0.1b
v6 x v13	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v6 x v15	33%	79.99 ± 0.3ab	12.66± 0.2 a-c	2.10 ± 0.1 b	5.50 ± 0.2ab
v6 x v17	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v6 x v19	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v6 x v20	33%	89.22 ± 2.1ab	11.33 ± 0.2 a-c	2.09 ± 0.2 b	5.49 ± 0.0ab
v14 x v4	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ±0 f
v14 x v5	44%	78.11 ± 1.7ab	11.66 ± 0.2 a-c	1.84 ± 0.1 c	4.44 ± 0.1 c
v14 x v7	56%	80.11 ± 1.4 ab	14.00 ± 0.2 ab	1.86 ± 0.1 c	4.36 ±0.0 c
v14 x v9	44%	76.11 ± 0.1 b	12.33 ± 0.1 a-c	1.84 ± 0.0 c	4.41 ± 0.1c
v14 x v12	56%	79.33 ± 1.1 ab	15.00 ± 0.2 a	1.85 ± 0.1 c	4.45 ± 0.1c
v14 x v13	44%	78.11 ± 1.7ab	11.00 ± 0.2 a-c	1.84 ± 0.2 c	4.31 ± 0.1c
v14 x v15	44%	78.99 ± 0.9ab	11.66 ± 0.2 a-c	1.84 ± 0.1 c	4.48 ± 0.1c
v14 x v17	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v14 x v19	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v14 x v20	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v21 x v4	56%	90.77 ± 2.4a	9.667 ± 0.1 a-c	1.46 ± 0.2 de	2.13 ± 0.0de
v21 x v5	56%	78.66 ± 2.5ab	8.000 ± 0.2 c	1.49 ± 0.1 d	2.31 ± 0.1 d
v21 x v7	44%	80.66 ± 2.5ab	7.333 ± 0.2 c	1.44 ± 0.2 e	2.08 ± 0.0 de
v21 x v9	56%	53.66 ± 1.2 c	9.333 ± 0.2 bc	1.49 ± 0.3 d	2.28 ± 0.1 d
v21 x v12	56%	80.66 ± 2.5ab	9.000 ± 0.2 bc	1.47 ± 0.2 de	2.01 ± 0.1 e
v21 x v13	56%	78.89 ± 3.5ab	9.000 ±0.1 bc	1.44 ± 0.2 e	2.09 ± 0.0 de
v21 x v15	67%	80.55 ± 0.8 ab	11.33± 0.3 abc	1.46 ± 0.3 de	2.16 ± 0.0 de
v21 x v17	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v21 x v19	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f
v21 x v20	0%	0 ± 0d	0 ± 0 d	0 ± 0 f	0 ± 0 f

Table 4. Correlation study among parameters of successful crosses in hybrid roses.

Parameters	Hip set %age	Days to hip maturity	Diameter of hip (cm)	Hip fresh weight (g)	Seeds per hip
Hip set %age	1				
Days to hip maturity	-0.26358	1			
Diameter of hip	-0.17395	-0.028086	1		
Hip fresh weight	-0.671	0.17822	0.12358	1	
Seeds per hip	-0.15763	0.2311	0.030417	*0.68203	1

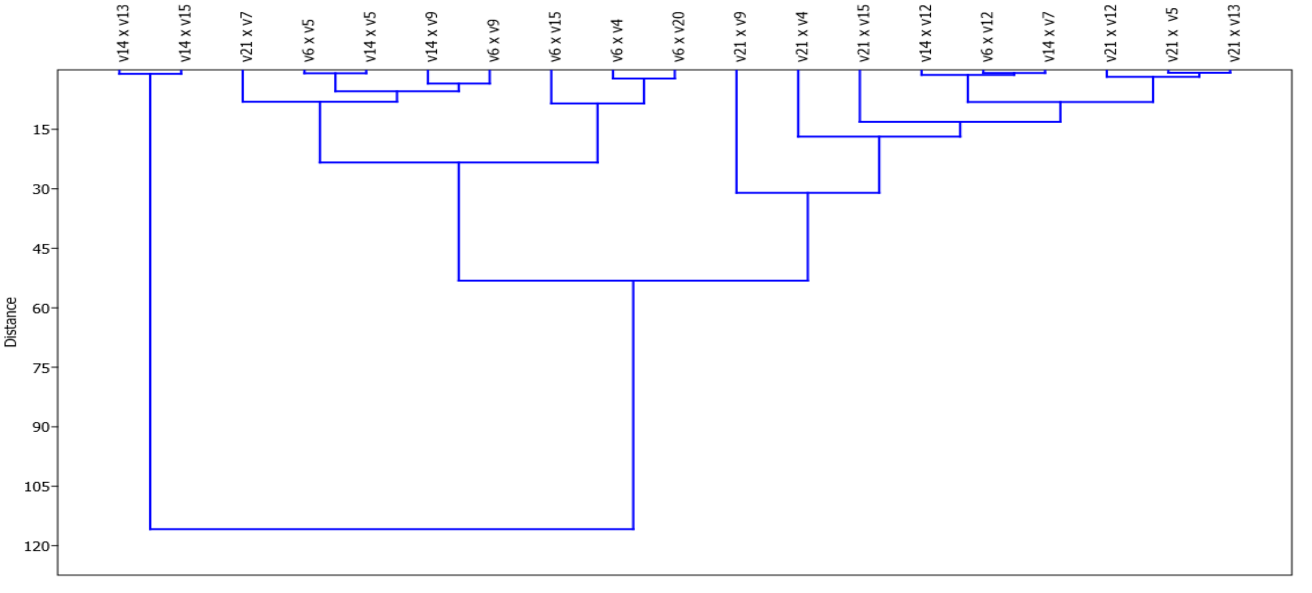

Fig. 6. Tree diagram showing grouping of different crosses in *Rosa species* regarding parameters in breeding experiment.

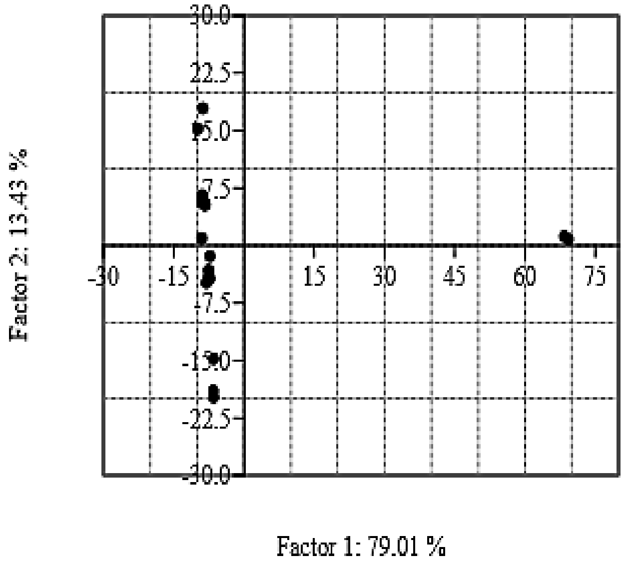

Fig. 7. Plot designed for description of principal components based on means of different crosses among hybrid roses on this the basis breeding parameters.

Discussion on *in vitro* Pollen behavior for success of breeding: *In vitro* evaluation of pollen behavior can be helpful in increasing the success of breeding in the field conditions. All varieties were examined for their prominent characteristics. Selection of varieties on the basis of seed bearing and pollen donor parents were carried out earlier in different *Rosa species* (Visser *et al*., 1977; De Vries & Dubois, 1987; Crespel *et al*., 2015) which possessed different fertility level among species (Zlesak, 2009). In the present work, pollen viability of all varieties was evaluated to check the fertility rate and then further selection was made for breeding experiment. The maximum pollen viability percentage was observed in variety Gruss-an-teplitz (67.4 %), followed by Angel face (64.6 %). The pollen viability percentage of all varieties used in this study remained in between 28.6 % (Candy stripe) to 67.4 % (Gruss-an-teplitz). This pollen viability made the selection of parents easy. Interspecific hybridization produced heterozygosity with varying level of ploidy and prominent behavior of recessive alleles (Visser *et al*., 1977). In his work on pollen morphology variations in *Rosa species*, Ercisli (2007) recorded pollen viability percentage among species (31.88 to 48.6%) and in hybrid tea roses (27 to 61 %). Pollen grain viability percentage fluctuates as it is mainly dependent on varieties ploidy level and pollen efficiency (Visser *et al*., 1977) as well as collection time (Gudin *et al*., 1991).

Quantity of pollen per anther may vary in *Rosa species*, depending on variety, species, age, nutritional application and growing environmental conditions (Ercisli, 2007). In this study, number of anthers per flower varied among each variety. Maximum number of anthers was recorded (136) in variety Helen naude and minimum (25.67) in Gruss-an-teplitz. Indistinguishable variations regarding number of anthers (83-260) was also observed by Zuraw *et al*., (2015). In modern roses, Gunes *et al*., (2004) reported variability in number of anthers in *R. villosa* (81.4) and *R. Elliptica* (148.1). Similar variation in number of anthers per flower was also observed in hybrid tea and floribunda roses by Ercisli (2007) and Zlesak (2009).

Pollen germination test of these varieties were also done to check the growth of pollen tube so that fertilization process may occur easily. The pollen germination in all varieties showed different behavior while grown on different concentration of sucrose. The maximum pollen germination was observed (54.23%) in sucrose 15% media. As sucrose level increase up to 20%, germination of pollen also decreased. Pollen germination percentage was recorded 54.23 to 6.99% in all varieties. Richer *et al*., (2007) recorded pollen germination 43.18 to 5.17 % in *Rosa Majalis* and *Rosa Canina* which authenticated present findings. Germination capacity may vary in each variety depending on growing environment with pollen germination 16-38% in different concentration of sucrose. Similar results were reported by Visser *et al*., (1977) in hybrid tea roses (14-47%) to find out the suitable parents for hybridization.

Less germination percentage was observed at both 10% and 20% sucrose media as compared to 15% in all rose varieties. Pollen growing at 20 % sucrose media (Farooq *et al*., 2016) along with combination of Agar media, maximum germination was noted in scented roses *R. damascena* (57.6%) which were reduced in *R. centifolia* and *R. indica* while growing alone or with agar combination (Farooq *et al*., 2016). In this study maximum pollen grain germination was observed in 15 % sucrose level along with 2% agar. In two genotypes of *Rosa* species, Ercisli, (2007) reported pollen germination 32.16 to 11.21 % which endorsed this work. Sucrose with the combination of boric acid always shows positive results in roses (Pearson & Harney, 1984; Marchant *et al*., 1992). Similar results were reported by Farooq *et al*., (2013) in his work on *R. bourboniana* and Gruss-an-teplitz while growing in 1% agar and 15 % sucrose media and found pollen germination up to 60.80% and 52.20%, respectively. The study on pollen tube growth (Al-Jibouri *et al*., 1987) and pollen viability (Pearson & Harney, 1984) give different results this may be due to genetic differences (Jacob & Pierret, 1998). Pollen availability and pollination in ornamnetals is mainly adhered by weather and influence breeding success in plants (Yao *et al*., 2019).

The variation in pollen tube length (184.2 µm at 20x magnification) and pollen viability percentage (23 to 45%) was observed in different species (Werlemark, 2000; Ueda & Akimoto, 2001). Pollen viability decreases as temperature increases (Visser *et al*., 1977) and fertility of gametes also alter as physiological changes occur in plant growth (Gudin *et al*., 1991). The pollen diameter in different concentration of sucrose varies but varieties overall performance was best with nominated number of hips after crosses in Pothwar condition. This performance may be varied due to limited supply of water and nutrients. The commercially adapted varieties may vary in morphological attributes as grown at different environmental conditions. Candy stripe is one of the most attractive variety in commercial flower market as like First prize and Jude-the-obscure. In experimental area, these varieties performance was recorded meager as compared to other varieties (Khan *et al*., 2019). These varieties are considered good in Faisalabad and Pattoki region for their flower attractiveness, mix color geometry and scented petals. The varieties Hot cocoa, Bridal pink and Morstylo were at par with each other in pollen diameter having size of 23.46 µm, 23.40 µm and 23.26 µm, respectively. The varieties Magic lantern and Broceliande pollen diameter were observed more fertile but Broceliande variety was selected for breeding parent due its attractive and regular bloom in growing season. Both Midas touch and Anee Marie Trechslin varieties also produced fertile pollen with diameter 27.53 µm and 25.80 µm were selected for regular flowering and attractive color bloom. These varieties performance was excellent in Pothwar region. Due its attractive color bloom, Midas touch used as seed bearing parent and Anee Marie Trechslin used as pollen donor parent. These varieties produced consistent flowering. Pollen diameter may be considered as fertility predictor, but variation in pollen diameter of species and hybrid teas (21.3 µm-40.8 µm) also observed (Pipino *et al*., 2011; Ueda & Akimoto, 2001). Pollen germination in species (29-51 to 52-82 µm) and hybrid tea roses (32.31 to 52.99 µm) may helpful in success of hybridization (Ueda & Akimoto, 2001). Pollen germination and pollen tube growth of hybrid tea at 15 % sucrose concentration performed well (Visser *et al*., 1977) and has positive correlation with pollen diameter (Pipino *et al*., 2009). Shriveled pollen was observed in tetraploid as compared to diploid (2n gametes), ploidy information may be important for success of hybridization through crossing (Zlesak, 2009). Differences in genomic size may make ploidy identification difficult through flow cytometry because diploid and tetraploid DNA content variation may occur due to triploids (Yokoya *et al*., 2000).

Discussion on conventional crossing success: Variation in hip setting percentage was observed in executed cross combinations. Out of total 30 cross combinations, 19 cross combinations (63.33%) remained successful in hip setting. Variations were found in hip setting and seed germination percentage after crossing among triploid, diploid as compared to tetraploid but with low hip set (14%) was seen when triploid used as a female parent (Cock *et al*., 2007). The maximum hip set percentage was observed in Gruss-an-teplitz crosses followed by Bora bora crosses in this work. Difference in hip set percentage among *Rosa* species may be influence by hormonal control on embryo and hip formation (Cruden, 1989; MacPhail, 2007; Nybom, 2007). A rose hip is a berry-like structure formed after pollination and subsequently petals shedding occurred. Pollination during the flowering month of April, May and June (De Vries & Dubois, 1987) can give successful achievement in hybridization as pollen shed on stigma at right time which results in hip formation just after couple of weeks (Crespel & Gudin, 2003). In *Rosa* species, comparison of varieties on their morphological attributes and their resulted seed with their associated parameters evaluated earlier (Najda & Buczkowska, 2013; Nadeem *et al*., 2015; Farooq *et al*., 2016). Success rate, number of seeds, seed weight and hip development can be improved by application of repeated pollination practice (Chimonidou *et al*., 2007). Temperature range 23- 30^{0}C and stigma receptivity of female parent with fertile pollen can significantly play a role for successful pollination (Crespel & Mouchette, 2017). Environmental fluctuation, rain and nutrition deficiency can also inhibit plant growth as well as hip development at optimum temperature 25-30^{0}C (Nybom, 2007) which may vary according to research area. Variation in temperature can cause hip abortion during embryo development (Gudin *et al*., 1991) but low temperature may helpful for seed formation and hip setting in hybrid tea roses (Von Abrams & Hand, 1956).

In this study, hip setting varied significantly among all female parents. Gruss-an-teplitz showed overall maximum hip setting (67 %), followed by Bora bora and Midas touch. Midas touch hip setting percentage was seen less as compared to other female parent among all crosses. Variation in hip setting among all female parents may be hindered by genetic variability or other relevant pre and post-pollination factors. Rose flower cultivars whose longevity is affected by season, can perform better with summer production than winter production (Borch *et al*., 1995). Crossing between within the species can cause sterility as in case of triploid or diploid hybridization. Ploidy or doubling of chromosome e.g. tetraploid can overcome these difficulties (Semeniuk & Arisumi, 1968, Roberts *et al*., 1990). Whereas determination of valuable morphological characters, floral characters are considered most important than vegetative character in *Rosa* species (Nybom *et al*., 2004). Sealed storage of air-dried seeds at low temperature is highly recommended (Schopmeyer, 1974). The variability in morphological character of species makes taxonomic classification accuracy difficult due to spontaneous variation of characters as species may naturally hybridize. So, species can be identified from other section by their unique genetic basis rather than morphological description (Nybom *et al*., 2004; Wissemann & Ritz, 2007). This genetic basis may include meiotic system which shows maternal inheritance or basis of inheritance can leads to apomixis. The resulting progeny plant may or may not receive pollen parent genetic characters (Werlemark & Nybom, 2004).

Conclusion

Breeding among screened varieties seems to be promising due to suitability of climate. However, breeding efforts in an institution particularly in Pothwar climate, can be most attractive aspects in horticultural industry. The combination of superior traits varieties with Midas touch, Bora bora and Gruss-an-teplitz female parents has a potential for developing new hybrid lines. These varieties have significant seed setting percentage in addition with successful adaptation and hybridization. In future, this study might be useful for selecting new variants among *Rosa species* in Pothwar region.

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