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CROSS COMPATIBILITY IN VARIOUS SCENTED *ROSA* SPECIES BREEDING

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Rosa damascena, *Rosa gallica* L., *Rosa centifolia* L., *Rosa bourboniana* and Gruss an Teplitz are important species which are being used in the production of rose oil. The improvement of existing rose germplasm is necessary to sustain the public demand of rose oil globally. In the present study, five *Rosa* genotypes (*Rosa damascena*, *Rosa gallica* L., *Rosa centifolia* L., *Rosa bourboniana*, *Rosa indica* and Gruss an Teplitz) were crossed bred to check genotypic compatibility and seed setting potential under local climatic conditions. The results indicated that maximum fruit set percentage (83%) was exhibited by Gruss an Teplitz followed by *Rosa indica* (70.33%) and *Rosa damascena* (53.33%). Significant variations were recorded for time to maturity of hips, number of seeds per hip, and weight of hips in all crosses. Overall, Gruss an Teplitz and *Rosa indica* proved better female parents and yielded high values of number of hips per plant and fruit set percentage.
Keywords: *Rosa*, rose breeding, essential oils, pollination, seed setting.

INTRODUCTION

Roses are the index of civilization as the history of cultivation of roses dates back to about two thousand years. In the era of the Han dynasty (141–87 BC), roses were used to decorate the gardens of the royal palace in China (Guoliang, 2003). Similarly, roses go far back in history in West Asia and Europe, particularly in eastern countries, where people used to cultivate roses for oil extraction as well as for the purpose of beautification. The species mainly used for oil production are *R. damascena* Mill., *R. gallica* L., *R. moschata* Herrm. and *R. centifolia* L. (Tucker and Maciarello, 1988). In Pakistan, the rose oil is being produced mainly from *R. damascena* and *R. centifolia*, but *R. bourboniana* and Gruss an Teplitz are also being exploited for inferior quality rose oil (Farooq *et al.*, 2011; Jaskani *et al*., 2005; Akhtar *et al*., 2016). *R. damascena* produces flowers in the month of April while *R. centifolia* continues blooming throughout the year even under extreme temperature. The consumers of rose oil in perfumery and cosmetics industries demand the quality of rose oil with respect to its chemical and physical properties (Rusanov *et al.*, 2009). There is need to develop the new varieties of oil-bearing roses by maintaining the standards of scent molecules of rose oil (Baydar *et al.*,2016).

The capability to produce new rose cultivars is imperative for the breeders to continue and improve the market share. The genus, *Rosa* consists of 200 species and more than 18,000 cultivars (Gudin, 2000) but all these species and cultivars have been formed by the crossing of only few types (Maia and Venard, 1976). Among all *Rosa* species, about 20% species are considered as scented, 50% are low scented and the rest are non-scented (Schulz, 2003). The taxonomic variation in the *Rosa* species offers the opportunity of success in crossing (De Vries and Dubois, 1996).

Rose Breeders carry out a good number of crosses amongst tetraploid roses (garden and cut-flower roses) that represent compatible crosses (Spethmann and Feuerhahn, 2003). Successful cross breeding have been achieved between *R. damascena* and *R. gallica*, *R. centifolia* and *R. rugosa*. All the essential oil *Rosa* species are tetraploid (2n=4x=28), which providing better chance for successful crosses (Staikov and Kalaidzhiev, 1972; Staikov *et al.*, 1980). Due to ploidy difference, only 5-10% of wild roses have been used in breeding programs (Spethmann and Feuerhahn, 2003). Rose breeders always require better pollinators for crossing. Although there are a lot of steps required creating a new cultivar from pollination to seed maturation and then germination, but viability and good germination percentage of the pollen grains of the male parent increases the rate of successful cross pollination. The F-1 hybrids of *R. damascena* and *R. bourboniana* have been produced successfully and evaluated by morphological markers (Kaul *et al.,* 2009). A recent breeding project has been completed at Cyprus (2004-2007) with the objective to study the possibility of transferring fragrance of *R. damascena* to hybrid tea rose cultivars. The project is hoping for improved hybrid cultivars with the mixture of the characteristics of *R. damascena* and hybrid tea rose (Chimonidou *et al.*, 2007).

The present study was aimed to make crosses between the essential oil *Rosa* species to estimate the fruit set percentage. The characters of different essential oil *Rosa* species can be exploited by developing the hybrids, using the valuable information from this research work. These characters may be the continuous blooming in *R. centifolia* or nice aroma of *R. damascena* which could be tried to combine in a single hybrid in the future breeding research

MATERIALS AND METHODS

***Plant material*:** Plant material used for this investigation consisted of 3-years old five *Rosa* species. The plants were established in the research area of *Rosa* Project, Institute of Horticultural Sciences (IHS), University of Agriculture, Faisalabad, Pakistan (Latitude 31° 25' 0" N, longitude73° 5' - 1" E) (Table 1).

***Pollination*:** All the essential oil *Rosa* species gave better results for pollen viability in microscopic observations and germination test at 1% agar plus 15% sucrose in preliminary experiment during March-April (Figure 1A). Crosses between these species were done by emasculation and manual cross-pollination protocol. Each species was crossed with other, making twenty crosses using RCBD statistical design with three replications. Both seed and pollen parents were prepared at the same stage of maturity. Tetraploid roses, in general, exhibit self-compatibility (Chimonidou *et al.*, 2007), hence anthers were removed from the flowers of female parents with the help of forceps to avoid self-pollination (Dhyani *et al.*, 2005). Emasculation was done when flowers were in one-

Table 1. Plant material used for reciprocalcrosses.

Name of species	Date of introduction	Habitus	Ploidy level	Flower colour	Plant height	Blooming
Rosa damascena	Pre-1850	Bush	Tetra-ploid	Light Pink	2.0 m	Once
Rosa centifolia	Pre-1450	Bush	Tetra-ploid	Pink	1.5m	Repeat
Rosa bouboniana	Pre-1819	Bush	Tetra-ploid	Pink	2.0 m	Repeat
Gruss an Teplitz	Pre-1897	Bush	Tetraploid	Red	1.5m	Repeat
Rosa indica	Pre-1800	Bush	Tetraploid	Red	1.5m	Repeat

(A) (B) (C)

(D) (E) (F)

Figure 1. (A) Petals removal for detaching the anthers before pollen shedding (Gruss an Teplitz); (B) Collection of pollen sacs in petri dishes; (C) Microscopic observation of the *in vitro* pollen germination; (D) Flower buds covered with white paper bags to prevent from stray pollens; (E) *Rosa damascena* fruit (hips); (F) Achenes (Rose fruit seeds).

third to one-half open stage by detaching petals (Chimonidou *et al.,* 2007) followed by the removal of anthers with sterilized razor (Figure 1B). Anthers were collected in sterilized labeled petri dishes from the plants of *Rosa* species chosen as female as well as male parents (Figure 1C). To protect the stigmas of the emasculated flowers, the female parent flowers were enclosed in paper bags to exercise the pollination at the targeted time using magnify glass. Pollen was dusted on the emasculated plants with camel-hair brush and flower budswere again covered to avoid outsourced pollination (Figure 1D). After three days, paper bags were removed and pollination was repeated twice.

***Data collection*:** For each cross starting from cross pollination, data were recorded for fruit set (%), time of hip bearing (days from pollination to hips harvesting), number of seeds/ hip, hip diameter (cm) and hip weight (g).

***Data analysis*:** All the data were analyzed statistically by performing analysis of variance and the means were compared by using Duncan's multiple range (DMR) test at 5% probability (Steel *et al.,*1997). Correlation among the characteristics was analyzed according to Pearson's coefficient. Principle component analysis and cluster analysis were done by using Statistica7, Stat-soft. Inc. 1984-2007.

RESULTS

The maximum fruit setting was observed in crosses of Gruss an Teplitz (83.0-74.7%) followed by *R. indica* (70.3-58.0%) and *R. damascena* (53.3-11.0%) as a female parents (Table 2). A limited success in fruit setting (4.7%) was observed in the crosses of *R. bourboniana* (female parent) with all other *Rosa* species. *R. bourboniana* showed no success when crossed with *R. damascena*. As a female parent, *R. damascena* showed good fruit set percentage with *R. centifolia* (53.3%) followed by *R. indica* (51.0%) and Gruss an Teplitz (11.0%) but could not set any hip with *R. bourboniana.* All the values regarding time to hip ripening showed non-significant results (81.3 to 50.3) within all crosses. The maximum number of seeds/hip was produced by those crosses where Gruss an Teplitz (17.0-15.0) and *R. indica* (16.0-15.0) were used as female parents. While crosses of *R. centifolia, R. bourboniana* and *R. damascena* produced 6.2-5.4, 2.55-4.4 and 6.4-7.3 number of seeds/hip, respectively as female parents.

Maximum value for the diameter of hips was recorded in Gruss an Teplitz (1.7 cm) when crossed with *R. centifolia* while minimum value was found in *R. centifolia* (0.7 cm) crossed with Gruss an Teplitz. *R. damscena* also excelled for hip size when crossed with *R. indica* (1.4 cm), *R. centifolia* (1.3 cm) and Gruss an Teplitz (1.3 cm) (Figure 1E).*R. damscena* showed the highest value for hips weight (2.0 g) when crossed with *R. centifolia* and Gruss an Teplitz, while Teplitz and *R. indica* showed the second highest value for hips weight as 1.9-1.2 g and 1.4-1.3 g when crossed with all other *Rosa* species, whereas the lowest value was observed for *R. centifolia* (0.4 g) when crossed with Gruss an Teplitz. Overall *R. bourboniana* showed the least values for weight (0.5-0.5 g)

Table 2. Comparison of different parameters in reciprocal crosses of *Rosa* species.

Crosses	Fruit set (%)	Time to hip ripening (days)	No. of seeds/hip	Diameter of hip (cm)	weight of hip (g)
R. damascena × *R. centifolia*	53.3±3.8d	81.0±0.6a	11.0±0.6b-e	1.3±0.0bc	2.0±0.0a
R. damascena × *R. bourboniana*	0.0±0.0g	0.0±0.00b	0.0±0.0f	0.0±0.0e	0.0±0.0h
R. damascena × Gruss an Teplitz	11.0±2.0fg	81.3±0.9a	9.7±0.3de	1.3±0.0bc	2.0±0.0a
R. damascena × *R. indica*	51.0±5.9d	80.0±0.6a	10.8±0.3cde	1.4±0.0bc	1.7±0.0b
R. centifolia × *R. damascena*	7.0±0.0fg	72.3±0.9a	14.3±0.3a-d	1.4±0.0bc	1.0±0.0e
R. centifolia × *R. bourboniana*	0.0±0.0g	0.0±0.0b	0.0±0.0f	0.0±0.0e	0.0±0.0h
R. centifolia × Gruss an Teplitz	26.8±3.8e	70.0±0.6a	12.3±0.3a-e	0.7±0.0d	0.4±0.0g
R. centifolia × *R. indica*	16.3±2.0f	72.0±1.2a	12.7±0.67a-e	0.8±0.0d	1.8±0.0b
R. bourboniana × *R. damascena*	0.0±0.0g	0.0±0.00b	0.0±0.0f	0.0±0.0e	0.0±0.0h
R. bourboniana × *R. centifolia*	3.00±2.08g	53.00±26.5a	7.33±3.84e	0.53±0.3d	0.36±0.2g
R. bourboniana × Gruss an Teplitz	4.7±2.3g	55.3±27.7a	7.7±3.8e	0.6±0.3d	0.4±0.2g
R. bourboniana × *R. indica*	4.7±2.3g	50.3±25.2a	8.7±4.3e	0.5±0.2d	0.3±0.2g
Gruss an Teplitz × *R. damascena*	77.7±7.9ab	69.0±0.6a	17.0±0.6a	1.5±0.0abc	1.2±0.0de
Gruss an Teplitz × *R. centifolia*	80.0±4.0ab	70.0±0.6a	15.0±0.6a-d	1.7±0.1a	1.9±0.0ab
Gruss an Teplitz × *R. bourboniana*	74.7±3.8ab	72.0±1.2a	16.7±0.9a	1.6±0.1ab	1.5±0.2c
Gruss an Teplitz × *R. indica*	83.0±2.08a	72.7±0.9a	16.3±0.9ab	1.3±0.1bc	0.7±0.0f
R. indica × *R. damascena*	58.0±5.9d	68.3±0.3a	16.0±0.6abc	1.4±0.0bc	1.4±0.0cd
R. indica × *R. centifolia*	60.0±4.0cd	70.0±0.6a	15.7±1.2abc	1.2±0.1c	1.3±0.0cd
R. indica × *R. bourboniana*	61.7±2.7cd	70.7±0.3a	15.7±0.9abc	1.3±0.0bc	1.3±0.0cd
R. indica × Gruss an Teplitz	70.3±5.36bc	69.0±0.6a	15.0±0.6a-d	1.2±0.1c	1.3±0.1cd

Means sharing similar letter in a column are statistically non-significant (P>0.05); ± SE.

Table 3. Correlation between different parameters for cross breeding in *Rosa* species.

Parameters	Fruit set%age	Time to hip ripening	Seeds/hip	Diameter of hip	weight of hip
Fruit set%age	1.0000				
Time to hip ripening	0.6081**	1.0000			
Seeds/hip	0.9092**	0.8000**	1.0000		
Diameter of hip	0.8218**	0.8687**	0.9326**	1.0000	
weight of hip	0.6391**	0.7912**	0.7382**	0.8488**	1.0000

* = Significant (P<0.05); ** = Highly significant (P<0.01)

among all crosses. the value was 1.7g when crossed with *R. indica*. Gruss an The cross (Gruss an Teplitz×*R. damascena*) yielded maximum number of seeds/hip (17) while (*R. bourboniana*×*R. centifolia*) produced minimum number (7.3) of seeds/hip (Figure 1F). The crosses showed significant results for diameter of hips with maximum diameter of hips (1.7cm) for Gruss an teplitz×*R. centifolia*. The least value for this parameter found in cross *R. bourboniana*×*R. indica* with value of 0.5 cm.

All the parameters were correlated to estimate the degree of association with each other (Table 3). Fruit set percentage has strong and positive correlation with all other examined parameters (time to hip bearing (days), number of seeds/hip, hips diameter and hips weight. The maximum value (r =0.9326**) for positive correlation was found between number of seeds/hip and diameter of hips indicating a strong relation. While diameter of hips and weight of hips showed a strong correlation (r = 0.8488**) for each other. The weakest correlation (r = 0.6081**) was found between fruit set percentage and time to hip ripening. Cluster analysis performed on the whole set of data based on the parameters are illustrated in Figure 2. Dendrogram showed 5 main clusters at linkage distance of 40. Cluster I was comprised of *R. damascena*×*R. centifolia*, *R. damascena*×*R. indica*, *R. indica*×*R. damascena*, *R. indica*×*R. centifolia* and *R. indica*×*R. bourboniana*. Cluster II was comprised of Gruss an Teplitz×*R. damascena*, Gruss an Teplitz×*R. centifolia*, Gruss an Teplitz×*R. indica*, Gruss an Teplitz×*R. bourboniana* and *R. indica*×Gruss an Teptitz. Cluster III included three crosses which were *R. damascena*×*R. bourboniana*, *R. centifolia*×*R. bourboniana* and *R. bourboniana*×*R. damascena* while cluster IV had*R. damascena*×Gruss an Teplitz, *R. centifolia*×*R. damascena*, *R. centifolia*×*R. indica*, and *R. centifolia* × Gruss an Teplitz. The last cluster V wascomprised of *R. bourboniana*×*R. centifolia*, *R. bourboniana*×Gruss an Teplitz and *R. bourboniana*×*R. indica*. Dendrogram further

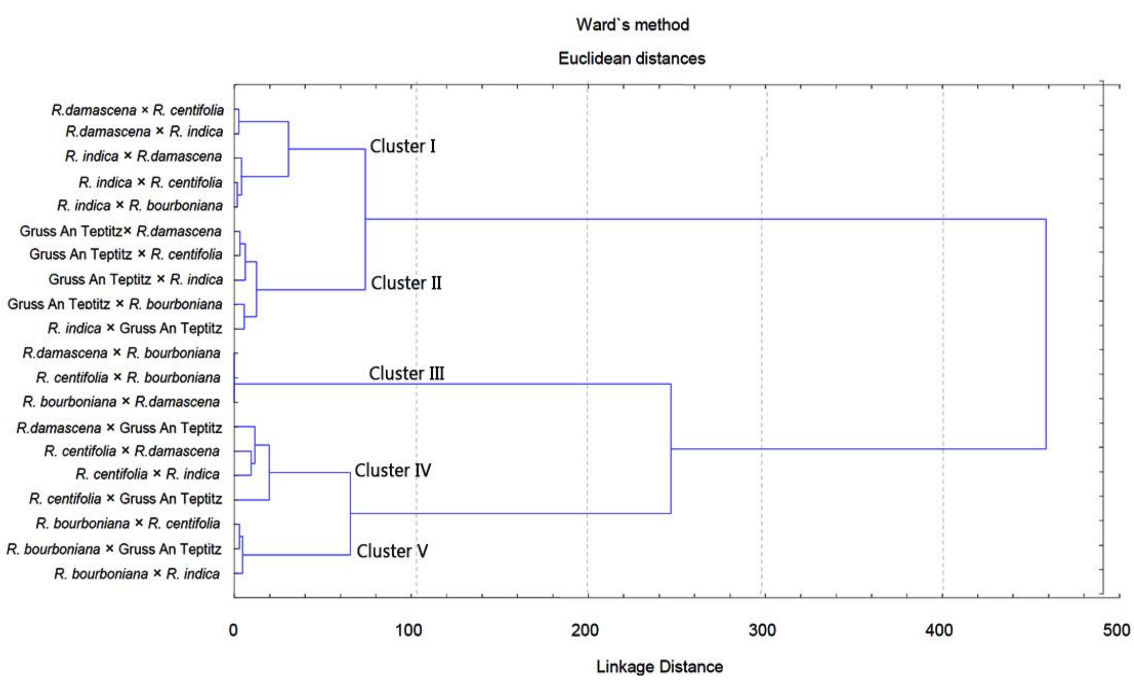

Figure 2. Tree diagram showing grouping among different crosses of essential oil *Rosa* species on the basis of examined characters in breeding trial.

subdivided these groups at linkage distance 10. The first two components of the PCA analysis explained 81.86 and 9.96% of the total variation by grouping the crosses of essential oil *Rosa* species presented in the Figure 3. The grouping of this plot confirmed the results of cluster analysis at linkage distance equal to10.

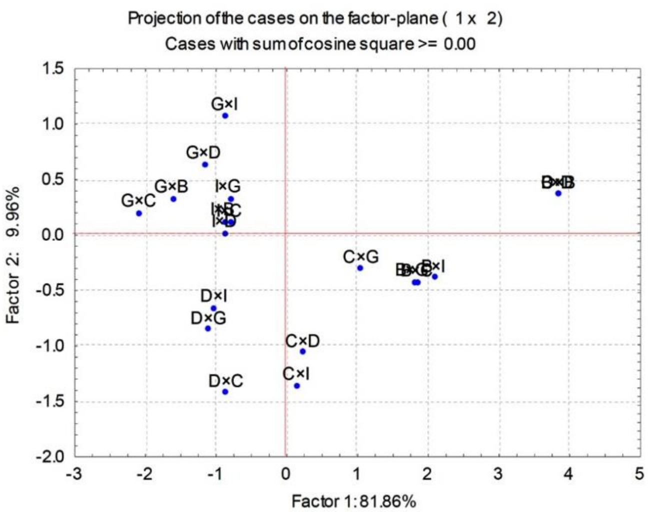

Figure 3. Plot of principal component based on means of different crosses among essential oil *Rosa* species on the basis of examined characters in breeding trial.

DISCUSSION

The key to success for potential pollination is achieved when pollens are accessed onto stigmas at very correct time (Crespel and Mouchotte, 2003). The finest time (month) for pollination is more important than the time of the day. May-June has been proved as the optimal time for pollination during the year for the Mediterranean region (De Vries and Duois, 1996). The repeated pollination practice improves the number of seeds, weight/hip and also weight of achene (Chimonidou *et al*., 2007). A rose fruit starts to appearin a couple of weeks if there is any success in fertilization (Crespel and Mouchotte, 2003). Temperature and relative humidity also effect the pollen germination. It is evident that significant improvement in pollen germination is achieved at 23-30°C, 60-65% relative humidity and low pH of female parent's stigmatic exudates (Crespel and Mouchotte, 2003). Our study showed that the maximum fruit setting was observed in Gruss an Teplitz (82.3-74.7%) followed by *R. indica* (70.3-58.0%) and *R. damascena* (53.3-26.7%). A limited success in fruit setting (4.7-0.0%) was observed in the crosses of *R. bourboniana* (as a female parent) with all other species proving that a considerable variation appeared in the values for fruit setting which is predominantly attributing to certain physiological and genetic factors. Lata (1971) and Pal (1969) reported more hips development in inter-specific crosses than in intra-specific. There are lot of issues regarding fruit setting, hybrid crash and unease occurred whereas very few (<1%) seedlings are female fertile (Svejda, 1974, 1976).

Agamospermy within *Rosa* species is already reported by a number of researchers (Ratsek *et al.,* 1941; Gustafsson and Hakansson 1942; Cole and Melton, 1986). Agamospermy is a convincing way of seed creation in a few *Rosa* species (Kroon and Zelinga, 1974; Wissemann and Hellwig, 1997; Werlemark *et al*., 2000; Crespel *et al.*, 2001; Werlemark and Nybom, 2001; Wrerlemark, 2003; Nybom *et al.*, 2006; MacPhail, 2007; MacPhail and Kevan, 2007; Nybom, 2007). The process of emasculation damages the flower and may get in the way with the hormones that could control endosperm, embryo and hip formation (Young, 1982; Stougaard, 1983; Cruden and Lyon, 1989; Stone *et al.,* 1995). Some studies demonstrated that even with emasculation, accidental selfing may have occurred at a stage before expected anthesis (Werlemark *et al.*, 1999; Macphail, 2007; Nybom, 2007).

The pollen grains after landing on the stigmatic surface of the ovary, absorb water and sugar, are germinated by producing a tube, which grows down the style to the embryo sac. The pollen tube penetrates the embryo sac, where one male gamete unites the egg to form zygote. That later on, by mitotic division, becomes the embryo. Embryo development appears to control the development of hips on roses (Gudin, 2000). The vital physiological and environmental factors, involved in manipulation of *Rosa* breeding, must be optimum to have successful results. Another factor, pollen incompatibility, prevents fertilization in addition to some genetic reasons, slashing pollen tubes from growing into styles subsequently leading to limited fruit set. All these notable factors mentioned in above paragraph vary even from flower to flower, thus affecting the fruit set in roses (Flory, 1950).

Temperature plays a pivotal part in hip development. The optimum temperature for rose breeding is reported 20-25°C while for hip development 25-30°C (De Vries *et al.*, 1996). In the area of our trial, the temperature prevalence was 40-45°C during the months of May-June (Pakistan Meteorological Department, 2006) which could be the possible reason for hip abortion and low number of hip development being considerably influenced on rate of embryo development and the thickness of the endocarp (Gudin *et al*., 1991). Similarly, low temperature and less light intensity significantly improve the hip development in hybrid tea rose (Von Abrams and Hand, 1956).

Maximum number of seeds/hip was produced by Gruss an Teplitz and *R. indica* while *R. centifolia, R. bourboniana* and *R. damascena* produced 6.2-5.4, 4.4-2.6 and 7.3-6.4 number of seeds, respectively when crossed with all other *Rosa* species. The variation in seed production is attributed to genetic and/or physiological reasons based on compulsory phenomena (pollination, fertilization and embryogenesis), consequently, embryo abortion should be a reason for statistical difference in the number of seeds. Our studies depicted very encouraging results regarding fruit setting in *Rosa* species used in this experiment as a source of rose oil. However, for a successful and sustainable cross breeding in essential oil roses must be initiated under protected/controlled conditions to avoid the climate stress and to get the maximum fruit setting and hip development**.**

Conclusion**:** Oil-bearing *Rosa* species are used in the perfumery and cosmetic industry. This study aimed to reveal the compatibility among locally grown *Rosa* species used for oil extraction. Gruss an Teplitz and *R. indica* were proved as good compatible female parents yielding maximum fruit setting. While *R. damascena* (female parent) also showed good compatibility results with *R. centifolia*. There is a need to evaluate their breeding potential through seed germination and progeny performance for the desirable characteristics under the various climatic conditions.

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