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Pollen Biology of *Grewia optiva* Drummond Genotypes: An Important Agroforestry Tree of North Western Himalayas

Saresh N.V.[1]*, Archana Verma[2], Asu Singh Godara[1], Dharmendera Meena[1] and Arjun Lal Bijarnia[1]

[1]*Agricultural Research Station (Agriculture University Jodhpur) Keshwana, Jalore 343 001, India*

[2]*ICAR-Central Arid Zone Research Institute, Jodhpur 342 003, India*

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Abstract: *Grewia optiva* is an important agroforestry tree of Himalayas because of its utility as fodder, fuel and fibre. Therefore, for breeding and conservation of the species the study of the components of pollen biology is important. In the present investigation it was observed that pollen grains of *Grewia optiva* were isopolar radio symmetric normal and fertile and size ranges from 49.00 to 74.37 µ. Positive correlation was observed among anther and flower levels (0.985), anther number and pollen, production per anther (0.686) and anther number and pollen production per flower (0.800). Pollen viability rates significantly differed among freshly collected pollen grains and stored pollen grains at 4°C and -20°C. The percentage of fruit set were found to decrease with the collection time of pollen grains, at the beginning of anther dehiscence it was 68-79%, after 6 h 39-53% and 24 h of anthesis 26-38%. The present experiment showed that 20% sucrose with BKM (Brewbaker and Kwack's medium) was the optimum medium for the germination of pollen grains. Germination percentage was maximum in genotype BI-4 (20.40%) and minimum in SO-3 (5.20%). The stigma became receptive 14-20 hours after pollination and the species is found to be self-compatible. The variation in size, pollen production, viability and germination per cent was because of genetic differences among the genotypes and external factors like temperature, humidity etc. The findings provide important insight to understand the reproductive biology of *G. optiva* and to develop planned breeding programme for the species.

Key words: Pollen biology, *Grewia optiva*, pollen viability pollen germination.

In order to meet increasing human needs for timber, firewood, fodder, fibers and other forest products four restoration strategies were developed that is conservation forestry, agroforestry industrial forestry and environment forestry (Pandey, 2007). These strategies have been vigorously implemented in various parts of India. Most of the programs use improved quality planting material obtained from rigorous genotype selection. For developing successful tree improvement programe it is necessary to study and understand the pollen biology of the candidate species. Hybridization is one of the potential method for improving planting materials in forestry.

Reproductive success of hermaphroditic plants depends on the quantity and quality of the gametes and off-springs produced. Pollen grains contain microgametophytes of seed plants which upon pollination germinate and produce pollen tubes that grow through the pistil to effect fertilization and seed set. Production and dissipation of pollen have direct impact on the quantity and quality of the seed produced. Before the pollen used for hand pollination it is necessary to study the pollen viability which determines the ability to deliver sperm cells to embryo sac (Shivanna *et al*., 1991). Therefore pollen studies plays a vital role in tree improvement programes as it determines genetic variability influenced by gene flow and heterozygosity of the population.

Grewia optiva is naturally distributed in Indian subcontinent: Bhutan, Nepal, Pakistan and in India, it is distributed in areas of Himachal Pradesh, Jammu and Kashmir, Punjab, Sikkim and Uttar Pradesh up to an elevation of about 2000 m (Brandis, 1906). It is sparingly found in forests areas and is mostly cultivated along agricultural fields (Singh, 1982). It belongs to family Tiliaceae and it is a very preferable tree by farmers on account of its utility as fodder, fuel and fibre, especially during winter period. There is a need to develop large scale

*E-mail: sareshnv@gmail.com

breeding programs for its genetic improvement, conservation and sustainable utilization. The detailed study of reproductive biology and pollen biology of the family Tiliaceae is confined to genus *Corchorus* (Patel and Datta, 1958; Rathcke *et al*., 2005; Perveen and Qaiser, 2007) *Tilia* (Anderson, 1976) and a very few species of genus *Grewia* (Hashmi and Qaiser 1990; Zietsman, 1991; Perveen and Qaiser, 2007). The information available for *G. optiva* is mainly on the reproductive and floral biology, (Pant *et al*., 1997; Verma *et al*., 2011) however for pollen biology it is lacking. In order to practice effective genetic improvement of *G. optiva* the knowledge of floral biology with pollen biology is essential. Therefore, in the present study ten *G. optiva* genotypes from five district of Himachal Pradesh were screened for better understanding of pollen biology which includes the pollen morphology, viability and germination.

Materials and Methods

Plant material and study site

Ten genotypes of *G. optiva* were collected from five different regions for study. Genotypes along with location, code and other details are given in Table 1.

The study was conducted for three successive flowering seasons between April 2010 to April 2012. To determine the pollen structure, production, viability, germination and pollen tube growth rate of genotypes branches having maximum number of flowers from each genotype were selected in the peak flowering season. Flowers were collected in paper bags and pollen biology studies were conducted out in laboratory of Department of Tree Improvement and Genetic Resources UHF, Nauni (Solan). Petals and sepals from the collected flowers were separated and the anthers were placed in sterile petri-dishes having butter paper for 6-10 hrs to release the pollen grains. Then pollen were collected and observations were made on the pollen biology often *G. optiva* genotypes by following methods:

Pollen structure and pollen shedding

The flowering branches were tagged and anthers were collected to examine morphological changes under microscope in order to determine the pattern of pollen shedding. Size of pollen grains was measured under light microscope using ocular and stage micrometer.

Pollen production

To determine the number of pollen grains per flower one mature undehisced anther per flower was removed from ten flowers each of *G. optiva* genotypes. Anthers were placed on a slide and dabbed with a squashing needle until all the pollen grains were released on to the slide. A drop of acetocarmine stain (2 µl) was put on the dehisced pollen and covered with cover slip. Pollen grains in each anther were counted with the help of photograph captured under a compound microscope (40X objective and 10X eyepiece). From this value pollen production per anther and pollen grains per flower were calculated (Bernardello *et al.,* 1994).

Pollen-ovule ratio (P/O ratio)

The pollen - ovule (P/O) ratio was determined by the number of pollen grains per flower divided by total number of ovule per flower. Buds and flowers were fixed in 70% ethanol. Ovule quantity was calculated using

Table 1. Location and details of G. optiva genotypes used for study

District	Genotypes	Code	Latitude	Longitude
Solan	Gaura	SO-1	30°90′N	77°09′E
Solan	Nauni	SO-2	30°86′N	77°16′E
Solan	Deog	SO-4	31°10′N	77°67′E
Solan	Kailar	SO-8	31°19′N	76°71′E
Solan	Dharja	SO-3	30°91′N	77°03′E
Sirmaur	Madhobag	SI-15	30°71′N	77°21′E
Sirmaur	Kalagat	SI-6	30°51′N	77°9′E
Chamba	Shahu	CH-2	32°56′N	76°10′E
Bilaspur	Kuthera	BI-4	31°56′N	76°48′E
Hamirpur	Hamirpur Kanal	HA-4	31°67′N	76°53′E

Anderson and Symon's method (Anderson and Symon, 1989).

In vitro pollen germination

The fresh pollen from each flower was placed in a petri-dish containing varying concentration of sucrose (10, 20, 25 and 30%) and Brewbaker and Kwack's Medium (BKM) (Brewbaker and Kwack, 1963) with various sucrose concentrations (20, 25, 30 and 35%) to detect the optimum level required for the species established. The optimum medium was determined by germination trial in different germination media. Periodic observations were recorded by counting the number of germinating pollen as well as the total number of pollen per microscopic field and calculated in percentage.

Pollen storage

Fresh pollen from the day of anthesis was collected for pollen storage. The anther was excised which is about to dehisce, gently brushed into butter paper and collected into eppendorf tubes and stored at -20°C in dessicator. All the vials were stored for 12 months and at the end the pollen viability was studied.

Pollen viability

In-vitro testing the pollen viability: Variation in pollen viability capacity with pollen grain age was investigated by staining experiments *in-vitro*. Aceto carmine staining method was used to test the viability of fresh and stored pollen grains.

In-vivo testing of pollen viability: Pollen viability was tested on hand pollination experiments carried out on twenty flowers for the pollens collected at different duration. In the evening hours the emasculation was carried out a day before the flower opening and after emasculation it was bagged. Stigma were pollinated with different duration of pollen that is 06 and 24 h and the pollinated flowers were bagged. Daily observations were made up to fruit set. The percentage of fruit set in pollinated flowers was calculated by counting the number of fruits present in the pollinated flowers. From this the pollen viability under *in-vivo* conditions was recorded.

Self-incompatibility studies

The flower buds expected to open next day were bagged before anthesis and left as such without external interference. This is done for each genotype under study and the data regarding the seed set was observed.

Stigma receptivity

Visual observation of stigmatic surface: The change in the appearance of stigma was observed from 24 hours before opening of bud till it withered completely. The shiny watery light green stigma was considered receptive while dull and brown was accounted non-receptive.

In-vitro germination of pollen grains on stigma: The stage at which pollen germinated on the stigma surface was taken as the stigma receptive stage. For this freshly shed pollen from other flowers was applied to the stigma surface with the help of a camel brush. For studying stigma receptivity in unopened buds, buds were carefully opened with the help of fine forceps to expose the stigma and then pollen applied. These stigmas were cut from the style after pollen application at 12, 24 and 72 hours interval and fixed in FAA (1: 1: 18 v/v formalin: glacial acetic acid: 70% ethanol).The germination of the pollen grains on the stigma was confirmed under dissection microscope.

Statistical analysis

Means ± SE were calculated for all measurements. The among genotype variation for pollen size, no of anther per flower, pollen per anther and pollen per flower were assessed by ANOVA. Correlation coefficients (r) between pollen size, anther number, pollen number per anther and pollen number per flower was calculated at $P<0.05$ and $P<0.01$.

Results and Discussion

Pollen dehiscence, morphology production and pollen ovule ratio

The anthers were mature at the time flowers started anthesizing. The surface of the anthers was smooth and a line appeared on the surface of the anthers before dehiscence. The dehiscence of anthers started from the point which is attached with the filament and goes along the periphery toward the centre and spread the pollen grains. The process of dehiscence of anther consumes 10-20 minutes duration. The observations on anthers dehiscence, is in line with the observations of Pant (1991) and Verma *et al.* (2011), who indicated the opening

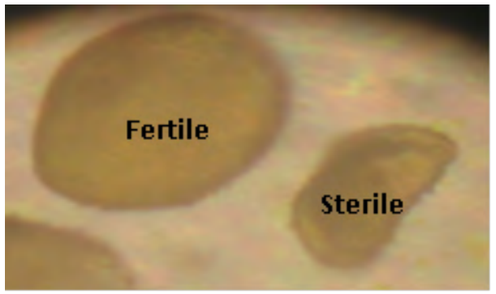


Fig. 1. Fertile and sterile pollen grains of *Grewia optiva.*

of flower and pollen dehiscence in similar fashion between 7.00 to 9.00 AM in *G. optiva*.

In mass the pollen grains exhibits a sulphur yellow color when observed under microscope. The individual pollen grain appears light yellow in color. The size of normal and fertile pollen grains' was 49 to 57 µ and sterile pollen grains were smaller ranging from 26.0 to 46.0 µ (Fig. 1). Significant (<0.05) variation was observed among different genotypes. The genotypes SO-2 (74.37 µ) and CH-2 (68.26 µ) had bigger pollen grains and genotype HA-4 (57.16 µ) and SO-3 (58.83 µ) had smaller among all the genotypes with mean pollen size of 65.21 µ. (Table 2). The genotypes were differing significantly in pollen size and the average pollen size for healthy pollen was 65.21 µ. Variation in pollen size was reported by Shrivastava *et al.* (1987) in *Moghania chappar*, Jain (2001) in members of Acanthaceae. Nagarajan *et al.* (1998) in tamarind, Wani (2005) in *Bauhinia variegata*, Dhir *et al.* (1982) in monoecious trees like *Populus deltoides* and Vaknin *et al.* (2021) in *Moringa oleifera* and *M. peregrina*.

Average number of pollen grains were 286.15 ± 31.81 (ranging from 225.50-362.70) (Table 2) in fully developed and mature anthers differing significantly (P<0.05) among some of the genotypes. The mean number of pollen grains per flower was 30089.56 ± 3522.27 (ranging from 23393.50-39228.50). Pollen number per flower was highest in SO-1 (39228.50) followed by SI-6 (38139.10) and the lowest in BI-4 (23393.50). Similarly, the pollen number/anther was higher in SO-1 (362.70) and lowest in BI-4 (225.50) (Table 2, Fig. 2). *G. optiva* contains eight ovules (four ovaries each contain two ovules). The mean number of pollen grains per ovule was 3761 thus the pollen-ovule ratio was 3761: 1. Pollen size is affected by the number of pollen grains a flower can support. In our study pollen number per flower was ranging between 24739 (HA-4) - 39228 (SO-1), whereas, average anther per flower was 104.8±39.06. Pollen per anther was significant among the various genotypes with an average of 286.15±31.81. Correlations between various parameters of pollen biology revealed a significant positive correlation between pollen per anther and pollen per flower (0.985), anther number and pollen production per anther (0.686) and anther number and pollen production per flower (0.800) (Table 3). Contrary to our observation, negative correlation in these traits was reported by Aguilar *et al.* 2002; Yang and Guo (2004). A negative correlation between number of pollen

Table 2. Pollen size (µ) anthers per flower and pollen production among Grewia optiva genotypes

District	Genotypes	Pollen size(µ)	Anther/Flowers (No's)	Pollen/Anther (No's)	Pollen/Flower (No's)	Pollen: ovule (No's)
Solan	Gaura (SO-1)	63.82	108.10	362.70	39228.50	4904
	Nauni (SO-2)	74.37	102.60	271.70	27835.50	3479
	Deog (SO-4)	64.38	102.60	275.10	28429.20	3555
	Kailar (SO-8)	67.15	112.50	326.10	36425.10	4553
	Dharja (SO-3)	58.83	104.10	303.40	31446.10	3931
Sirmour	Madhobag (SI-15)	64.38	103.70	249.90	25900.40	3237
	Kalagat (SI-6)	67.15	112.50	339.10	38139.10	4767
Chamba	Shahu (CH-2)	68.26	101.90	249.00	25358.30	3170
Bilaspur	Kuthera (BI-4)	66.60	104.70	225.50	23393.50	2924
Hamirpur	Hamirpur Kanal (HA-4)	57.16	95.30	259.00	24739.90	3092
Mean		65.21	104.80	286.15	30089.56	3761
SE±m		3.84	39.06	31.81	3522.27	
CD $_{0.05}$		11.33	77.6	63.19	6997.60	

Table 3. Correlation coefficients (r) between pollen size, anther number, pollen number per anther and pollen number per flower

Parameters	'r' value
Pollen size and pollen per anther	-0.056
Pollen Size and pollen per flower	0.039
Anther no. and pollen per anther	0.686**
Anther no. and pollen per flower	0.800**
Pollen per anther and pollen per flower	0.985**

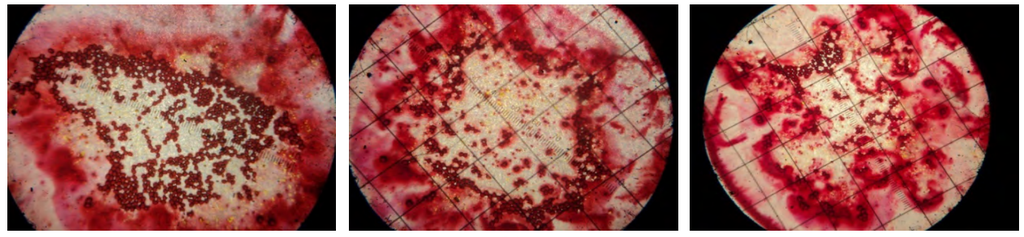
*P ≤ 0.05; **P ≤ 0.01

per flower has been well documented at both inter- and intraspecific levels in many plant groups (Mione and Anderson, 1992; Knudsen and Olesen, 1993; Stanton *et al.* (1994); Vonhof and Harder, 1995; Worley and Barrett, 2000; Sarkissian and Harder, 2001; Yang and Guo, 2004; Hulwale *et al.,* 1995). This relation has been interpreted as a simple trade-off between pollen size and number due to the limited resources available to the flower. Thus for a given species the competitive advantage of larger pollen grains may counterbalance the numerical advantage of small pollen (Sarkissian and Harder, 2001). Genotypic differences for pollen production have been reported for *Prunus armeniaca* by Alburquerque *et al.* (2004), Davarynejad *et al.* (1995) and Mahanoglu *et al.* (1995).

Pollen viability

In-vitro pollen viability

Pollen viability rates significantly differed among the genotypes tested. Data presented in Fig. 3 shows the notable difference in pollen viability percentage of freshly collected pollen grains and pollen grains stored for one year at 4°C and -20°C. Data revealed that viability percentage was higher for freshly collected pollen grains and gradually decreased in storage conditions (Fig. 3). Similar observation was recorded by Hanna and Towill, (1995), they observed that exposure of pollen with high water content to low temperatures can also lead to intracellular ice formation cell death and loss viability and germination. In case of freshly collected pollen, genotype SI-15 and SO-8 (82%) had maximum viable pollens and genotype BI-4 and SO-3 (75%) had least viable pollen. The mean pollen viability for genotypes was 68.6% varying between 60 to 73.50%. There was 50% decrease in pollen viability in pollens stored at 4°C than that of pollens stored at -20°C and fresh pollen. The viability per cent was ranging between 48.50% (SO-8) to 38.00% (HA-4). The viability of fresh pollen grains was 78.45% and the pollen stored at 4°C and -20°C were 43.05 and 68.6% respectively. The genotypes were showing significant differences for pollen viability in all the storage conditions. The variation in viability is because of genetic differences among the genotypes and other factors like temperature, humidity, organic solvents etc. appear to be most important factors for the maintenance of pollen viability. The findings are in conformity with the study of Verma *et al.* (2011) where pollen grains preserved at -20°C pollen grains retained their viability up to 72% for prolonged period than those stored under other conditions and also variation in viability was observed due to genotypic differences. This is somehow unexpected since usually the decline in pollen germination is much faster at -20°C (Hanna and Towill, 1995). In vitro pollen germination of fresh pollen was 57.1% which was maximum and it was progressively reduced with conservation time and temperatures. The results indicate by Lora *et al.* (2010), that pollen collected and stored at sub-zero temperatures at the beginning of the cherimoya blooming season can be used along the whole blooming



(a) (b) (c)

Fig. 2. Pollen production per anther (a) SO-1 genotype (b) SI-6 genotype (c) BI-4 genotype.

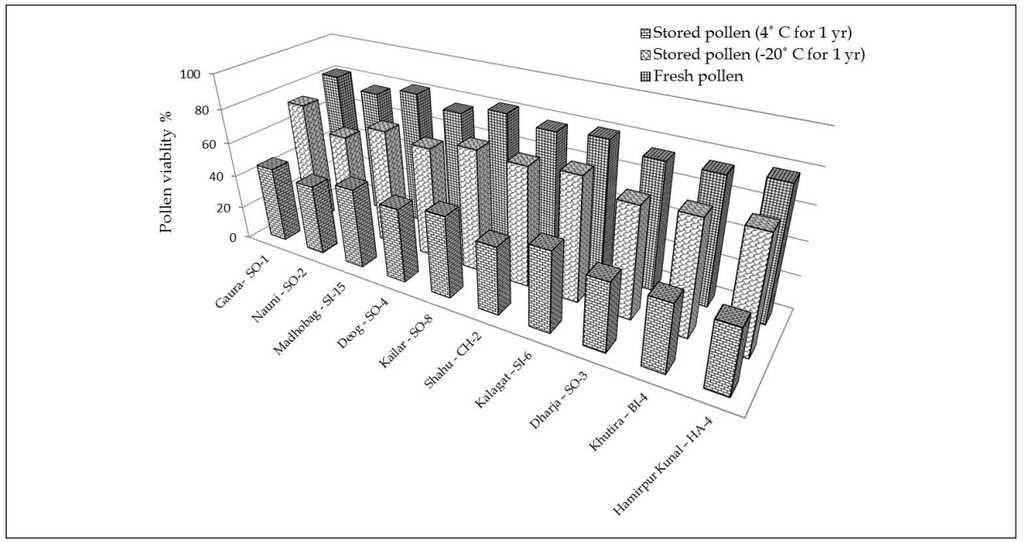


Fig. 3. Per cent viability of fresh and stored pollen grains of ten genotypes.

season avoiding the need of collecting fresh pollen daily.

In-vivo pollen viability test

The *in-vivo* testing of pollen grains viability based on the fruit set was analyzed.The percentage fruit set was found to decrease with the collection time of the pollen grains. The pollen grains collected at the beginning of anthesis showed maximum viability (68-79%). The pollen grains collected after 6 h (39-53%) and 24 h (26-38%) of anthesis showed decreased viability and hence fruit set was also declined (Fig. 4). The viability per cent in pollens collected at 0 h after anthesis, highest in SI-6 (79%) followed by SO-1 (78%) and least in BI-4 (68%) genotype whereas, SI-6 (53%) and SO-8 (51%) showed maximum viability in collected at 6 h after anthesis and least in SO-4 (39%) genotype. The result obtained

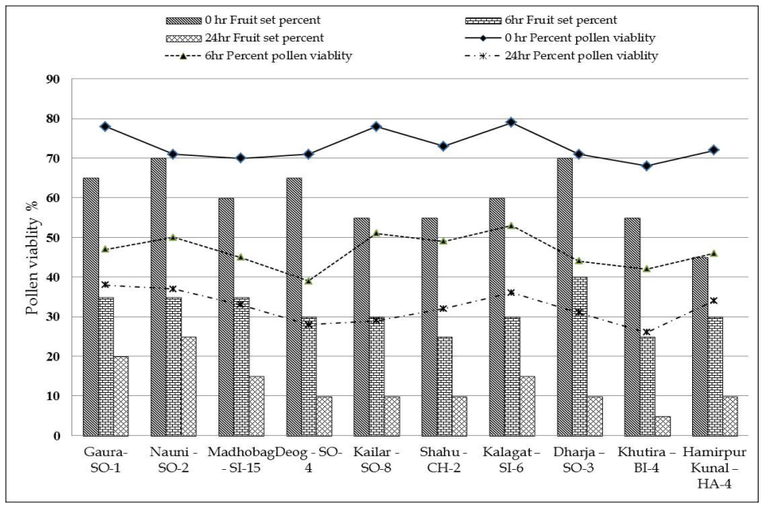
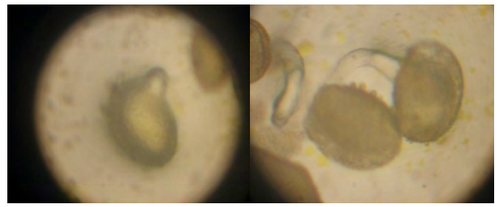


Fig. 4. Pollen viability per cent and fruit set per cent for pollen collected at different durations.



A B

Fig. 5. Pollen germination in Grewia optiva on BKM with 20% sucrose concentration A. Pollen tube initiation stage B. Pollen grain with longer pollen tube.

for pollens collected 24 h having 38% pollen viability in which was maximum in SO-1 genotype and least viability was recorded in BI-4 (26%) genotype. Overall, similar trend was noticed with respect to fruit set percentage, with reference to pollen collected at 0 h of the anthesis the fruit set was highest in SO-3 and SO-1 (78%) least was in SO-8, CH-2 and BI-4 (55%), whereas genotype SO-3 (40%) and SO-1, SO-2 and SI-15 (35%) showed maximum and CH-2 and BI-4 (39%) genotype showed least fruit set with respect to pollen collected at 6 h of anthesis. Similar observation was noticed in *G. optiva,* the viability was decreased with the time (Verma *et al.,* 2011). Thangaraja and Ganesan (2008) also recorded decreased viability with the time of pollen in treatments particularly and it was in the pollen collected after 15 h of anthesis.

Pollen germination

In different genotypes pollen germination was tried on different media like sucrose, dextrose, agar, stigmatic extract, boron in sucrose solution ranging from 5 to 30% but none of these stimulated pollen tube growth. Only in 20% sucrose solutions three pollen tubes growth was observed but tend to burst. So different levels of sucrose concentration i.e. 20, 25, 30 and 35% along with that of Brewbaker and Kwack Media (BKM) containing boric acid-100 mg l^{-1} calcium nitrate-300 mg L^{-1} magnesium sulphate-200 mg L^{-1} and potassium nitrate-100 mg L^{-1} were tried. The results revealed that 20% sucrose with BKM was the optimum medium for the germination of pollen grains of *G. optiva* all the genotypes responded for this combination only (Fig. 5). Pollen germination percentages were significantly differed for various genotypes with mean pollen germination of 12.64% for all genotypes. Germination percentage was maximum (20.40%) in genotype BI-4 followed by genotypes SO-1 i.e. (17.20%) and HA-4 (15.20%). Minimum germination of pollen was recorded for genotype SO-3 (5.20%) (Fig. 6). Artificial germination of the pollen grains is a test of pollen fertility which is important for undertaking any breeding program. The medium components required for pollination of different plant species varies (Vasil, 1960). The present investigation showed 20% sucrose

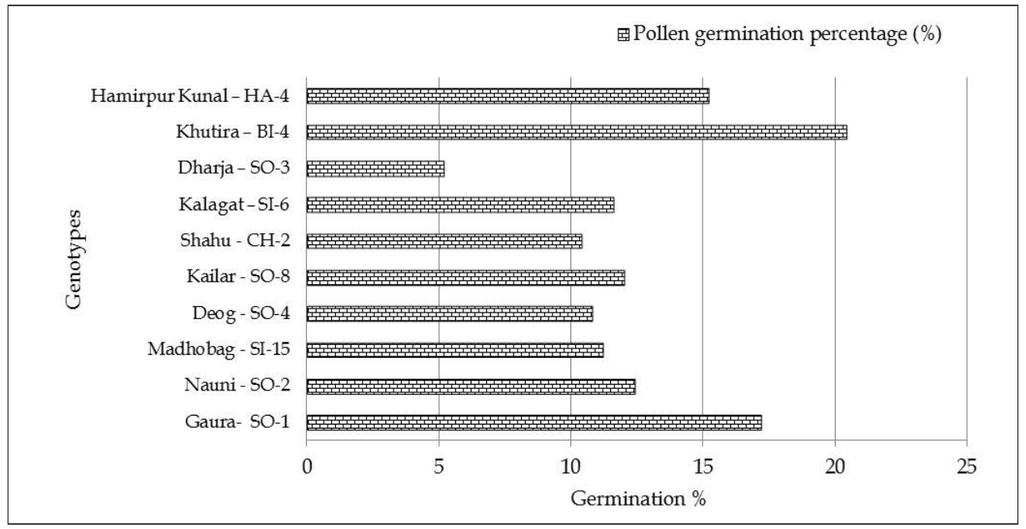


Fig. 6. Pollen germination percentage of different genotypes.

with BKM was the optimum medium for the germination of pollen grains of *G. optiva* for all the ten genotypes. Thangaraja and Ganesan (2008) also observed similar results in studies on pollen viability in *Terminalia paniculata,* where 90.10% pollen germination was obtained in 30% sucrose with Brewbaker and Kwack's medium (BKM). No germination was observed in distilled water. Contrary to this Tangmitcharoen and Owens (1997) reported pollen germination in teak using 10% sucrose with Brewbakers' solution. Contrary to our results Sharafi *et al*. (2011) reported significant differences among the genotypes in pollen germination and pollen tube growth rate in Loquat (*Eriobotrya japonica* Lindl) and Fakhim *et al*. (2011) in cultivars of peach.

Stigma Receptivity

Visual observation: In order to study the stigma receptivity by visual observation, stigma of different age groups (i.e. 24 48 and 72 hours after anthesis) were observed with the help of hand lens. The stigma was considered receptive when the lobes of stigma became fully relaxed, shining and watery green in color. The stigmas became receptive between 36 to 72 hours after anthesis (7 AM to 1 PM) and remain receptive for more than 72 hours in all the genotypes. The length of receptive period of each genotype was determined by controlled pollinations at regular interval for six days. The loss of receptivity became evident with the change in colour of the stigma lobes from watery green to dull black or brown. However, in *Dalbergia sissoo* Chauhan *et al*. (2004) observed that stigma

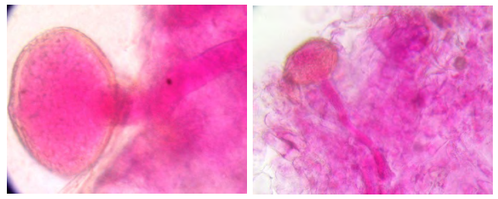


Fig. 7. Pollen tube germination on the stigmatic surface.

became receptive few hours before anthesis and remained receptive for few hours after anthesis. Wani (2005) also found that stigma was receptive in *Bauhinia variegata* from 2 hours before anthesis upto 12 hours after anthesis. The stigma is reported to be receptive at the time of anthesis in many tree crops such as peach, apricot, sweet cherry, apple and kiwi. Though the receptive period can vary with species or cultivar and requires delayed maturation of the stigma post-anthesis (Sanzol and Herrero, 2001).

In-vitro stigma receptivity: The microscopic observation showed that artificially pollinated stigmas were usually laden with abundant pollen. Differential staining confirmed the onset of receptivity in different genotypes of *Grewia* by pollen germination (Fig. 7). Pollen germination was observed in stigmas that were of age group between 36 to 72 hours. Formation of pollen tube occurred between 14 to 20 hours after pollination and loss of receptivity became evident by brown discoloration. Similar studies were carried out by Pant *et al*. (1997) in *G. optiva* where the receptivity of stigma ranged from 24 hours before anthesis to 12 hours after anthesis. However Tangmitcharoen and Owens (1997) observed that in teak within 2 h after

Table 4. Self-incompatibility in different genotypes of G optiva

District	Genotypes	No. of flowers selfed	No of flowers showing seed set	Selfing percentage
Solan	Gaura (SO-1)	22	10	45.45
	Nauni (SO-2)	25	8	32.00
	Deog (SO-4)	24	7	29.17
	Kailar (SO-8)	27	6	22.22
	Dharja (SO-3)	18	5	27.78
Sirmour	Madhobag (SI-15)	28	9	32.14
	Kalagat (SI-6)	16	5	31.25
Chamba	Shahu (CH-2)	17	5	29.41
Bilaspur	Kuthera (BI-4)	20	9	45.00
Hamirpur	Hamirpur Kanal (HA-4)	30	10	33.33
Mean		22.70	7.40	32.78
SE±m		1.54	0.65	2.30
CV		21.49	27.91	22.15

pollination 8% of the pollen tubes have reached the micropylar end of the ovule and pollen tubes first enter the embryo sac at 8 h.

Self-incompatibility: Self-incompatibility studies in the selected genotypes showed that genotypes were self-compatible, showed seed set and varying degree of selfing percentage. The data pertaining to self-incompatibility studies is presented in Table 4.The selfing percentage varied between 22.22% (SO-8) to 45.45% (SO-1). The genotypes showed significant difference ($P<0.05$) in the selfing percentage. In genotype SO-1 total 22 flowers were selfed out of which 10 flowers showed seed set with 45.45 selfing percentage. Although some of the flowers had no pollen germinated on their stigma. These results are in conformity with Verma *et al.* (2011), recorded 21.66% fruit set after selfing and Pant *et al*. (1997), recorded 40-42% selfing. High rate of fruit abortion occurs after self-pollination but some self-pollinated flowers can still develop into fruits (Tangmitcharoen and Owens, 1997). The selfing percentage was found to be maximum in genotype SO-1 (Gaura) that was 45.45%. According to the classification of Dafni (1992) the *G. optiva* is highly self-compatible species (mean fruit set was 32.78%). The reason for this may be the floral structure of the flowers as anthers are almost adhered to the stigma and the dehiscence of anthers took place before anthesis.

Conclusion

The pollen biology study of ten *G. optiva* genotypes revealed that the genotypes exhibited standard pollen germination and viability, except some decrease. The genotypes BI-4 SO-1, SO-8, SO-4 and SO-2 showed good germination viability and pollen-ovule ratio but all the genotypes not performed equally for each parameter. However the genotypes with good pollen germination and viability were selected for future hybridization and breeding programs. The species were found to be highly self-compatible. The results presented here are important for improving our understanding regarding their production biology of *G. optiva*, which would be helpful for breeding and conservation of the species.

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