DISCUSSION

The study of floral morphology and the blooming behaviour in this agro-climatic condition shows that the florets are highly heteromorphic in nature. Anthesis occurs earlier in the morning, whereas its style emergence generally occurs later in the evening or night depending upon the temperature and photoperiod. The study on its florets' blooming behaviour in different seasons of different years attending different photoperiods and temperatures showed that the gap between anthesis and style emergence was always dependent upon the above mentioned two factors and their interaction. In low temperature and short photoperiod, the anthesis occurred generally late in the morning but the style emergence much earlier in the evening and thus the time gap in this period was observed to be shortest, as seen in the year 1979-'80. In the said year, when blooming occurred at the end of December, the anthesis occurred between 9:00 and 10:00 a.m. and the style emergence between 4:00 and 4:30 p.m. and the gap between these two phenomena was only 6 to 7 hours. In the same cultivation year when the temperature was lower than the previous one but the photoperiod was slightly longer, the time gap between these two phenomena had increased by one hour approximately. In the month of March of the same year when the temperature was slightly higher but the photoperiod much longer, it was approximately 12 hours. The time gap between anthesis and style emergence was always observed in such a fashion to be dependent upon
photoperiod. It was also observed that this gap was not only dependent upon photoperiod but also on the temperature and it was quite vivid, when the time gap in the year 1978-'79 was compared with the same in the year 1980-'81. In the months of March and April as the average daily temperature differed in two years, the style emergence time also differed. As the months were constant the photoperiods were constant but the difference in daily temperature in two years caused earlier or late style emergence. Higher temperature caused late style emergence further and it was distinct when style emergence in the month of April of the year 1978-'79 cultivation season was compared with the same in the year 1980-'81.

Heteromorphic nature of these florets always facilitates cross pollination by giving the insects sufficient time for pollen transfer from one floret to the other. On the other hand these factors like temperature and photoperiod further give the insects more time to visit them and thus increase the probability for higher cross pollination. This would continue as long as the latter factors do not in any way hamper the pollination or the longevity of pollen grain or fertilisation.

The study on the pollen grain longevity in a number of years attending different temperatures and at the same time different temperatures in different months of a particular year, on the other hand, showed that generally freshly shed pollen grains germinated very quickly both in vivo and in vitro and this quick and high
germination rate was maintained only for a very few hours in vitro. This rate of germination gradually came down as the time passed and attained zero at 6.30 p.m. in all the seasons of a year or years; whereas style emergence after January was observed in this geographical area always to be after 7 p.m. or 7.30 p.m. i.e. at the time of style emergence most of the pollen grains were experimentally found to be nonviable in vitro. But when experiments with the same pollen grains were done in vivo i.e. when 10 or 12 hours old pollen grains were dusted on freshly emerged upper stigmatic surface, the same showed germination, though low in number. Such observations thus indicated that these 12 hours or more old pollen grains still maintained viability even after 6.30 p.m. But it was observed that the lag phase between pollination and germination of these old pollen grains was always very long and more than 16 hours in case of selfing and only three hours in case of crossing whereas freshly shed pollen grains, when dusted on the 12 hours or more hours old style both in selfing and crossing showed germination very quickly and within 3 hours after its dustings. The experiments thus conducted for years together revealed that the pollen grains probably attained stage of dormancy as the time passed and most of them attained this stage after 6 p.m. in all the seasons and this dormancy was assumed to be broken by the absorption of some substances secreted late in sufficient quantum in case of selfing on the upper stigmatic surface. But in crossing the pollen grain induced the upper stigmatic surface to secret this substance in sufficient quantum quickly causing rapid germination of
pollen grain. The presence and nature of these substances should be tested and verified by further experiments.

In this connection, it would be worthwhile to mention the work of Burlov (1973), and Kováčik et al. (1977) in regard to longevity of sunflower pollen grains. They showed that pollen grains longevity in sunflower could also be judged chemically besides germination in agar culture. In the work of Kováčik et al. (1977) it was most interesting to note that pollen fertility declined from 100% after 1 day of storage in ambient conditions to 78% after 5 days and then to 6% and zero after 6 and 7 days respectively.

The germination of pollen grain in vitro in different temperatures of different seasons of different years also showed that the rate of its germination was highly dependent upon temperature and it was quite vivid in the year 1980-'81. Here it was observed that with the rise of temperature, the rate of germination fell rapidly and dormancy was attained earlier.

In this context, it would be better to mention the findings of Herrero and Johnson (1980) in maize who showed that high temperature during maize pollination resulted in poor kernel set and the cause of this poor seed set was experimentally shown by the author to be due to rapid fall of pollen longevity of some genotypes in high temperatures, whereas some of the genotypes did not show any variation in germination rate in the same high
temperature. Anthony et al. (1980) also showed how temperature stress reduced the percentage of viable pollens for all cultivars of Phaseolus vulgaris.

Besides that, the experiments on pollen grain germination in vivo also showed that the upper stigmatic surface of freshly emerged style was not sufficiently sticky and so it could not possess a large number of pollen grains stucked on it at that time; whereas after twelve hours or more the same stigmatic upper surface were observed to be highly sticky and possessed a good number of old pollens and a huge number in case of newly shed pollen grains. Thus it was revealed that the upper stigmatic surface attained stickiness late. It was also observed that freshly shed pollen grains sticked more in number at the same time on the same old style. Such observation, on the other hand, indicated that freshly shed pollen grains were more sticky than the older ones. The combination of these two factors namely late attainment of stickiness by the upper stigmatic surface and high stickiness power of freshly shed pollen grains, probably caused huge number of newly shed pollen grains sticked on 12 or more hours old upper stigmatic surface.

In this context, it would be worthwhile to mention the findings of Woittiez (1979) on Petunia and Begonia spp. where it was shown that stickiness is mainly determined by the wetness of the pollen or stigma. The worker also showed that during style ageing the moisture status alters and this can cause the pollen
to fall off, depending on the wind force and the mass of the pollen grains.

Regarding the receptivity of the stigmatic surface it was observed that even at the time of anthesis, the non-emerged style were also fully receptive and was able to give good germination to the freshly shed pollen grains and in this case only four hours time gap was sufficient for pollen grain germination, even in selfing (bud pollination with new pollen in 1978-'79). The earlier attainment of receptivity by the non-emerged style was further experienced by the seed setting experiment in the year 1979-'80 through bud pollination at the time of anthesis and also at noon. Thus it was proved that attainment of stickiness and receptivity by upper stigmatic surface are two separate phenomena and they are not probably related to each other. In this regard it would be worthwhile to mention the findings of Heslop-Harrison and Barber (1975) who showed that mature stigmas of sunflower pollen were of dry type.

The earlier attainment of receptivity by the non-emerged style had also been shown in other crops like Brassica oleracea, a highly self incompatible species, by many workers like Odland and Noll (1950), Roggen (1972), Carter et al. (1975), Yun-Pu Yin et al. (1980), through their success in seed setting by bud pollination technique.
From the above discussions in respect of sunflower, it might then be concluded that newly shed pollen grains probably possessed some type of substances in it, which is essential for its faster germination and its later growth through style even in selfing. As the time passes, the substance is anyhow destroyed or consumed and this results in no germination in vitro. On the other hand, late germination of old pollen grains in case of selfing and early germination in case of crossing on the freshly emerged stigmatic surface indicates that the said germinating substance is also produced on the upper stigmatic surface and the old pollen grains dusted on it might slowly absorb that from the upper stigmatic surface and germinate. The loss or destruction of pollen grain germinating substance in it is assumed to be the cause of pollen grain dormancy as observed in vitro experiments in different temperatures of different seasons in different years. It was also revealed through the experiment in 1980-'81, that this probable loss or destruction of the germinating substance causing earlier dormancy was rapid in higher temperatures.

Quicker germination of old pollen grains on freshly emerged style in crossing in comparison to selfing, on the otherhand, indicated that pollen grain surface might possesses some type of different substance which act in the lock and key manner on the stigmatic upper surface and quickens the secretion of germinating substances on the upper stigmatic surfaces. This lock and key arrangement is probably activated only when the genetic make up of pollen grain and stigmatic surface will be of different types.
In this context it would be worthwhile to mention the physiological classification of incompatible species by Lewis (1954) where it had been shown that in the species having heteromorphic type of floral morphology might possess any type of physiology between the two—namely, complementary stimulant and oppositional inhibitor.

The physiology of sunflower pollen grain thus indicated that insect visit is essential not only for the easy movement of freshly shed pollen grains towards the older styles, but also, for one plant to other having different genetic make-up in respect to specially self-incompatible allele. Thus, not only the heteromorphic nature of florets but the physiology of pollen grain and stigmatic upper surface and their genetic make-up play roles in the high cross pollination behaviour of this crop. All these latter phenomena together, thus, to some extent, govern the self-incompatibility nature of the said crop. From the above experiments it was also revealed that here upper stigmatic surface also plays a major role in regard to the germination of any type of pollen grains.

The role of upper stigmatic surface in the self-incompatibility reaction in sunflower was further clarified in the seed setting experiments where different selfing mechanism was mainly applied. Here it was observed that this upper surface not only controlled the germination of pollen grain, but also its further growth through it.
The pollen grain viability experiments in vitro also showed that among the huge pollen grains dusted on the best agar medium even just after their shedding, only a very few germinated, most of them did not show any symbol of germination. Similar observations were also obtained in vivo (1977-’78). It might be, that these non-germinating pollen grain secreted some anti-substances during its growth, that might inhibit the germination of others and such mutual inhibition was probably one type of competition rather than growth of a huge number of pollen tubes through a narrow stigler canal and reflected economic utilisation of stored food resource by the former.

The experiments on pollen grain germination and their penetration through the upper stigmatic surface in different types of mating, namely selfing and crossing in the year 1977-’78 showed that in crossing, pollen grains were not only quicker in germination but also rapid in penetrating the stigmatic upper surface; whereas in selfing the reverse phenomenon was observed i.e. very late to germinate and most of them did not penetrate the upper stigmatic surface. As a result of which after 12 hours of dusting, 21% of stigmatic surfaces were fully devoid of pollen grains and the rest only with bulged pollen grains; whereas in crossing, just after 4 hours of pollen grain dusting, 100% new stigmatic surfaces were found to possess old pollen grains and among them 57.4% with germinated pollen grains having normal pollen tube growth. In crossing involving new pollens and old styles, 90.7% of stigmatic surfaces
possessed germinated pollen grains after 4 hours of dusting and after 7 hours it was 100% and in the latter cases the number of germinated pollen grains with normal tube growth were also more in number than that shown in case of old pollen and new style.

Thus the observation revealed that:

(1) the germination of pollen grains and their further growth were greatly inhibited in selfing, in contrast to crossing and this inhibition occurred mainly on the stigmatic upper surfaces. The low germination of pollen grains and their less penetrance through the upper stigmatic surface in selfing or in incompatible mating was also shown by Vithanage and Knox (1977).

On the basis of this observation i.e. hindrance at the upper stigmatic surface, it is assumed that incompatibility phenomenon in sunflower might be of sporophytic type (Linskens, 1963). The great role of stigmatic upper surface in the incompatibility reaction was also later proved through the experiments on seed setting by different types of selfing procedure. The presence of sporophytic type of incompatibility in sunflower was also suggested by Habura, 1957 (cf. Pimuth, 1957), Stojanova, 1969; Vithanage and Knox, 1977; and Naskar, 1979).

(2) A great fall in the number of germinated pollen grains per style in case of old pollen grains on new style thus confirmed the results on viability experiment in vitro that there was surely loss of some substances present in freshly shed pollen grains for
their germination and so they attained a stage of dormancy which was later broken to some extent in vivo through the absorption of the same from the upper stigmatic surfaces in both selfing and crossing. In selfing the process was slow but in crossing it was quick.

(3) Fertilization of ovules are anticipated to be mainly caused by the freshly shed pollen dehisced 12 hours later to the emergence of style.

The results of the high rate of pollen grain germination and its faster growth rate in GA in vitro indicated that in crossing or compatible mating, some GA like substances might be induced to secrete rapidly by the said pollen on the stigmatic upper surface and in the styler tissue which not only increased the percentage of germination but also resulted in rapid growth through stylar canal. It might be that due to the same reason 90.7% of stigmatic surfaces were observed to possess germinated pollen grains, after 4 hours and 100% after 7 hours with normal tube growth, whereas in selfing with the same old pollens, the more or less similar type of observations were made only after long hours of dusting.

It might be noted that here GA was applied in very low concentration (5 ppm). The application of GA$_3$, on the other hand though at higher concentration (50 ppm), was also observed to be followed by Miller and Fick (1978) for pollination control. Anyhow, the role of the same in low concentration in vivo should be verified in the future programme.
To overcome the self incompatibility phenomenon in sunflower, different selfing procedures were adopted in different years attending different photoperiods and temperatures, as production of selfed seed is essential in developing inbred lines for the production of hybrid seeds and in exploiting hybrid vigour commercially.

During the adoption of different selfing procedures it was revealed that even simple bagging (natural selfing) was quite helpful and gave a good amount of seed set per capitulum if the said procedure was practiced during short day length and at low temperature as seen in the year 1979-'80, up to the middle of January. This high seed set was probably possible because during the short photoperiod and low temperature the gap between the anthesis and style emergence time was shorter and during this short gap the pollens shed in the morning did not reach the stage of dormancy at the time of style emergence even *in vitro* and due to low temperature the pollen grains were also slow to reach the stage of dormancy. So fresh collection of germinating substances by these old pollen grains from the upper stigmatic surface did not probably arise. But the same selfing procedure, when adopted in later period of the cultivation season, did not give good seed set in any year and that was probably due to longer photoperiod that caused late style emergence which was always observed to occur after 6.30 p.m. and this was repeatedly observed in years together to be the last boundary time for pollen grains to attend the stage of full dormancy.
It was also observed that during this short photoperiod and low temperature any selfing procedure that caused easy pollen grain movement from florets to florets with or without injury on the upper stigmatic surface resulted in better seed set than natural selfing as seen in the year 1978-'79, 1979-'80 and 1980-'81, in the case of soft brushing at the time of anthesis and noon, soft brushing at the time of style emergence, steel brushing at anthesis, steel brushing at noon, forced pollination after opening the non-emerged stigma at morning and noon. It was also observed that with the increase in the photoperiod, the time gap between anthesis and style emergence increased and the seed set in all the selfing procedures gradually declined.

The different selfing procedures adopted in different seasons attending different photoperiods and temperatures also showed that mild injury to the upper stigmatic surface or removal of stigmatic upper portion always increased the seed set per capitulum than natural selfing, but heavier injury to the style might cause some less seed set and it was quite distinct in the year 1980-'81. Here it was observed that selfing with steel brush involving the old pollen and new stigma gave always better seed set than decapitation followed by pollination involving the same pollen and the same style. The experiment of the same year also showed that freshly shed pollens were always more active than old pollens and it was distinct when the pollinations with two different types of pollen grains, fresh and old were performed separately after decapitation.
of stigma. It is necessary to mention that the said technique of styler excision technique or decapitation was first followed by Straub in the year 1947 (cf. Brewbaker and Mazumdar, 1954) in Petunia infIata L. Anyhow, in the case of new pollen grains the seed set through decapitation was more in number in all the seasons. The higher activity of fresh pollen grains in regard to seed-setting was also observed in different seasonal temperatures in the same year when soft brushing in the morning at the time of anthesis was only practised and the same was compared with natural selfing. In the latter process there was no agent present that would cause movement of fresh pollen grains from the new florets to the 12 hours old style; whereas in the procedure of soft brushing at the time of anthesis, the difficulty in pollen movement was nullified and this pollen transfer was probably the cause why high seed setting in the said procedure occurred. This high seed setting in soft brushing at the time of anthesis, on the other hand, again proved the presence of some substance in the fresh pollen that not only helped in its germination but also in its growth through the style. The said year's experiments also showed that even mild injury at the time of anthesis involving new pollen grains and old style could not bring any more sharp improvement over soft brushing at the time of anthesis. The seed setting in this type of selfing (steel brushing at the time of anthesis) was not even equivalent to the pollination of decapitated style involving the same pollen and style, rather it was lower in all the temperatures. Not only that the said steel brushing also gave much lower seed set than the
steel brushing at the time of style emergence in all the temperatures. The above type of low seed set in steel brushing at the time of anthesis, where very high seed set was anticipated, might be due to gradual accumulation of pollen tube growth inhibiting substance below the upper layer of stigma. The said inhibiting effect could only be nullified to some extent by the removal of stigma as seen in case of decapitation of old stigma at the time of anthesis followed by pollination with fresh pollen grains. In this context, it would be worthwhile to mention that the said steel brush pollination technique for overcoming incompatibility in other crops was also followed by Roggen and Vandijk (1972) in *Brassica oleracea* and showed that the seed set in this process was more or less equivalent to bud pollination.

The higher seed setting percentage in bud pollination in the year 1978-’79 and 1979-’80 involving new pollens and pre-emerged stigma in comparison to natural selfing in the corresponding years and the similar high seed set in the same years in the selfing after brushing at the time of style emergence indicated that up to the time of style emergence when the latter occurs within 12 hours after anthesis, pollen tube growth inhibiting substances does not probably accumulate in sufficient quantum on the upper stigmatic surface. As time passes, the concentration gradually increases and reaches the lower zone and probably so, inhibition of pollen tube growth also occurs at higher rate even after steel brushing in the morning thereby the seed setting in the latter procedure did
not differ sharply from seed setting in soft-brushing in morning and this inhibition in morning could to some extent be nullified by the decapitation of the same 12 hours old style at the time of anthesis as discussed previously.

In this context, it would worthwhile to mention the findings of Gomai and Hinata (1971) and Nishio and Hinata (1977, 1980) who reported that the stigmas of each homozygous self incompatible inbreds have a specific and unique antigen which are identified as glycoproteins and the specific glycoproteins are only observed in mature stigmas but not in young ones.

Thus the above selfing procedures on seed setting experiment showed how stigmatic upper surface plays its role in the incompatibility phenomenon. The said procedures also showed the importance of pollinating agent in pollen grain movement from new rows of florets to the old florets that bloomed on the previous day and from one capitulum to the other. The latter phenomena also proved the presence of some growth substance in freshly shed pollen grains in sufficient quantum, essential not only for its germination but also for its growth through the style and that ultimately resulted in higher fertilization and better seed setting even in selfing.

The various selfing procedures adopted in different natural temperatures and photoperiods also revealed that with the increase in temperature or photoperiod the seed setting always declined as seen in the year 1980-'81. Such fall in seed-setting in sunflower
at much higher temperature even in open pollination was also reported by Naskar (1972) and Vranceanu et al. (1979). It might be that low relative humidity at high temperature was the cause of such low seed set in general as shown by Henry (1980) in *Dffenbachia maculata*. In this context it would be worthwhile to mention the work of Ascher and Peloquin (1970) who showed that in incompatible mating incompatibility reaction was accelerated with the increase in temperature and probably that might also be another cause why seed setting in selfing was so low and even nil at high temperatures. The finding of Pinthus (1959) in sunflower in respect of effect of temperature on seed setting in selfing was also worthy to mention. Similarly Flaschenriem and Ascher (1980) showed that winter environments increased self seed yield in self-incompatible *Petunia hybrida*.

The stronger inhibition of pollen tube growth *in situ* at higher temperatures shown by Lewis (1952) and Straub (1958), should also be mentioned in this respect. All these analyses of different workers thus give the clue why seed set in sunflower in different types of selfing at higher temperatures was quite low or nil. In this respect it would be worthwhile to mention the findings of Vranceanu et al. (1979) in sunflower. He showed that high temperature (24° - 35°C) during flowering caused a serious set back in the percentage of fertile seeds as a result of reduction in the pollen density and pollen tube growth.
The result of sib-mating in the year 1978-'79, on the other hand, revealed that it, in general, gave higher seed set than any selfing procedures so far adopted, though in some cases very low seed set were obtained. In this context, it would be worthwhile to mention that the pollen grain behaviour in sibmating in regard to its germination. It was observed that even the old pollen grains were very quick to germinate on foreign styles. All these results in sibmating thus indicated that lock and key type reaction might be present between styles and pollen grain coming from two plants of different genetic make up and in only proper assemblage secretion of pollen grain germinating substance is rapidly initiated on the stigmatic upper surface and the same complementary product might also stop the secretion of pollen tube growth inhibiting substance in the style. As a result the pollen tube growth are probably very rapid and results in high fertilization or the complementary product nullifies the inhibition at the embryo development after proper fertilisation and thus causing high seed set. This should be verified by further experiments in later periods.

More or less similar type of hypothesis was first proposed by East in 1926, 1929 and later by Lewis (1952), Linskens (1960), Makinen and Lewis (1962) and Lewis et al. (1967) through their antisera experiments. Nashrallah and Wallace (1967) demonstrated the presence of antigenic substance that were specific to each S-genotype in the tissue of the pistils of cabbage. Using radioactive tracers Linskens (1958, 1959, 1960) was able to show that
the proteins of the pollen and style formed a complex in selfed pistils. In regard to nature of the incompatibility reaction Lewis et al. (1967) and Stadny and Linskens (1965) showed that intact pollen grains and pollen tubes diffuse out different proteins including enzymes such as amylase and invertase. Pandey (1967) suggested that each S-allele is associated with a specific combination of peroxidase iso-enzymes. In this regard Linskens (1965) and Linskens and Tupy (1966) after observing the differences in amino acid pool of styles of incompatible plants after selfing and crossing suggested that antibody production is inhibited by repressor produced by an enzyme identical to the antibody, the antigen by combining with the enzyme prevents the production of the repressor and so promotes the production of the antibody.

High reciprocal differences in seed set in sibmating also indicated the presence of sporophytic type of incompatibility as described by Lewis (1954).

Open pollination gave maximum seed set in all the seasons of different years as pollination in this case was frequently done by mixtures of pollen grains of different genetic make-up, as a result of which there lies no question of pollen tube growth hindrance through the style and this ultimately led to high fertilization and high seed set. In this respect it would be worthwhile to mention the finding of Naskar (1979) who definitely showed how pollen mixture could increase seed set in sunflower. Empty seeds in
the capitulum of sunflower even after open pollination have been described to be due to different reasons other than self-incompatibility by many workers; for example, Mohsov (cf. Khanna, 1972) and Khanna (1972) described it to be due to insufficient nutrient supply (either phosphorus or potash) to the developing seeds, Vranceanu et al. (1979) to be due to high temperature, Barbier and Amid (1966), Free and Simpson (1964), Free (1970), Langridge and Goopman (1974), Cirnu et al. (1978) and Basavanna (1979) due to lack of insect pollination and Mohsov (cf. Khanna, 1972) due to inadequate water supply.

On the basis of all these results in different types of selfing in different years attending different temperatures and photoperiods, the following suggestions were made to develop inbreed lines:

1) attempt should always be made to grow sunflower in such a fashion so that flowering must come in the shortest photoperiod and at low temperature,

2) some procedures must be adopted for easy pollen grain movement towards the upper surface of stigma,

3) among the different selfing procedures, steel brushing at the time of style emergence is most easy to practise and gives best seed set after which soft brushing at the time of anthesis could be named, as the latter process requires minimum skill and the time of pollination is quite favourable, though the seed set by the said procedure is to some extent lower than the former one.