# Precocious germination of pollen grains in anthers of Soybean (*Glycine max* (L.) Merr.)

**Satwinder Kaur, Harsh Nayyar[1], R.K. Bhanwra and Sanjeev Kumar**

Department of Botany, Panjab University, Chandigarh 160 014, India

**Abstract**

While examining the pollen fertility in different genotypes of soybean (*Glycine max* (L.) Merr.), precocious pollen germination was noticed in all the ten anthers at bud, half-open flower and fully-open flower stages. Genotypic variation existed for this trait and out of the six genotypes evaluated, 'Palam soya' showed the least germination (17%) while 'Harit soya' had the highest germination (90%) at half-open stage. At fully-opened stage, all the pollen grains appeared to be germinated prior to their release from anthers. Stigma receptivity was relatively more in genotypes having higher *precocious* pollen germination indicating a positive correlation between the two. Some preliminary observations on this phenomenon, hitherto unknown in soybean, are reported here that may have significant implications in soybean breeding.

***Keywords:*** anthers, *Glycine max*, soybean, precocious pollen germination,

---

[1]Corresponding author: P.O. Box 1217, Panjab University, Chandigarh, 160 014,

email: nayarbot@pu.ac.in; harshnayyar@hotmail.com

Soybean (*Glycine max* (L.) Merr.) is an important pulse crop that is a source of edible vegetable oil and high protein feed supplements for livestock. Its seed is also used for wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987). Precocious germination of pollen grains in anther loculi is reported to occur in cleistogamous flowers (Maheshwari, 1962). It has also been found to be present in some chasmogamous flowers like *Lathyrus sativus (*Verma and Grewal, 1971), *Trifolium dubium* Sibth. (Sharma and Koul, 1996), *Catharanthus roseus* (Mishra and Kumar, 2001) and *Trifolium fragiferum* L. (Dhar *et al*. 2002).

While investigating the pollen fertility in different genotypes of soybean, we noticed *precocious* pollen germination in anther loculi and found genetic variation with respect to this phenomenon. To the best of our knowledge, this phenomenon is unreported in soybean and we present here our observations in this context with some comments on its possible causes and significance. These observations might have significant implications in soybean breeding.

## Material and Methods

Six genotypes of soybean (*Glycine max* (L.) Merr.) namely 'Brag', 'Harit soya', 'Lee', 'Palam soya', 'Panjab-1' and 'Shivalik' were raised in earthen pots (30 cm height, 25 cm diameter, 14.72 L volume) having a mixture of air dry soil, sand and farm yard manure in ratio of 2:1:1 (v/v). The soil was loam with a pH of 7.1 and available N, P, and K at 54, 43 and 158 kg $ha^{-1}$, respectively. The seeds after inoculation with *Rhizobium* were planted in each pot in second week of June and after emergence, the plants were thinned to 2 per pot. During onset of reproductive phase, about 100 buds and flowers (half open

and fully open stages) were harvested from each genotype at 10 am and subjected to analysis of pollen fertility and stigma receptivity. The anthers and stigma were carefully removed and examined for pollen germination, pollen load on stigma, pollen tube growth and stigma receptivity. Anthers were stained with 0.5% acetocarmine solution for pollen tests. Percentage pollen germination was determined on the basis of at least 200 grains per replicate. Mean pollen tube length was taken as the average of 45 randomly chosen tubes per replicate. For *in vivo* studies, stigmas of flowers were stained with acetocarmine and the number of germinated and non-germinated pollen grains were counted. The stigma receptivity was examined with esterase test using α-naphthyl acetate as a substrate in the coupling reaction with fast blue B (Mattson *et al*., 1974; Turano et al. 1983). Stigmas were immersed in the working solution, and incubated at 37 $^{o}$C for 15 min. A positive test for esterase was indicated by a deep purple stain. Staining intensity was then scored on a 1-5 scale (1, low and 5, high). Scanning electron microscopy studies were conducted according to Postek *et al*. (1980). The flowers were collected at the time of bud, half-open flower and fully opened flower stages. Photographs presented here refer to half-open flowers of 'Bragg' genotype. The dehydration of flowers was done for 30 min each in a series of alcohol concentrations as 50, 60,70,80, 90 and 100%. After dehydration, the flowers were subjected to critical point drying using the liquid carbon dioxide with CPD7501 critical point dryer. After CPD, stigmas were placed on the aluminum stub having double adhesion tape, and conducted with gold with JFC 1100 sputter. The stigmas were examined under JSM 1100 JEOL scanning electron microscope at different magnifications.

## Results and Discussion

The soybean flower is a standard papilionaceous flower with calyx of five united sepals; zygomorphic corolla of carina, alae, and vexillum; androecium of ten diadelphous 9+1 stamens; and gynoecium of a single carpel. The stigma is knob-shaped (Figure 1a). It has two series of stigmatic hairs arising from proximal region, which encircle about two-third of the stigmatic surface. Precocious pollen germination was noticeable in all the ten anthers examined (Figure 1b)**.** Mean germination values at bud stage ranged between 17-90% among different genotypes (Table 1). 'Palam soya' showed the least value while 'Harit soya' had the highest germination at this stage. By the time, flowers reached the half-open stage, pollen germination was found to be complete in anthers of all the genotypes. The anthers with germinated pollen grains were found to make a cluster around the stigma at half-open stage (Figure 1c) and the germinated pollen grains were lying on the stigma (Figure 1d). The number of germinated pollen on the stigma varied from 80 ('Bragg') to 123 ('Lee') per flower (Table 1). Pollen tube growth (Table 1) varied from 121 µm (Panjab-1) to 222 µm ('Palam soya').

In our findings, a deviation appears to occur in the functioning of pollen grains from the normal manner. Conventionally in soybean, the anthers dehisce on the day of anthesis, pollen grains fall on stigma, germinate and within hours the pollen tubes reach the ovary and fertilization is completed (Johnson and Bernard 1963). But, in our studies, anthers dehisced even prior to anthesis (at bud stage) and released a varying proportion of germinated pollen grains on to the stigma indicating early maturity. At half-open stage of flowers, stamens appeared closer to stigma and a large number of germinated pollen grains were present on the stigma (Plate 1d). Similar situation existed at fully-opened

stage but there was reduction in count of germinated pollen grains (Table 1), possibly because of their shedding due to anthesis. Fertilization, however, occurred in 10-15 hours after anthesis that matched the earlier findings (Johnson and Bernard 1963). Stigma receptivity was relatively lower at bud stage (Table 1) but increased subsequently at half-open stage (Table 1) that appeared to coincide with release of pollen on stigma, as described above. Interestingly, stigma receptivity was more in those genotypes that also showed higher precocious germination pointing towards a positive correlation. Previous studies indicate that the stigma in soybean becomes receptive to pollen one day before anthesis and remains receptive for 2 days after anthesis (Peterson *et al*., 1992).

Soybean is cultivated as a 'kharif' season crop in the month of June in northern parts of India and its flowering phase in August coincides with monsoon rains. *Precocious* germination of pollen in anthers in our studies thus might be related to high relative humidity (78% ± 1.1) prevailing during the investigation that possibly acted as a trigger. Involvement of high moisture in this process was verified by application of mist of water on buds and flowers on dry days that appeared to induce *precocious* germination to some extent. In this context, our observations correspond with Dhar et al. (2002) who also correlated *precocious* pollen germination in one of the three species of *Trifolium fragiferum* L. with high relative humidity and high soil moisture. These authors also indicated a genotype-specific expression of this trait.

This phenomenon could be genetically governed as reported in *Catharanthus roseus* (Mishra and Kumar, 2001). In this plant, a monogenic recessive mutation in the white flower mutant, *Rpt* of this species has been found to control *precocious* pollen grain germination. Other possible reasons may include either poor control mechanisms

for germination at metabolic level, as it happens in case of vivipary where reduced abscisic acid levels have been implicated (Finkelstein *et al.,* 2002) or it could be a deliberate exercise in the plant to ensure selfing. On the other hand, in *Lathyrus sativus*. Verma and Grewal (1971) argued that this mechanism was an adaptive phenomenon for impeding and, consequently, delaying the discharge of pollen from anthers by obstruction caused due to germinated pollen, and thus hindering self-pollination These authors reasoned that due to smaller size of pollen, poor stainability and bloated pollen tubes, *in vivo* germinated pollen were ineffective for fertilization but could prevent the release of effective pollen grains. No such abnormalities could be noticed in our studies.

It is concluded that *precocious* pollen germination in soybean might be a mechanism to facilitate high degree of selfing. Since, it could be noticed even at bud stage, it might interfere in hybridization efforts for soybean breeding. These observations need further probing to find out the underlying metabolic mechanisms of this anomalous behavior.

**Acknowledgement**

First and second authors are thankful to UGC, New Delhi for fellowship and research project, respectively.

**References**

Dhar R, Sharma N and Koul A K 2002 Intra-population variation in site of pollen germination and pollination in *Trifolium fragiferum* L.; *Phytomorphology* 52: 323-330

Finkelstein R R, Gampata S S L and Rock C D 2002 Abscisic acid signaling in seeds and seedlings; *Pl. Cell* 14: S15-S45

Johnson H W and Bernard R L 1963 *The Soybean* (Academic Press: New York, USA).

Maheshwari J K 1962 Cleistogamy in angiosperms; in *Proc. Summer School Bot Darjeeling,* pp. 145-155 (eds) P Maheshwari, B M Johri, and I K Vasil (Ministry Sci. Res. Cult. Affairs: New Delhi, India) pp 145-155.

Mattson O, Knox R B., Heslop-Harrison J and Heslop-Harrison Y 1947 Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions; *Nature* (London) 247: 298-300

Mishra P and Kumar S 2001 A monogenic recessive mutant with precocious *in situ* pollen germination in *Catharanthus roseus* L.; *JMPAS* 23: 277-279

Peterson C M, Mosjidis C O H, Dute R R and Westgate M E 1992 A flower and pod staging system for soybean; *Ann. Bot*. 69: 59–67

Postek M T, Howard K S, Johnson A H. and McMichael K L 1980 *Scanning Electron Microscopy, A Student's Handbook* (Ladd Research Industries Inc : Williston, USA)

Sharma N and Koul A K 1996 *In situ* pollen germination and selective ovular abortion in *Trifolium dubium* Sibth.; *Curr. Sci.* 77: 598.

Smith K J and Huyser W 1987 *Soybeans: Improvement, Production, and Uses* (American Society of Agronomy : Wisconsin, USA)

Turano M J, Baird L M and Webster B D 1983 Characteristics of the stigma of chickpea; *Crop Sci* 1033-1036.

Verma S C and Grewal S S 1971 Precocious germination of pollen in *Lathyrus sativus* and its significance; *Phytomorphology* 21: 362-367

**Table 1.** Precocious pollen germination (%), pollen load (number), pollen tube growth (µm) and stigma receptivity in different soybean genotypes. Germination was observed at bud, half-open and anthesis stages. Figures in parenthesis indicate stigma receptivity (1-5 scale). Data represent mean values ± S.E. Differences between genotypes are significant at P<0.05.

| Genotype | Bud stage | Half-open flower | Fully-opened flower | Pollen Load at half-open stage | Pollen Tube length ( *in vivo*) at half-open stage |
|---|---|---|---|---|---|
| Bragg | 27.6 ± 1.2 (1.4 ± 0.6) | 100 (3.8 ± 0.3) | 42.9 ± 1.2 (3.7 ± 0.2) | 80 ± 1.2 | 136.8 ± 7.8 |
| Harit Soya | 89.6 ± 1.6 (4.1 ± 0.3) | 100 (4.3 ± 0.4) | 86.0 ± 1.8 (4.2 ± 0.3) | 86 ± 2.1 | 152.1 ± 8.4 |
| Lee | 29.5 ± 1.3 (1.6 ± 0.2) | 100 (2.8 ± 0.3) | 45.1 ± 2.1 (3.9 ± 0.6) | 123 ± 2.4 | 166.6 ± 6.5 |
| Palam Soya | 17.1 ± 1.7 (1.2 ± 0.2) | 100 (3.7±0.24) | 34.5 ± 2.3 (3.8 ± 0.12) | 87 ± 2.3 | 222.9 ± 7.3 |
| Panjab-1 | 79.4 ± 1.8 (3.4 ± 0.3) | 100 (3.9 ± 0.2) | 92.2 ± 2.4 (3.9 ± 0.15) | 112 ± 2.5 | 121.6 ± 7.2 |
| Shivalik | 77.1 ± 1.5 (3.5 ± 0.13) | 100 (3.9±0.18) | 89.8 ± 2.3 (3.7 ± 0.12) | 100 ± 1.8 | 202.6 ± 6.8 |
| L.S.D. (P<0.05) | 3.8 | 1.2 | 2.8 | 4.6 | 3.8 |

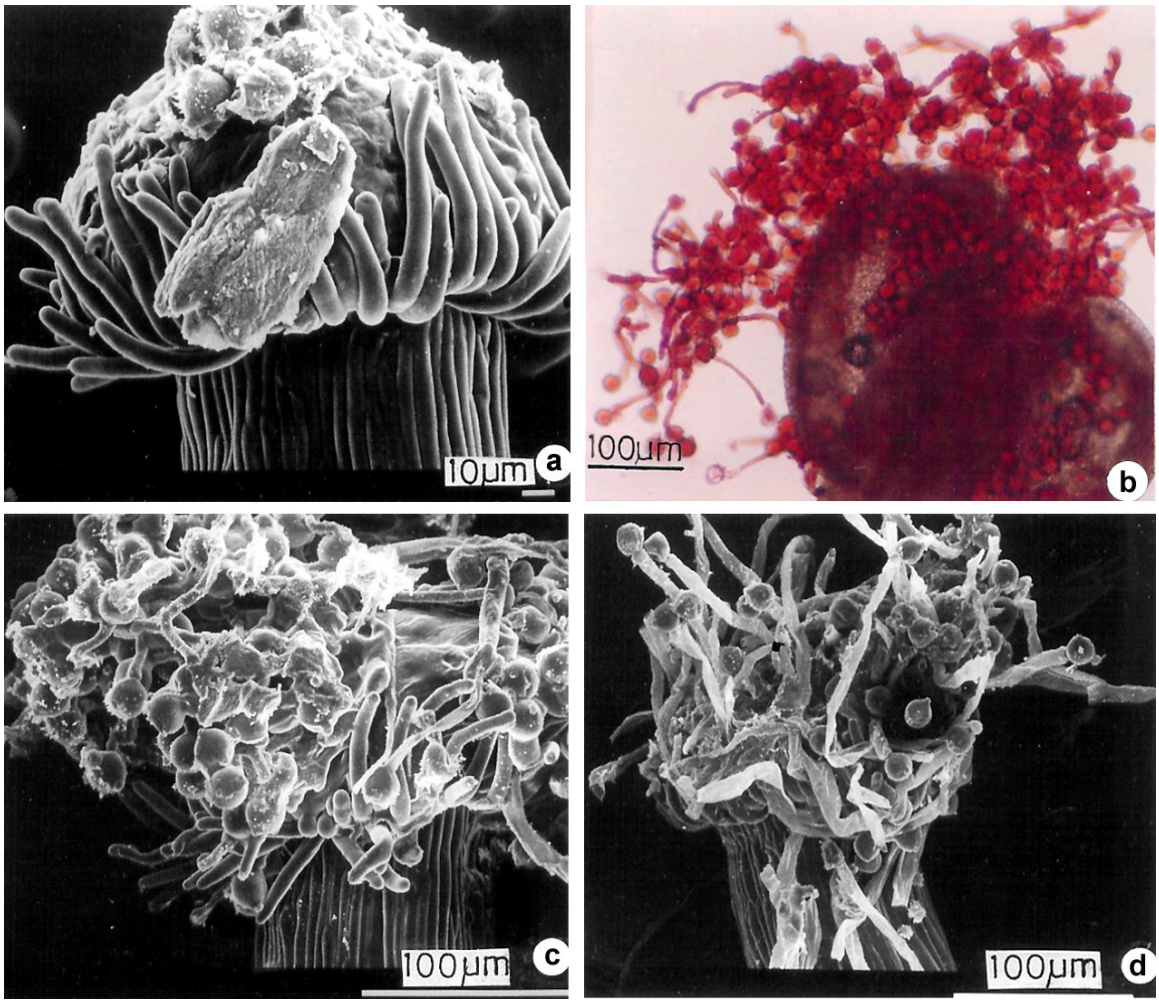


**Figure 1 a-d:** a- Stigma surface; b- Precocious pollen germination in anther loculi, c, d- germinated pollen on stigmatic surface at half-open flower stage of 'Bragg' genotype.