Journal of Agroecology and Natural Resource Management
Print ISSN: 2394-0786, Online ISSN: 2394-0794, Volume 2, Number 1; February-March 2015 pp. 51-56

http://www.krishisanskriti.org/janrm.html

The Significance of Pollen Viability and Stigma Receptivity on seed Setting

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Abstract—*Pollination is the primary step in seed formation. Availability of more pollen grains and abundance of flower visitors is responsible for higher yield set. Stigma provides initial nutrients and guidance cues for pollen grain germination and tube growth. Seed set was higher on spikelets with double exerted stigma (20%) than with single exerted stigma. Results indicated that pollen viability is mainly affected by drought/dehydration, heat stress and UV-B radiation. Low autogamy and self-incompatibility are the two major genetic reasons for poor seed setting and filling. Environmental factors greatly influence the seed setting by way of not only affecting crop itself but pollinators also and thus pollination. Temperature and moisture conditions should be favorable to maintain continuous vigorous growth throughout the growing and seed setting period for optimum and maximum seed yields. 548 genes were identified to express specifically on stigma papillar cells and of rice. The total number of genes expressed in mature Arabidopsis pollen varied from 992 to 1587. Thus, the knowledge of pollen viability and stigma receptivity and the way it is affected by environmental factors help's in hybrid formation, which will further aid to higher seed set and ultimately yield.*

Key words*: Pollen viability, stigma receptivity, seed setting, external environment, self incompatibility, gene effect*

1. INTRODUCTION

Pollination is the primary step in seed set, and events starting at pollination and terminating at fertilization in wide crosses involve complex and harmonious interactions including cell-cell recognition and cellular signaling (Mascarenhas 1993, de Graft *et al*, 2001). Failures in pollination are thought to be widespread in plants and a common cause of low seed set (Wilcock and Neiland, 2002).

Pollen quality is often equated to pollen viability, i.e., the proportion of pollen grains that are viable. Viability is the rapid growth of the pollen tubes carrying the male gametes, through the tissues of the stigmas and the styles. Pollen of a very large number of species contains two nuclei at the time of dispersal. One of these nuclei divides to form two male gametes by about the time the pollen tube reaches the ovary. In several other species such as those of the grasses (cereal crops included) the pollen contain three nuclei, as the male gametes are already formed by the time of dispersal. Pollen of such species has notoriously short viability, less than 10 minutes in rice to about two hours in some others (Rao, 2008).

The angiosperm stigma is an efficient structure with both morphological and physiological adaptations that enable pollen capture, hydration and germination. The stigma surface may play a vital part in controlling interspecific hybridization and in regulating compatibility relationships within species. Stigma receptivity is a crucial stage in the maturation of a flower which may greatly influence the rate of self-pollination, pollination success at different stages in the flower life cycle, the relative importance of various pollinators, transfer, and the chances of gametophytic selection. Receptive stigmas are characterized by high enzymatic activity. The presence of several enzymes is found to coincide with this developmental (Knox *et al.,* 1986; Dafni 1992; Kearns and Inouye 1993).

Pollen viability and stigma receptivity enhancing is necessary as it ultimately leads to seed set, which is important feature especially in relation to hybrids. Good pollen viability and stigma receptivity will lead to high yield, which is the ultimate aim in the production of hybrids. The significance of pollen viability and stigma receptivity on seed setting are discussed below.

2. POLLEN VIABILITY

2.1 Effect of Pollen viability

Pollen viability is mainly affected by drought,/dehydration, heat stress and UV-B radiation. Knowledge of pollen viability and the way it is affected by environmental factors may help predicting percent seed setting.

1.2 Factors influencing pollen viability

Pollen viability may be affected at different stages of development, from early in the anther till late on the stigma. The literature on pollen viability that appeared until 1974 has been reviewed by Stanley and Linskens (1974). The accumulated data indicated that pollen viability was influenced by relative humidity, temperature, atmospheric composition and oxygen pressure.

2.2.1 Humidity and temperature

The response to high or low humidity may differ between species and is usually associated with the intrinsic hydration state of the pollen at dehiscence (Nepi *et al*., 2001). Temperature can affect pollen grains during transport and germination on the stigma, but also during development in the anther. From several studies it appears that mature pollen grains are generally rather resistant to temperature stress applied after dehiscence, but detailed investigations have been carried out in a few instances only.

Table 1: Factors affecting pollen viability

Species	Treatment/Stress	Analysis	Effect	Reference
Brassica juncea	Exposure of mature dehisced pollen to heat stress	Germination, seed set	Severe heat stress decreases pollen viability	Rao et al. 1991
Capsicum annum	Dev under high tempearature: with and without high CO2	Pollen germination	High temperature inhibits pollen fertility	Aloni et al. 2001
Oryza sativa	Drought resistant	Grain set	Grain set drops by 20%	Sheoran and Saini, 1996
Solanum tuberosum	Pollen germination under UV-B radiation	Pollen germination, pollen tube length	Pollen tube length but not pollen germination	Torabinejad et al., 1998

2.2.2 UV-B radiation

UV-B radiation (280-320 nm) is normally present in the sunlight that reaches the earth’s surface, and it is therefore likely that mechanisms are present in plants to protect them against the damaging effects of UV-B.

2.2.3 Transport

One of the crucial events in the fertilization process of higher plants is the transfer of pollen grains from the anther to the stigma, often of another flower. In the case of wind pollinators, the transport of pollen trough the atmosphere will probably have similar effects as exposure to the environment, which was shown to rapidly decrease viability of maize pollen (Luna et al., 2001; Aylor, 2003, 2004). Honey bees are often seen as the most efficient pollinators.

2.3 Other factors affecting pollen viability

Elevated CO_2 levels protect pollen viability against heat stress during development (Aloni *et al*., 2001). Pollen from flowers with a history of ant occupancy had significantly lower germination on virgin recipient stigmas than pollen from unoccupied control flowers (Candace Galen & Brian Butchart, 2003).

2.4 Assessing pollen viability

Table 2 lists some of the most commonly used methods, a more extensive overview has recently been provided by Dafni and Firmage (2000).

Table 2: Methods to determine pollen viability

Method	Advantage	Disadvantage
Various methods (Baker's reagent, benzidine test, peroxidase indicator)	Variable, dependent on test	Variable, usually applied for few species only
Vital stains (FCR, TTC)	Very fast, applicable to virtually all species. Tests cytoplasmic enzymes and in some cases integrity of plasma membrane.	False positives reported repeatedly, not always correlation with seed set
In vitro pollen germination	More accurate, easy protocol for most species	Not all species germinate easily, optimum germination medium may differ between species. Generally more reliable than vital stains.
In vivo pollen germination	Simulates natural pollination, more valid than in vitro germination	Laborious. Compatibility mechanisms may prevent germination in certain species
Seed set	Most natural and reliable method to examine pollen viability	Laborious. Hand pollination may lead to over-estimation of pollen viability

The fastest way of analyzing pollen viability is using vital stains. The most commonly used vital stain is the fluorochromatic reaction (FCR) which reveals esterase activity in pollen with an intact plasma membrane (Shivanna and Heslop-Harrison, 1981).

Another frequently used method to assess pollen viability is *in vitro* germination. Because pollen grains of many species will easily germinate in a medium that contains boric acid and an osmoticum, this method is widely used (Taylor and Hepler, 1997). Finally, pollen viability may be measured after pollination, by analyzing germination on the stigma or seed set derived from that pollination.

2.5 Expression profile of pollen

Two different approaches were used to determine the overall gene-expression pattern of pollen in *Arabidopsis*: Affymetrix ATH1 8K GeneChips and serial analysis of gene expression (SAGE). The GeneChip papers report that 10% or 40% of the genes expressed in *Arabidopsis* pollen are pollen-specific (162 or 387 genes in and, respectively); the SAGE paper states that 83% of the pollen expressed gene tags (1,251 tags) are pollen-specific. The reports give estimates of the total number of genes expressed in mature Arabidopsis pollen that are just as different, ranging from 992 to 1,587. Some of the pollen-specific genes not only are unique to pollen but also are among the genes that are most highly expressed in pollen, so they worth further investigation (José António da Costa-Nunes and Ueli Grossniklaus, 2003).

3. THE STIGMA

The stigma is the receptive surface of the style that collects the pollen and enables its hydration and germination. Each stigma remains receptive to pollen for a few days at most, and in some species may be functional for only a few minutes after pollination. Characters can only be studied from fresh, mature stigmata, and the study of fixed or dried stigmata is inadequate.

Identification of the functional and adaptive significance of variation in flower morphology is fundamental to our understanding of the processes that shape patterns of seed production and floral evolution (e.g., Nilsson, 1988; Campbell, 1991; Johnston, 1991; Schemske and A gren, 1995; Galen and Cuba, 2001; Aigner, 2004). The position of the stigma within the flower is a key aspect of flower morphology, which influences the efficiency of pollen transfer (Campbell *et al.,* 1996; Cresswell, 2000; Nishihiro *et al.,* 2000) and the likelihood of within flower self-pollination (Karron *et al.,* 1997; Motten and Stone, 2000; Elle and Hare, 2002).

3.1 Assessing Stigma Surface Morphology

Observations of stigma surface morphology and secretions can often be made with a dissecting microscope or hand lens. However, the scanning electron microscope (SEM) is extremely valuable for observing and recording stigma morphology because of its high resolution, large depth of field and ability to examine untreated stigmata..

3.2 Stigma Receptivity

Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen. Details of stigma receptivity have been studied in only a limited number of species (Shivanna, 2003). Optimal receptivity is variable and can be from a few hours after flower opening as in teak (Tangmitcharoen and Owens, 1997), to a few days after anthesis as in oak (Kalinganire et al., 2000) and Silene alba (Young and Gravitz, 2002).

3.3 Methods for accessing stigma receptivity

1. Evaluation of seed set after pollination at different time interval relative to anthesis.
2. Detection of stigmatic secretions on the papilla surface (e.g., Dumas et al, 1983; *Knox et al*., 1987).
3. Movement of stylar lobes in some species (e.g., malvaceae, caryophyllaceae, etc.) (Knox et al., 1986).
4. Cytochemical detection of nonspecific enzymes on the surface of the stigma (e.g, ATPase, peroxidase, etc).

In vitro pollination analysis is the best studied system (maize) allows an expanded understanding of the fertilization process:

1. Using radioactive labels added to pollen, such as ^{32}P, allowing autoradiographic analysis of pollen tube growth: 6-8 hours was required for crosses between A632x W117.
2. Identification of biochemical markers of female receptivity. (Dupuis and Dumas, 1990).

3.4 Factors affecting stigma receptivity

3.4.1 Temperature and humidity

The effects of temperature and relative humidity on stigma receptivity in a cytoplasmic male sterile wheat line were estimated by measuring per cent seed set on plants raised in controlled environments at Canberra. Three levels of temperature and two levels of humidity (75-90 per cent, 40-60 per cent) were combined with four times of pollination (1, 3, 5, and 7 days after flowering). It was found that the effects of temperature and humidity were additive. It was found that the moderate temperature-low humidity combination had higher seed set as 77.4 percent and lowest seed setting was recorded as 0.4 percent at high temperature and high humidity combination (Imrie, 1966).

3.4.2 Self incompatibility

Self-incompatibility (SI) is used by many flowering plants to prevent self-fertilization and thereby generate and maintain genetic diversity within a species. Sporophytic self-incompatibility systems are associated with dry, papillate stigmas; most gametophytic systems with wet stigmas. In both gametophytic and sporophytic multi-allelic systems, it is clear that SI involves a complex biochemical signalling system between the pollen grain (and/or the tube) and the pistil (Elleman and Dickinson, 1994).

3.4.3 Stigmatic inhibition

The relationship between pollen cytology and pollen behavior was discovered by Brew Baker (1957, 1959) showed that the 17 genera known to display stigmatic incompatibility at the time were trinuceate, whereas 33 of the 36 genera recorded to exhibit pollen-tube inhibition in the ovary produced bi-nucleate pollen grains. Pandey (1970) attributed to the relationship that the second mitotic division is directly associated with the determination of self-incompatibility phenotype. The other explanation could reside in the differences in the localization of the pollen substances involved in recognition (Nettancourt, 2001).

3.5 Receptivity of exerted stigmas in rice

Experimental evidences suggest that exerted stigma traits, in particular its receptivity in CMS lines would increase outcrossing rates. Analysis was done on Xie-quig-zao cultivars and selections on partially male sterile lines with exerted stigmas and Er-jiu-qing with non exerted stigmas for stigma receptivity to alien pollen at Linshui, China (1984). Seed set was in direct proportion to percentage exerted stigma (PES). Seed set was higher on spikelets with double exerted stigma (20%) than with single exerted stigma (Xu Yun-Bi and Shen Zong-Tan, 1988).

3.6 Genome-wide gene expression profiling reveals conserved and novel molecular functions of the Stigma in Rice

Rice stigma specific or -preferential gene expression profiles were generated through comparing genome-wide expression patterns of hand-dissected, unpollinated stigma at anthesis with seven tissues (seedling shoot, seedling root, mature anther, ovary at anthesis, seeds 5 d after pollination, 10-d-old embryo, 10-d-old endosperm, and suspension-cultured cells) by using both 57 K Affymetrix rice whole-genome array and 10 K rice cDNA microarray. 548 genes was identified which expressed specifically or predominantly in the stigma papillar cells of rice. Real-time quantitative reverse transcription-polymerase chain reaction analysis of 34 selected genes all confirmed their stigma-specific expression. The expression of five selected genes was further validated by RNA in situ hybridization.

4. SEED SET

Seed set is affected by many factors, including pollen loading, environment, environmental variability, incompatibility mechanism in the pistil, and others. The effect of temperature, and relative humidity on many of the attributes of plant flowering (the speed of dispersal of pollen, matured pollen, vitality of pollen grain, pollination and fertility and increased of seeds), while affecting of wind speed and sunshine duration on the movement and the activity of pollinators, especially honey bees and its role in floret pollination, which leads to increase seed production. Sexual reproduction in many flowering plants involves self incompatibility (SI), which is one of the most important systems to prevent inbreeding. In many species, the self-/nonself-recognition of SI is controlled by a single polymorphic locus, the S-locus. The sporophytic type of self-incompatibility mechanism is one of the genetic reasons for poor seed setting in sunflower.

Following are major factors causing seed setting and filling problem:

4.1 Genetic

4.1.1 Low autogamy

Low autogamy is one of the genetic reasons for poor seed setting and filling in sunflower. Therefore, evaluation of hybrids and their parental lines for their autogamy becomes necessary before releasing any genotype or hybrid. Rathod *et al*. (2002) in one of the autogamy study in sunflower reported that hybrid produced significantly more autogamous seeds over better parent. The datas are presented graphically in Fig.1.

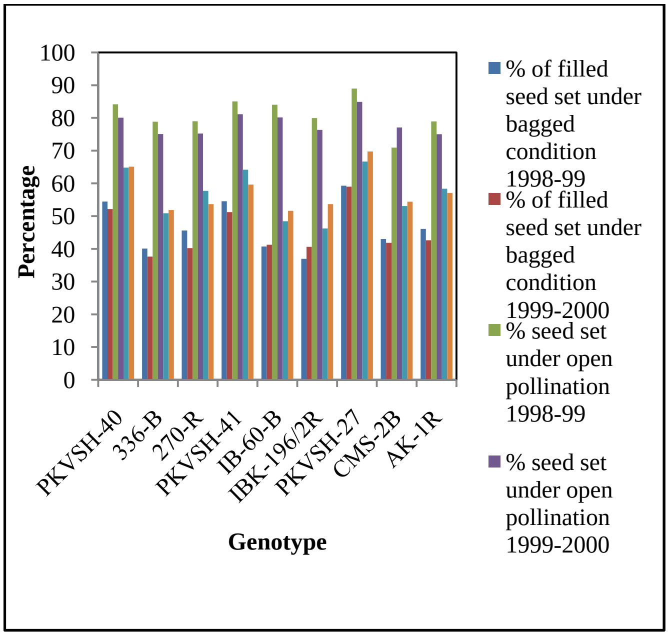

Fig. 1: Autogamy studies of sunflower genotypes under bagged and open conditions

4.1.2 Self-incompatibility

Self-incompatibility is the inability of fully functional pollen grains to fertilize and seed set on selfpollination. There are two types of self-incompatibility in homomorphic angiosperms, genetically speaking. One is the *gametophytic* type-the other, *sporophytic*. In the gametophytic system pollen behavior (i.e., function or failure in a particular mating) is determined gametophytically by the S allele in each grain. The incompatibility inhibition regularly occurs at some stage during pollen tube growth in the pistil of gametophytic species. In the sporophytic system pollen behavior is sprophytically determined (imposed by the maternal genotype) and inhibition commonly occurs at the stigma surface so as to inhibit pollen germination or drastically curtail pollen tube growth.

Identification of self-fertile lines is one of the means for improving seed setting and productivity. Vara Prasad *et al*. (2006) in one of this type of study reported that hybrids are generally more vigorous, uniform, self fertile and resistant to many pests and diseases.

4.2 Physiological

There is a degree of wastage of photo assimilates in plants due to photorespiration which can otherwise be utilized for building yields. Poor seed development may result from insufficient assimilate supply (source limitation).

4.3 Environmental factors and their management

4.3.1 Moisture stress

Mohan Reddy *et al*. (2003) reported that there was maximum decline in LAI and dry matter accumulation in sunflower subjected to moisture stress at flowering stage resulting reduction in yield

4.3.2 Intercepted solar radiation

A reduction in intercepted photosynthetically active radiation (PAR) during a short period of seed filling could affect weight per seed.

4.3.3 Season

The mean seed set under self-pollination was highest in rabi, followed by spring and kharif. Under open pollination, it was highest in kharif followed by spring and rabi.

4.4 Agronomic management

4.4.1 Pre sowing treatments

Seed invigoration treatment helps to improve the germination and vigour of the seed and ultimately it establishes a good field stand and yields higher.

4.4.2 Planting time and planting design

Among several crop production practices, planting date decides the correct expression of a genotype for all morphological characters and physiological processes.

4.4.3 Staggered sowing

Umesh *et al.* (2007) reported that *staggered* sowing of male parent seven days early (S2) resulted in the increase in per cent seed set and filling as a result of better synchronization in parental lines.

4.4.4 Fertilization

Nanja Reddy *et al*. (2003) reported that soil application of boron (2 kg/ha) ray floret stage increased the seed yield by 53%. Bhagat *et al* (2005) reported that seed yield was significantly increased with increasing levels of sulphur. Zinc foliar application improved not only the boldness and vigor of seeds in zinc-deficient plants, but also the seed zinc content in zinc-deficient seeds as well as the sufficient ones (Girish Chandra Pathak1, Bhavana Gupta1, Nalini Pandey,).

4.4.5 Irrigation

Judicious application of irrigation and nitrogen is essential to achieve higher benefits, especially under limited resource conditions.

4.4.6 Insecticides uses and pollination

The use of insecticides to control pests on agricultural crops is indispensable. Often economically important non target insects such as honeybees are killed in the process of pest control.

4.4.7 Integrated nutrient management

Integration of organic manures and biofertilizers with chemical fertilizers is more emphasized not only to boost the production from limited land resources but also for its sustainability.

5. CONCLUSION

The variation in flowering phenology and pollen yield individually and annually along with temporal separation in anther dehiscence and pollinator’s visitation cause pollen limited reproduction, which ultimately influences the reproductive success. Availability of more pollen grains and abundance of flower visitors is again found to be responsible for higher yield set.

Interaction between temperature and relative humidity increases the impact of each of them individually on the anthesis dehiscence i.e, the pollen viability, tripping and fertilization as well as wind speed, while the moderate increase of movement and activity of pollinators, thus contributing to the success of the tripping and fertilization. Increasing temperatures speed up the maturation and anthesis dehiscence, while working on the lack of pollen grain viability. Pollen’s having higher hydration levels are metabolically more active, hence allowing fast pollen tube extrusion, thereby leading to faster fertilization and seed set. Increasing in temperature and relative humidity reduce the viability of pollen grain.

Exserted stigma showed higher receptivity within 1-2 d after flowering. Seed set was in direct proportion to percentage exerted stigma (PES). Seed set was higher on spikelet's with double exerted stigma (20%) than with single exerted stigma. The exserted panicle is fragile and can be easily damaged by environmental conditions. Eg. water stress, wind, physical interruption, etc, during flowering period.

Strategies for improvement of seed setting:

To enhance pollination and crop yield, the timing and placement of honey bees, is an important parameter for effectiveness of seed setting in developmentally advanced flowers. Selecting restorer lines with stronger pollen viability and germination rate than the maintainer of the specific cross combinations will be an effective measure for enhancement of seed setting. Identification of self sterile lines will also help in getting desired hybrids of high genetic purity. With recent advancement of molecular biology and genetic engineering directed genetic manipulations to increase the period of pollen viability and stigma receptivity will contribute immensely in studying other pollination behavior of the crops. Moreover better understanding of metabolic pathways for pollen viability and stigma receptivity will enlighten the breeders to develop breeding lines with high pollen viability and stigma receptivity.

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