Study of pollen viability for the development of genetic engineered plants of Solanaceae family

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Abstract

Pollen viability is important for growers, breeders and development of genetic engineered plants. Information on pollen viability is a pre-requisite for carrying out a meaningful crop improvement programme. The present investigation was carried out in the Department of Biological Science, M.G.C.G.V., Chitrakoot, Satna (M.P.). Five plants of Solanaceae family namely *Datura stramonium, Solanum xanthocarpum, Capsicum annum, Solanum nigrum*, and *Withania somnifera* were used for study the pollen viability. Stain tests have advantages as indicator of pollen viability because they are faster and easier than pollen germination. Pollen viability was assessed by acetocarmine stain (2%). The percentage pollen viability ranged from 67% to 84%. The highest percentage of pollen viability was recorded in *Datura stramonium* (84%), which revealed that this genus has highest reproductive power as well as power of division and the lowest percentage of pollen viability was obtained in *Withania somnifera* (67%), revealed that this genus become scarcer and endangered. From the results it is concluded that information of pollen viability is an important for the study of reproductive biology and ensuring safer crosses performed to generate new hybrids and /or increase the fertility.

Key words- Pollen viability, Solanaceae, Acetocarmine, Chitrakoot, Genetic engineered plants.

Introduction

Solanaceae is a family of flowering plants that contains a number of agricultural plants as well as toxic plants. The name of the family comes from the Latin word Solanum "the nightshade", which means "quitting", refers to sedative effects associated with many of the species due to the presence of alkaloids. The Solanaceae is a large varied family of trees, shrubs and herbs including 98 genera and estimated species number is between 3000-4000. The family has a worldwide distribution, being present in all continents except Antarctica. The greatest diversity in species is found in South America and Central America. Most members of the Solanaceae are erect or climbing, annual or perennial herbs, but shrubs are not uncommon and there are a few trees.

Solanum xanthocarpum commonly known as bhatkateli or yellow berried nightshade, a prickly herb is an important plant species in Ayurveda and Folklore medicine since time immemorial. Although *Solanum xanthocarpum* is important traditionally but lack of experimental research in a hindrance for its exploration in modern system of medicine. It is occurred in Ceylon and Malacca through South–East Asia, Malaya, Tropical Australia and Polynesia, very important found throughout Indian plains from Sea sore to hills up to 1000 m height.

Solanum nigrum commonly known as makoi, or locally just "black nightshade" and small fruited black nightshade, is a species in Solanum genus. It is a fairly common herb or short lived perennial shrub, sometimes purple-green, hairy with glandular, simple, and prickles absent. It grows well under different climatic conditions of the country, but prefers a moderate climate. The plant is sedative to frost and shade, along the roads, fences and in neglected places. It is commonly found in Sri Lanka, China, India, South America, Zimbabwe, and some European countries.

Datura stramonium commonly known as dhatura or jimsonweed and originated in North America. The genus was derived from "datura" an ancient Sanskrit word for the plant and stramonium is originally from Greek, Strychnos (nightshade). It is cultivated in Germany, France, Hungary, and South America and throughout the world. It is indigenous to India and grows abundantly throughout the Himalayas from Kashmir to Sikkim.

Capsicum annum commonly known as mirch or chili growing at tropical and sub- tropical regions of New World. Capsicum is derived from the Greek word "Kapsimo" meaning to bite (Basu and De, 2003). *Capsicum annum* is originated in Central and South Americas, and then spread to Europe, Africa and Asia, especially to India, China and Japan. (Bosland P.W., 1996). It is cultivated in almost all the tropical countries, East Africa, West Africa and India are the regions producing the drug on commercial scale. In India it is grown in Andhra Pradesh, Uttar Pradesh, Gujarat, Maharashtra, Assam, and Tamilnadu.

Withania somnifera commonly known as "Ashwagandha" or Indian ginseng is considered a wonder herb having assorted Ayurvedic, Unani and indigenous medicinal properties. It occurs in Eastern and Western Ghats, Andaman and Nicobar Islands, Sri Lanka, Myanmar, Pakistan, Malaysia, Thailand, Java, Asian and African tropics. In India it is found in Central Indian states of Madhya Pradesh, Rajasthan, and parts of Punjab, Himachal Pradesh and Uttar Pradesh.

Pollen viability is generally considered as ability of pollen grains to germinate and deliver the sperm cells to the embryo sac to complete the compatible fertilization. Pollen can preserve its value for long time if it kept dry. Similarly Nabel & Rulty, 1937 reported that pollen is capable of compatible fertilization even after long periods of storage. Basic information on the meiotic behavior and estimation of pollen viability are useful for germplasm characterization and identification of genetic variability, biodiversity, and evolutionary processes of the species (Palm- Alva et al, 2004). The amount and quality of pollen produced by a flower is an important component of fitness. Pollen quality is often represented to pollen viability i.e. the proportion of pollen grains that are viable (Stanley and Linskens, 1974, Heslop- Harrison et al, 1984). During the plant maturation, the pollen viability can be affected by several endogenous and exogenous factors; such as the stage of flower development (Lacerda et al, 1994), high temperatures (40°C) (Giodano et al, 2003), and low temperatures (15°C) (Chira, 1963), nutritional status of the plant (Howlett, 1936), Luminosity (Goss, 1971), agricultural pesticides and other chemicals (Mac Danieles and Hildebrand, 1939, Dubey and mall, 1972).

According to Dantas et al (2005) and Tuinstra and Wedel (2000), assessment of pollen viability is a preliminary and indispensable condition for genetic crop breeding. For these authors, information of pollen viability is an important for the study of reproductive biology and for the development of genetic engineered plants, as it enables a greater success rate of crosses. The need for assessing viability of pollen used in artificial pollution and in breeding experiments is also important in the understanding of sterility problems and hybridization programs (Gupta and Murty, 1985), fruit breeding programs (Oberle and Watson, 1953), and evolutionary ecology (Thomsan et al, 1994).

Pollen viability can also provide a measure of the reproductive fitness of hybrids, i.e. their ability to serve as pollen donors in natural populations (Mraz et al, 2005, Mckenzie et al, 2008, Mao et al, 2009), and it can also serve as an evaluation criterion of the post- pollination barrier between species (Rieserberg et al, 1999, freqre et al, 2005; Jarolimora, 2005; Mraz & Paule, 2006, Ackermann et al, 2008).

Material and methods

The study was conducted in the Department of Biological Science, M.G.C.G.V., Chitrakoot, Satna (MP) from September to January, 2018. To study the pollen viability of selected members of Solanaceae family, flowers or flower buds were collected in the morning (from 05:00 AM to 07:00 AM), immediately after anthesis, and kept at high humidity in a closed vessel with a wet paper towel (Lee et al, 1985) to avoid pollen dehydration. Flower buds were fixed in Carnoy's solution in the ratio of 3:1 ethanol: glacial acetic acid for 24 hours at room temperature and stored in 70% alcohol in a freezer at 3°C.

Selected five medicinal plants belonging to Solanaceae family were tested for pollen viability status by using the acetocarmine staining method. Using dissecting forceps, scalpel and a needle, anthers of selected members were opened to allow extraction and subsequent transfer of pollen dust on to a microscopic glass slide in a drop of acetocarmine stain. Mature anthers were crushed and pollen grains mixed through with the acetocarmine stain. Cover slips were gently placed on to different slides for each species and sealed with nail polish. The slides were then observed under a light microscope. For each plant species, three slides were prepared. For each slide five randomly selected fields were observed under the 10X objective (100X magnification). To determine the pollen viability, dark stained pollen grains were recorded as fertile and viable (it has the potential to germinate), and unstained or very lightly stained pollen grains were considered as sterile or non viable (it has no potential to germinate). Pollen viability was calculated by dividing the number of viable pollen grains by the total number of pollen grains counted in the field of view.

Formula used

$$\%\ \textbf{Pollen viability} = \frac{\textbf{Number of viable pollen grains}}{\textbf{Total number of pollen grains analysed}} \times \textbf{100}$$

Results

The pollen viability was analyzed in selected five plants of Solanaceae family. The pollen viability results obtained using an acetocarmine method is described on table 1. and illustrated in figure 1. The percentage pollen viability was 84% for *Daturra stramonium*, 81% for *Solanum xanthocarpum*, *Capsicum annum* had 77%, *Solanum nigrum* had 71%, while *Withania somnifera* had 67%.

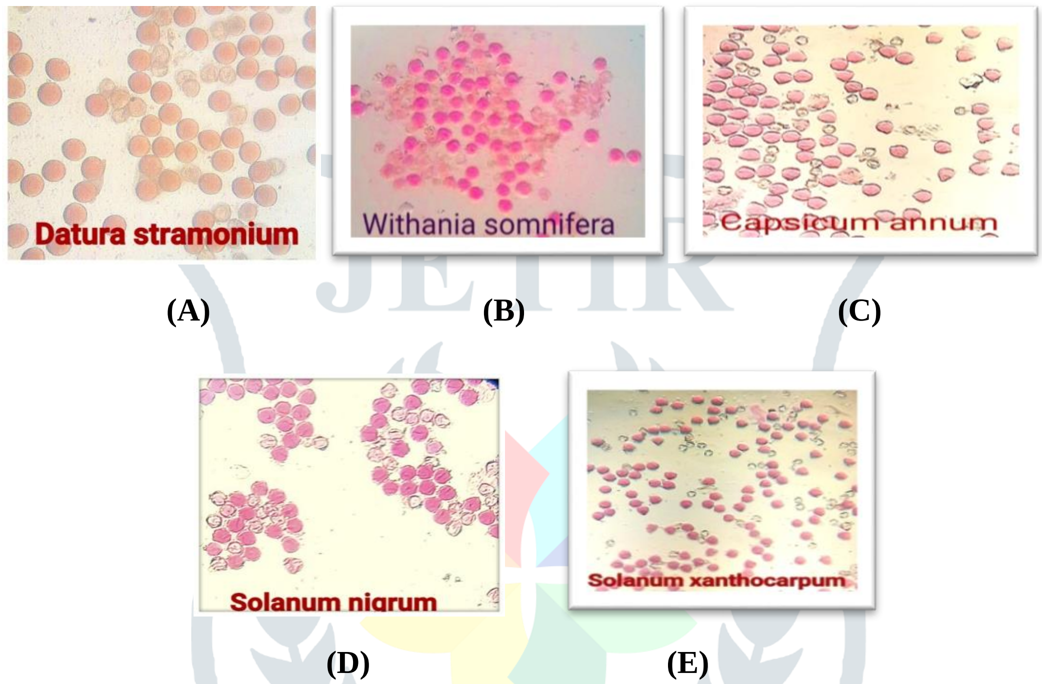

Figure 1. Showed viable (darkly stained) and non viable (light stained or empty) pollen grains of selected members of Solanaceae family (A). *Datura stramonium* (B). *Withania somnifera* (C). *Capsicum annum* (D). *Solanum nigrum* (E). *Solanum xanthocarpum*

From the results it is concluded that the highest percentage pollen viability was recorded in *Datura stramonium* (84%) and *Solanum xanthocarpum* (81%). The lowest percentage pollen viability was obtained in *Withania somnifera* (67%). The remaining two plant species *Capsicum annum* showed 77% and *Solanum nigrum* showed 71% pollen viability.

Table 1. Showed percentage pollen viability of selected members of Solanaceae family

S. No.	Local name	Botanical name	No. of pollen grains examined	No. of viable pollen grains	No. of non viable pollen grains	Percentage of pollen viability
1	Datura	*Datura stramonium*	100	84	16	84%
2	Bhatkateli	*Solanum xanthocarpum*	100	81	19	81%
3	Chilli	*Capsicum annum*	100	77	23	77%
4	Makoi	*Solanum nigrum*	100	71	29	71%
5	Ashwagandha	*Withania somnifera*	100	67	33	67%

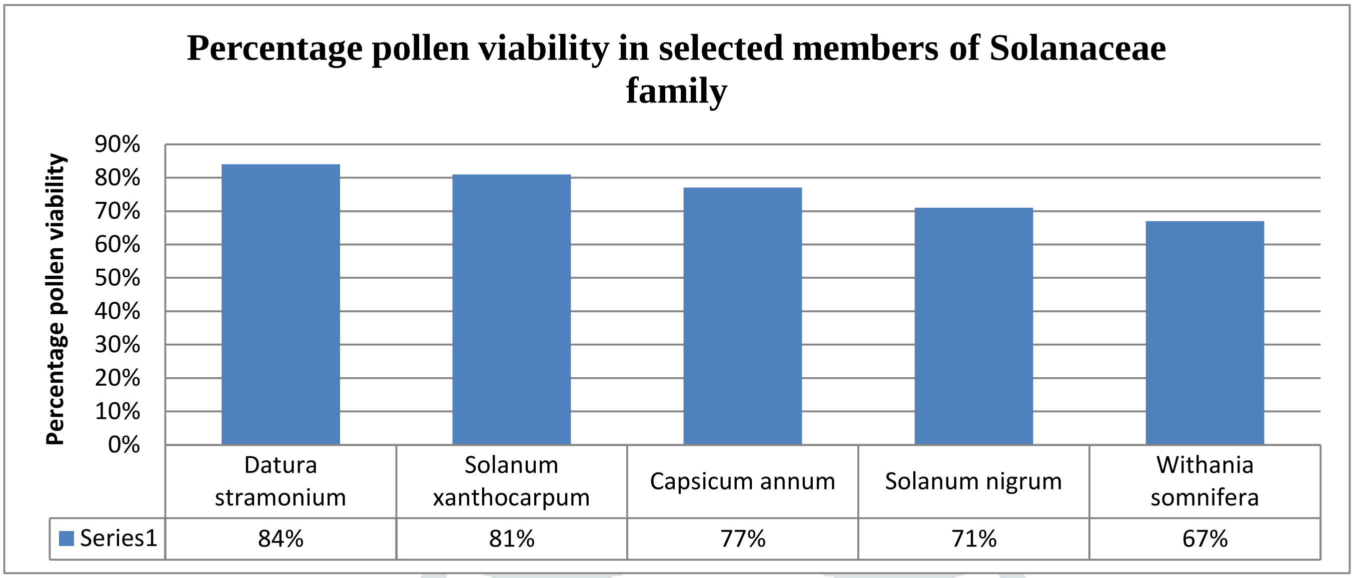

Figure 2. Showed percentage pollen viability in selected members of Solanaceae family

Conclusion

Pollen viability may play a critical role in the efficiency of hybrid formation. However, since pollen of different species and sometimes even varieties show different responses to environmental factors. It is not feasible with the current knowledge of predict the contribution of pollen in hybrid formation for most species. The information on pollen viability is a pre- requisite for carrying out a meaningful crop improvement programme, particularly when a trait of interest is to be incorporated from a wild source to cultivated forms.

Data on the viability and development of pollen grains are fundamental for studies of reproductive biology and genetics breeding of these selected plants of Solanaceae family, ensuring safer crosses performed to generate new hybrids and/or increase the fertility. From the results it is concluded that the highest percentage of pollen viability was recorded in *Datura stramonium* which revealed that this genus has highest reproductive power as well as power of division while *Withania somnifera* possessed lowest pollen viability, revealed that this genus might scarcer and endangered. Thus, the results obtained in this study provided important information that can be used for planning crosses by identifying the best male parents and the best time to collect pollen grains to maximize the potential of production of viable seeds and the establishment of breeding programs of these plants of Solanaceae family.

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