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Studies on Pollen Fertility and *in vitro* Pollen Germination in Medium duration Genotypes of Pigeonpea (*Cajanus cajan* (Millsp.) L.)

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ABSTRACT: Pollen is like any living organism and depicts a crucial stage in the life cycle of plants, as viable pollens are necessary for sexual reproduction in plants. The quality of pollen is assessed based on viability and vigour. Viability refers to the ability of the pollen to deliver functional sperm cells to the embryo sac following compatible pollination. The quality of the pollen should always be studied under defined conditions as the behavior and survival of the pollen grains are easily altered by the genotype and environmental conditions. A complete knowledge on pollen viability will help in proper understanding of pollination and fertilization in plants. Bearing this in mind, the present study investigated thirty genotypes for Pollen fertility and pollen germination. The highest mean performance for percentage of pollen fertility was recorded by KUPL20010 (95%) followed by KUPL19012 (93%), Co5 (93%), KUPL20011 (92%). The above-mentioned genotypes can be utilized as parents for hybridization programmes for developing high-yielding varieties. The lowest performers of these traits are KUPL20007 (42%), KUPL20005 (53%), KUPL20003(57%) and KUPL20026 (58%) can further be utilized to develop male sterile parents which can be used in large scale hybrid development programmes. The lowest mean performance for pollen germination were recorded by KUPL20017 (10.46%) followed by KUPL20024 (21.00%) and KUPL20019 (23.22).

Keywords: Pigeonpea, redgram, Pollen fertility, Aceto-carmine, Pollen viability.

INTRODUCTION

The problem of malnutrition and undernourishment in people is rapidly increasing and the consequences are alarmingly high, with one-third of malnourished children globally being Indian, and almost one-third of the Indians are thought to be malnourished. Pulses being one of the cheap and affordable sources of nutrition for people, it occupies a significant role in Indian agriculture. Within these nutritious and protein-rich crops, pigeon pea occupies a dominant position and has various advantages over other pulse crops.

Pigeonpea [*Cajanus cajan* (L.) Mills paugh] is a perennial shrub belonging to the Leguminosae family having a diploid chromosome number of 2n = 22, native to India. Popularly known as red gram, tur, arhar, gandul and pois d' Angole. Among all the other legumes pigeonpea is unique as its floral morphology allows both self as well as insect-aided cross-pollination and their extents vary from place to place. Other than high nutrition (protein, vitamin B and other essential amino acids such as methionine, lysine and tryptophan) content,this crop is hardy, widely adapted to a wide range of environment and cropping systems and alsoperforms well even under various biotic and abiotic stress condition. It is highly drought tolerant, mainly grown as a rainfed crop and requires less fertilizer. It enriches soil through symbiotic nitrogen fixation, recycles the soil nutrients, and adds organic matter and other nutrients that make it an immaculate crop for sustainable agriculture in the tropics and subtropics. The farmer is not only benefited from quality food but also the hardy shrubs provide fuel wood, fodder for cattle, low cost of cultivation and less crop risk due to wide adaptability and tolerance.

The production of pigeonpea has to be increased to meet the rapidly growing demand. The per capita availability of protein in the country is 28 g/day, while the WHO recommendation is 80 g/day. The overall production can be enhanced by increasing the area under cultivation i.e., horizontal expansion in area, which is practically not possible owing to demand in agricultural land and growing population. Therefore, concerted efforts are needed toincrease the yield potential of the crop. One of the possible methods to increase the productivityof the crop is through effective pollination. Effective pollination is a prerequisite for fertilization and seed set in most plants, information on pollen biology, including pollen viability, pollen germination and pollen tube growth is required for any rational

approach to increase productivity (Bolat and Pirlak 1999; Cruzan, 1990; Shivanna, 2003). Lack of compatibility affects the yield of the plants and therefore, pollen and pollination studies aid breeders in figuring out those cultivars that are compatible with one another (Kamrani, 2012). The duration during which pollen grains maintain their viability after being released can vary significantly. Under favorable circumstances, the majority of pollen remains viable for several days to a week. However, certain species of pollen are highly susceptible to drying out, losing their viability within just one hour under dry conditions (Shivanna and Rsam 1993).

Choudhary *et al*. (2011) stated that native pollen tubes have been observed to grow below the stigma comparatively slowly, indicating the presence of a self-incompatibility barrier. Pollen grains of a wide variety of species germinate successfully in sugar solutions. Sucrose is probably the best and most commonly used source of carbon and energy for pollen. It provides and maintains a proper osmotic environment for the germination of pollen and continued growth of pollen tubes. Supplementing the medium with boron germination and pollen tube growth stimulates (Gill, 2014).

MATERIALS AND METHODS

The local landraces collected from the different agro-packets of Tamil Nadu were evaluated for three years and used as experimental materials after selection (Table 1). The selected lines were raised at South Farm, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore during the *Kharif* 2021 season. All the intercultural operations were carried out as per the recommendations.

Table 1: Experimental materials used for the study.

Sr. No.	Experimental materials	Sr. No.	Experimental materials	Sr. No.	Experimental materials
1.	KUPL 20001	11.	KUPL20011	21.	KUPL20021
2.	KUPL20002	12.	KUPL20012	22.	KUPL20022
3.	KUPL20003	13.	KUPL20013	23.	KUPL20023
4.	KUPL20004	14.	KUPL20014	24.	KUPL20024
5.	KUPL20005	15.	KUPL20015	25.	KUPL20025
6.	KUPL20006	16.	KUPL20016	26.	KUPL20026
7.	KUPL20007	17.	KUPL20017	27.	KUPL20027
8.	KUPL20008	18.	KUPL20018	28.	KUPL20028
9.	KUPL20009	19.	KUPL20019	29.	LRG 41
10.	KUPL20010	20.	KUPL20020	30.	CO 5

A. Studies on Pollen fertility

For studying pollen fertility, five fully grown, unopened flower buds were collected from a single plant and five plants from each entry were used for collection of buds. A single bud is taken at a time and dissected carefully to squeeze the anthers out. The squeezed anthers were then excised and squashed over the glass slide. Freshly, acetocarmine solution (2% concentration) was made through blending 2 g of carmine powder with glacial-acetic acid of quantity 45 ml and then made up to a final volume of 100 ml. The mixture was gently boiled before being filtered through Whatman No. 1 filter paper (Sharma *et al*., 2021). The smashed anthers were drenched with 2 % aceto-carmine solution and observed under a light microscope. The pollen fertility study was carried out in the Genetics and Plant Breeding Laboratory, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore. The data on pollen fertility were recorded over 510 microscopic fields for each bud. The count on fertile and sterile pollen were made and analyzed using GenStat 12 edition (Pooja *et al*., 2021).

Pollen fertility percentage (%) =

$$\frac{\text{No. of fully stained polled grains}}{\text{Total no. of pollen grains}} \times 100$$

B. Studies on pollen germination

Pollen viability/fertility tests produce results quicker than pollen germination tests but the latter is necessary to make conclusive remarks on pollen viability experiments. Pollen grains were collected randomly within the entries and separately for different entries in butter paper covers from the just opened flower buds. The E-Amino Caproic Acid (EACA) media used for pollen germination was prepared with 37.5 % Sucrose, 100mg^{1} Potassium nitrate, 15% Poly Ethylene Glycol (PEG) 4000, 200mg^{1} Magnesium Sulfate, 250mg^{1} Boric Acis, 300mg^{1} Calcium Nitrate, 1000mg^{1} E-Amino Caproic Acid (EACA) and 1% agar (Jayaprakash, 2018). The pollen germination studies were conducted in Varshini BioTech, Meena Estate, Sowripalayam, Coimbatore. The media was poured in the petriplates and the pollen grains collected from each entry were inoculated in the pertiplates separately and incubated at 37°C for 4 hours. The pertriplates were taken out of the Incubator and 2ml killing solution of glycerine, formaldehyde, glacial acetic acid and distilled water in 20:5:3:72 ratio (Sass, 1958) was added to the plates and observed under a Compound microscope. A minimum of 10 readings per plate were made and used for analysis (Rathod *et al*., 2021).

Pollen germination percentage (%) =

$$\frac{\text{No. of germinated pollen grains}}{\text{Total no. of pollen grains on the slide}} \times 100$$

C. Analysis of variance

The mean data was analysed using Analysis of variance (ANOVA) and mean differences were tested as per the method suggested by Panse and Sukatme (1954). The total variation in the data can be separated into different components and their significance was tested through

ANOVA (Table 2). The significance was tested based on the table provided by Snedecor and Cochran (1967).

Table 2: Analysis of variance.

Source of variation	df	MSS	Expectation
Replications (r)	r-1		
Genotypes (g)	g-1	GMSS	GMSS/EMSS
Error	(r-1) (g-1)	EMSS	

Where,
r = Number of replications; g = Number of genotypes; df = Degrees of freedom;
GMSS = Genotype mean sum of squares; EMSS = Error mean sum of squares

RESULTS AND DISCUSSION

The fertility of pollen collected from 30 pigeonpea landraces was tested using acetocarmine solution and germination of pollen was studied under EACA media with 37.5% Sucrose solution and the analysis using statistical tools was performed. The Analysis of Variance (ANOVA) showed significant variation for both the traits studied (fertility and germination) indicating that the genotypes included in this investigation exhibit sufficient variability for the characters studied. Dhiman *et al*. (2021) also recorded similar results, where significant deviation for pollen germination% was observed between the genotypes selected for study. The presence of a large amount of variability might be due to diverse sources of materials taken as well as environmental influence affecting the phenotypes. The mean performance of genotypes for pollen fertility and pollen germination were shown in Table 3. The highest mean performance for percentage of pollen fertility was recorded by KUPL20010(95%) (Fig. 1) followed by KUPL19012(93%) (Fig. 2), Co5(93%), KUPL20011(92%) (Fig. 3). The above-mentioned genotypes can be utilized as parents for hybridization programmes for developing high yielding varieties. The lowest performers of these traits are KUPL20007(42%), KUPL20005(53%), KUPL20003(57%) and KUPL20026 (58%) can further be utilized to develop male sterile parents which can be used in large-scale hybrid development programmes. The scrutiny of pollen viability and germination has practical applications in crop breeding, facilitating the development of cultivars characterized by increased yield, resistance to diseases and insect pests, and lower production costs (Sharma *et al.,* 2021).

The lowest mean performance for pollen germination was recorded by KUPL20017(10.46%) (Fig. 4) followed by KUPL20024(21.00%) and KUPL20019(23.22). These lines can be utilized as experimental materials to further exploit Self Incompatibility as these lines performed moderately well in the case of percentage of pollen fertility.

Table 3: Mean performances of genotypes for pollen fertility and Pollen germination.

Sr. No.	Experimental materials	Mean Pollen fertility (%)	Mean pollen germination (%)
1.	KUPL20001	65.00	18.64
2.	KUPL20002	90.00	38.33
3.	KUPL20003	57.00	67.84
4.	KUPL20004	72.00	69.36
5.	KUPL20005	53.00	60.47
6.	KUPL20006	85.00	59.92
7.	KUPL20007	42.00	71.03
8.	KUPL20008	67.00	55.71
9.	KUPL20009	90.00	68.26
10.	KUPL20010	95.00	56.63
11.	KUPL20011	92.00	72.13
12.	KUPL20012	93.00	41.78
13.	KUPL20013	86.00	65.46
14.	KUPL20014	89.00	56.40
15.	KUPL20015	87.00	55.75
16.	KUPL20016	66.00	28.06
17.	KUPL20017	73.00	10.46
18.	KUPL20018	84.00	54.28
19.	KUPL20019	69.00	23.22
20.	KUPL20020	76.00	35.69
21.	KUPL20021	89.00	54.85
22.	KUPL20022	65.00	42.28
23.	KUPL20023	86.00	65.28
24.	KUPL20024	59.00	21.00
25.	KUPL20025	76.00	45.85
26.	KUPL20026	58.00	43.58
27.	KUPL20027	87.00	68.32
28.	KUPL20028	89.00	65.00
29.	LRG 41	91.50	69.48
30.	CO 5	91.00	64.87
	General mean	77.47	51.66
	CV	4.39	2.28
	CD 5%	4.65	1.76

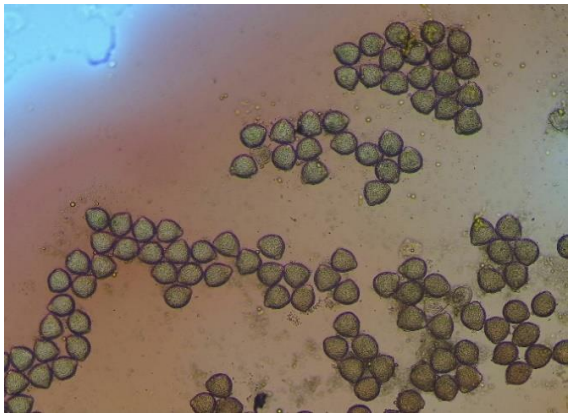

Fig. 1. Fertile pollen grains of KUPL20010.

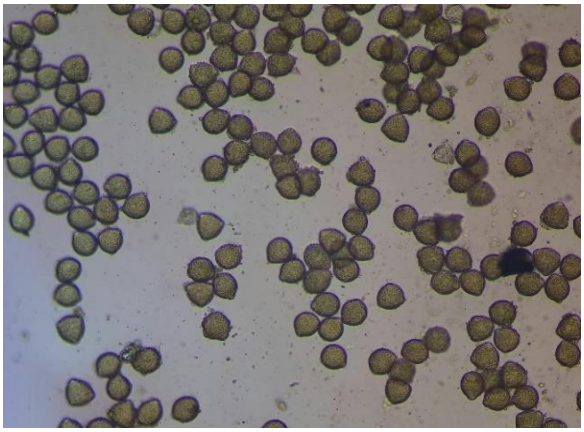

Fig. 2. Fertile pollen grains of KUPL20012.

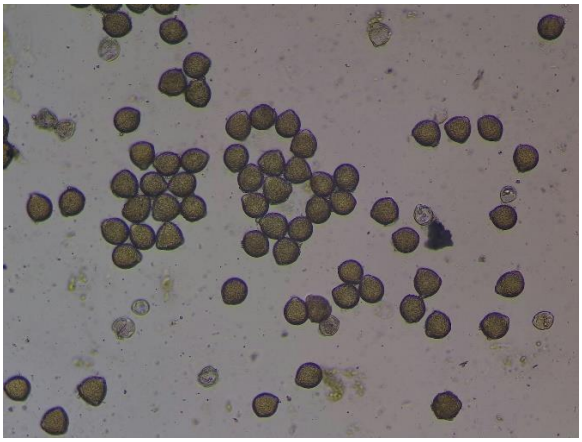

Fig. 3. Fertile and sterile pollen grains of KUPL20011.

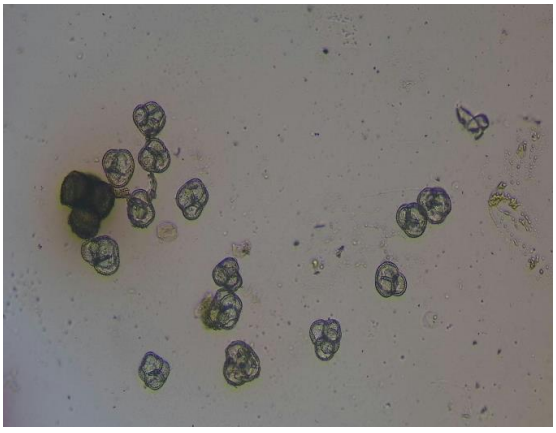

Fig. 4. Sterile pollen grains of KUPL20017.

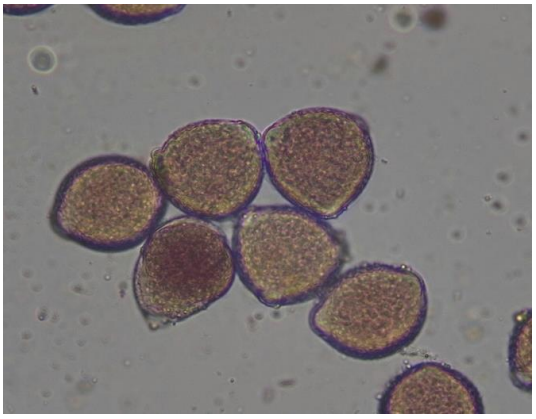

Fig. 5. Structure of fertile pollen grains at 100x microscopic view.

CONCLUSIONS

Pigeonpea being a mysterious crop among the pulses by its unexploitable genomes, baffling modes of pollination and inexplicable breeding behaviours. Further in-depth exploitation into its floral biology may provide some scope for developing high-yielding varieties and hybrids. Testing the viability of pollen through in-vitro pollen germination is a dependable approach. The outcomes of this study revealed that the lines KUPL20010, KUPL20012, and KUPL20011 can be utilized as parents for hybridization programmes for developing high-yielding varieties. Genotypes KUPL20007, KUPL20005, KUPL20003 and KUPL20026 can further be utilized to develop male sterile parents which can be used in large-scale hybrid development programmes. The other lines KUPL20017, KUPL20024 and KUPL20019 can be utilized as experimental materials to further exploit Self Incompatibility. Since there is a dearth of information regarding pigeonpea pollen characteristics, this study serves as a valuable reference for breeders seeking to create robust strains of pigeonpea with superior productivity and resistance to diseases and pests.

FUTURE SCOPE

Investigating the viability and germination characteristics of pigeonpea pollen can provide insights into its reproductive success. Understanding factors such as pollen longevity, pollen tube growth rate, and compatibility with different cultivars can contribute to better pollination management and higher crop yields. Utilizing pollen studies to identify desirable traits, such as increased pollen viability, germination rate, and compatibility, can contribute to the development of superior red gram cultivars. Breeding efforts can be focused on selecting parents with improved pollen characteristics to enhance crop performance, resilience to abiotic and biotic stresses, and overall yield potential. Exploring the potential of pollen as a source of DNA for genetic analysis and marker-assisted selection can provide a non-destructive and efficient method for assessing genetic diversity and identifying traits of interest in pigeonpea. Pollen-based molecular markers can facilitate rapid and accurate breeding strategies, leading to the development of improved varieties with desirable traits.

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Conflict of Interest. None.

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