Evaluation of Pollen Vitality in Different Sugarcane Genotypes Utilizing Various Techniques and Time Intervals

Wafaa E. Grad[1], Shimaa H. Elhoffey[1], Amr M. El-Sheikh[1], Mohamed A. Ghonema[1]

[1] Department of Breeding & Genetics, Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Egypt.

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Corresponding author1: Dr. Wafaa E. Grad
E-mail: **wafaa.grad@arc.sci.eg**

ABSTRACT

This study was conducted to contrast two staining techniques for determining pollen viability in sugarcane. At three diverse times (6:30, 8:00, and 9:30 a.m.), pollen from seven sugarcane genotypes was gathered, and its vitality was assessed using Lactophenol blue and Iodine stains. For every genotype, using a drop of the respective stain, three others were crushed on a glass slide. Pollen viability percentages were determined using an optical microscope. Variance analysis was used to analyze the factors (staining methods, genotypes, and times) and their interaction, and the means were compared. Lactophenol blue staining proved more sensitive than Iodine staining in detecting the natural decline in pollen viability in sugarcane. This study underscores the effectiveness of Lactophenol as a reliable medium for assessing pollen viability, offering a more accurate representation of the physiological state of pollen grains at various times (6:30, 8:00, and 9:30 a.m.) for different sugarcane genotypes. The genotypes exhibited variations in pollen grain viability percentage during time intervals. Genotypes G2004/27 and EH78-26-11 showed superior pollen grain vitality in both staining methods at early time, whereas genotype G2003/47recorded the lowest values at the later time.

Key words: sugarcane, pollen, viability, stain, time.

1. INTRODUCTION

Hybridization processes are commonplace for sugarcane breeders (*Saccharum* spp.), these procedures usually include crossing of extremely fertile sugarcane genotypes (as male) with male sterile kinds (as female) and then cultivating the fuzz, which are the actual sugarcane seeds. The degree of anther dehiscence seen via a hand lens may reveal a flowering variety's sexuality, but microscopic examination of pollen grains provides a more precise way to identify flowering sugarcane types as male or female (Olaoye *et al*., 2014).

Research on the induction of flowering has always been a primary focus of sugarcane breeding programs. Pollen viability is crucial for hybridization or crossing, typically expressed as the percentage of viable pollen grains determined by staining and direct counting methods. High pollen viability in the male parent is essential for successful crossing. Prior research has demonstrated that flowering is a complex process comprising various developmental stages, each characterized by distinct environmental and physiological requirements. Environmental conditions encompass factors including diurnal temperatures, day lengths, elevation, and the requirements for temperature and moisture (Coleman, 1963; Van Breemen *et al*., 1963; Clements and Awada, 1967; Gosnell, 1973; Moore, 1987; Moore and Nuss, 1987 and Araldi *et al*., 2010) rainfall patterns and distribution Olaoye (1996) sub-optimal photoperiods (Nayamuth *et al*., 2003 and Berding, 2005) increasing atmospheric CO_2

levels (Rosenzweig *et al*., 1995) and pollution levels. Pollen longevity is greatly influenced by environmental factors, particularly temperature and relative humidity. Depending on the species and conditions, viability can diminish within minutes to hours. Once shed, pollen is highly susceptible to dry air and high temperatures, which further shorten its viability and lifespan. Consequently, pollen has a limited lifespan, referred to as the "viability window." Notably, pollen from the Poaceae family is especially vulnerable and has a notably short lifespan (Barnabas and Kovacs, 1997).

However, in the genetic enhancement of sugarcane, cross-breeding is limited by the lack of flowering synchrony between parent plants. The primary methods to address this issue include staggered planting of accessions, employing darkrooms with controlled photoperiods, and the application of flowering inducers or inhibitors (Araldi *et al*., 2010).

Viable pollen grains are crucial for species dispersal, adaptability, and the survival of subsequent plant generations. They are also vital for targeted plant breeding, leading to crop improvement. Pollen viability encompasses various aspects of pollen performance, including stainability, germinability, and fertilization ability (Dafni and Firmage, 2000).

Moreover, the advancement of dependable techniques for assessing pollen's functional quality aids in tracking pollen vitality during storage, genetic research, pollen-stigma interaction studies, crop development and breeding, as well as incompatibility and fertility investigations, as noted by (Melekber Sulusoglu and Aysun Cavusoglu, 2014).

It is essential to understand the ability of the inflorescence to produce pollen for sugarcane breeding programs. In a cross between two genotypes, one genotype's inflorescence functions as the pollen donor (male), while the other acts as the pollen receptor (female) (Bull and Glasziou 1975; McIntyre and Jackson 2001 and Cheavegatti-Gianotto *et al*., 2011). The classification of inflorescence as either pollen donor or receptor is complemented by the importance of pollen viability for effective cross-pollination.

Pollen quality is vital for breeders. The aim of this study is to evaluate pollen viability among seven sugarcane (*Saccharum* spp.) genotypes. The viability was assessed using two different stains, Iodine and Lactophenol Blue, at three distinct times: 6:30 a.m., 8:00 a.m., and 9:30 a.m.

2. MATERIALS AND METHODS

This study was conducted during the March 2022 growing season at the El-Sabahia Station in Alexandria, Sugar Crops Research Institute (SCRI), Agricultural Research Center (ARC), Egypt (31° 22' N, 29.94° E). Flowering induction occurred during the optimal cycle when daylight hours gradually decreased from 12:00 to 11:30 between late September and mid-October, aligning with favorable temperature and humidity conditions, as shown in Figure (1).

2.1. Source of genetic materials

Pollen grains were collected from seven synchronized genotypes (Table1 and Figure2) that flowered at the same age under natural conditions in late November 2022.

2.2. Pollen grain characterization

Inflorescences were collected from three stalks in each plot to evaluate pollen viability at three distinct times: 6:30 am, 8:00 am, and 9:30 am. This assessment utilized a pair of staining techniques, Iodine and Lactophenol blue. The chosen times correspond with the typical schedules of sugarcane breeding programs, which commence operations around sunrise.

The experiment was conducted in three repetitions, each involving an inoculated slide. Pollen grains from each slide were examined under an optical microscope (PB/OPTI1, England) using 10X and 40X objectives. The viability of pollen was assessed using Iodine and Lacto-phenol (cotton blue) staining

methods, following the protocols of (Radford *et al*., 1974; Machado Jr, 1987 and Asghari, 2000). For each genotype, three mature others had been crushed on a glass slide before staining with a few drops of each stain (Iodine 0.1 N and Lactophenol blue) and sealed with a cover slip to prevent displacement of the pollen grains. With Iodine staining, viable pollen grains appeared brownish, while non-viable ones were yellowish. In the lacto-phenol staining method, viable pollen turned blue, whereas non-viable pollen remained transparent. By using an optical microscope (PB/OPTI1, England) and the scanning technique, the percentage of viable pollen for each staining procedure was determined until 100 pollen grains per slide were evaluated.

2.3. Statistical analysis

The effects of staining procedures, genotypes, timing, and their interactions were assessed by analysis of variance and the F-test ($P<0.01$). The total viable pollen percentage was converted to angle values in degrees ARC-Sin (Evwin *et al*., 1966). Mean comparisons were conducted using the LSD test at a 5% significance level (Waller and Duncan, 1969) with Minitab® 21.4.2 software.

3. RESULTS AND DISCUSSION

The examination of pollen grains from different genotypes, gathered at various times and subjected to multiple staining techniques, revealed that they could be distinguished as viable or unviable based on their structure and features (Figure, 3). Viable pollen grains were large, perfectly round and darkly colored, indicating fertility, whereas unviable pollen grains were transparent, hollow and lacked color indicating infertility.

Pollen grains that stained dark brown were considered alive, in contrast to the yellowish ones deemed unviable by the Iodine staining method (Figure 3a). Additionally, viable pollen grains were round and absorbed the blue stain (Lactophenol or cotton blue), while unviable ones remained transparent (Figure 3d). Viable pollen grains were further categorized into sulcate (Figure 3b) (with numerous pores visible under a light microscope) or colpate (Figure 3c) (with two apertures evident after viability testing). This classification aligns with the findings of Olaoye *et al*. (2014), who categorized pollen morphology of genotypes as either sulcate or colpate using Lactophenol stain blue.

The study demonstrated a highly significant impact on all assessed factors (Genotype, Time, staining technique) and their interactions, with the exception of the interaction between Time and Staining method. (Table 2).

Figure (4a) illustrates marked disparities in pollen grain viability across the studied genotypes. The highest viability was noted in Ph8013 and EH 78-26-11, succeeded by G.T. 54-9 and G2004/27, while G2006/41 and F161 exhibited reduced viability. On the other hand, The genotype G2003/47 showed the lowest viability. A significant discrepancy was noted between the two staining methods; as illustrated in Figure (4b), the Lactophenol staining method demonstrated superior values of pollen viability compared to the Iodine staining method. Additionally, the three distinct time points demonstrated notable differences in pollen viability, with the earliest time point (6:30 a.m.) displaying the highest viability and the latest time point (9:30 a.m.) showing the lowest percentage of viable pollen, as referenced in Figure (4c).

The interaction between the two staining methods and genotypes resulted in highly significant values (Figure 5a). The Lactophenol stain showed higher pollen viability than the Iodine stain for genotypes Ph 8013, GT 54-9, and G2003/47. Conversely, it showed lower values compared to Iodine for F161 and G2004/27 genotypes, while it yielded identical values for EH 78-26-11 and G2006/41 genotypes. Compared to Iodine staining, Lactophenol blue showed greater sensitivity in identifying alterations in pollen viability that naturally occur in sugarcane genotypes.

However, the Iodine staining method demonstrated higher stability and simplicity for classifying inflorescence at all evaluated times (6:30, 8:00, and 9:30 a.m.) likened to Lactophenol. Figure (5b) demonstrated a highly significant interaction between the three times and seven genotypes, with the earliest time showing the highest viability values and the latest time showing the lowest for various genotypes.

A negligible difference was observed between the two staining methods for pollen grains collected at each of the three times (Figure 5c).

Table 3 and Figure 6 demonstrate that the performance varied among different genotypes, with G2004/27 and EH 78-26-11 showing the highest pollen viability across the staining techniques at early time (6:30 a.m.), whereas G2003/47 showed the lowest viability. When comparing staining methods at each evaluation time, differences in pollen viability were noted between genotypes at 6:30, 8:00, and 9:30 a.m., for both Iodine and Lactophenol blue staining methods. Notably, at 8:00 a.m. and 9:30 a.m., there was a marked decrease in pollen viability for each genotype when assessed with either staining method, as indicated in (Table 3 and Figure 6).

Except for the G2003/47 genotype, which showed no variance in pollen viability at the later times due of its very low pollen viability at 6:30 a.m., the Lactophenol blue staining method was the only one to reveal variations in pollen viability over time among the evaluated genotypes. By tracking the decrease in pollen viability over time, the Lactophenol blue staining technique proved more sensitive.

The sensitivity of the Iodine staining method differed from that of the Lactophenol blue method. The Iodine staining consistently indicated similar pollen viability across time points, likely due to differences in the staining mechanisms. Iodine staining relies primarily on starch, which is predominantly found in viable pollen grains but can also be present in aborted pollen (King 1960 and Rodriguez-Riano and Dafni 2000). Conversely, the Lactophenol blue staining method binds to the cytoplasm of live pollen, which does not guarantee germination (Nepi and Franchi 2000). Nonetheless, this stain indicated a decrease in pollen viability over time (h), likely due to changes in the cytoplasm associated with loss of viability. This decline was particularly evident, especially in GT 54-9 and G 2004/27 genotypes that exhibited initially high viability, suggesting that even robust pollen can be susceptible to environmental stressors over time. The Lactophenol blue staining method not only allowed for the visualization of live pollen but also facilitated a deeper understanding of the physiological changes occurring within the pollen grains (Figure7). Kılıç *et al*. (2024) utilized five chemical staining methods, Iodine-potassium iodide, 2,3,5-triphenyl tetrazolium chloride, Lactophenol cotton blue, safranin, and acetocarmine to evaluate pollen viability. They observed that different chemical techniques had different efficacy depending on species and variation, which causes significant differences in outcomes even for the same species or variety. While other techniques could stain pollen that has already diminished in viability, others might stain pollen before maturity.

This study suggests that the decline in pollen viability was primarily due to reduced air moisture throughout the day, rather than temperature, which ranged from 22°C at 6:30 am to 24°C at 9:30 am in November. Low humidity likely caused desiccation, negatively affecting pollen hydration and reducing successful germination, which is essential for fertilization in flowering plants. Notably, the stable temperature during this period indicates that moisture content, rather than heat, is crucial for pollen health. These findings highlight the importance of monitoring environmental factors, especially humidity, to gain insight into plant reproductive success. Sufficient moisture is essential for initiation

and growth, as well as for timing emergence, anthesis, and regulating seed set. Flower opening and anthesis are influenced by relative humidity, since both processes often transpire few hours before to daylight, when the plant is well hydrated and relative humidity is high. Anthesis occurs when the relative humidity decreases near sunrise (de Calvino, 1925; Mclntosh, 1930 and Dutt *et al*., 1938). Sugarcane pollen viability is short-lived, significantly affected by humidity and temperature, with a half-life of just 12 minutes after dispersion (Sartoris 1942; Scarpari and Beauclair, 2008). Higher humidity levels increase pollen longevity, whereas in conditions of rapid desiccation, pollen has a half-life of merely 20 to 30 minutes (Moore, 1976). Low humidities at anthesis leads to poor seed set (Nuss, 1979). In temperate South Africa, pollen fertility has been shown to be limited at temperatures below 21°C (Berding, 1981). Due to the short lifespan of sugarcane pollen grains, common in many Poaceae family plants (Hanna and Towill, 1995), it is recommended to conduct pollen tests at 5:00 a.m. Currently, the plants are turgid, and the anthers are more fully exposed, resulting in greater pollen viability (Moore, 1987). After this period, survival becomes difficult due to water loss and the maintenance of dehydration in natural conditions (Nepi and Pacini, 1993 and Lisci *et al*., 1994). It is to begin breeding programs early, before advisable sunrise, as the high number of inflorescences to evaluate makes it challenging to limit the pollen viability test to a few hours. In Alexandria, Egypt, favorable temperature and humidity conditions have been conducive to flower growth, especially during the optimal cycle when daylight hours gradually reduced from 12:00 to 11:30 between late September and mid-October (Ghonema, 2017). Iovane *et al*., (2022) found that pollen viability significantly declines when exposed to high humidity and temperatures. Moreover, in light of climate change, it is essential to assess how rising temperatures affect sensitive reproductive traits like pollen viability and to anticipate potential reductions. Additionally, the impact of temperature increases on pollen thermotolerance should be evaluated along with other environmental factors, such as humidity.

To improve crossbreeding, it's crucial to consider pollen viability, as neglecting it can result in misclassifying inflorescences as male or female, leading to increased self-fertilization and the production of unviable seeds, which is undesirable in sugarcane breeding programs. The categorization of sugarcane inflorescences as male or female is affected by regional climate, which may vary owing to climate change. Olaoye *et al*. (2010) indicated that flowering behavior and the sexuality of sugarcane inflorescences whether male or female alter in response to climatic changes.

Finally, the study found that the Lactophenol staining intensity decreased over time, indicating a gradual loss of cytoplasmic integrity, which is crucial for pollen functionality. These results underscore the importance of timely pollen collection and application, particularly in breeding programs where pollen viability is essential for successful fertilization. The findings of the current study highlight Lactophenol efficacy as a reliable medium for assessing pollen viability, providing a more accurate representation of the pollen grains' physiological state. In contrast, the conventional Iodine staining method can yield inconsistent results, as it depends on the presence of starch, which does not necessarily reflect pollen's overall viability. Consequently, these findings suggest that future research on pollen viability should include Lactophenol staining to enhance the reliability of results and to obtain a better understanding of the reproductive capabilities of the plant species being investigated. Moreover, to ensure the vitality and longevity of pollen grains across all genotypes, it is advisable to initiate the hybridization process early under favorable

natural flowering conditions of temperature and humidity. This research could help develop strategies to lessen the impact of climate variability on plant reproduction.

4. REFERENCES

Araldi, R.F., Silva, M.L., Ono, E.O. and Rodrigues, J.D. (2010). Flowering in sugarcane. Cienc. Rural [online] 40 (3):694-702.

Asghari, J. (2000). Estimation of pollen viability of metsulfuron treated dyers woad (Isatistinctoria) for herbicide efficacy evaluation. Journal of Agriculture, Science and Technology 2 (2): 85–93.

Barnabas, B. and Kovacs, G. (1997). Storage of pollen. In: KR Shivanna and VK Sawney (ed.) Pollen Biotechnology for Crop Production and Improvement Cambridge University Press. U.K. pp 293-314.

Berding, N. (1981). Improved flowering and pollen fertility in sugarcane under increased night temperatures. Crop Science 21: 863-867.

Berding, N. (2005). Poor and variable flowering in tropical sugarcane improvement programmes. Diagnosis and resolution of a major breeding impediment. XXV Jubilee Congress of International Society of Sugarcane Technologists.

Bull, T.A. and Glasziou, K.T. (1975). Sugar cane. In Crop physiology:Some case histories, ed. L.T. Evans, 51–72. Cambridge: University Press.

Cheavegatti-Gianotto A., de Abreu H.M.C., Arruda, P., Filho, J.C.B., Burnquist, W.L., Creste, S., di Ciero, L., Ferro, J.A., de Oliveira Figuerira, A.V., de Soussa Filgueira, T., de Fatma GrossiDe-Sa´, M., Guzzo, E.C., Hoffmann, H.P., de Andrade Landell, M.G., Macedo, N., Matsuoka, S., de C. Reinach, F., Romano, E., da Silva, W. J., da Silva Filho, M.C. and Ulian, E.C., (2011). Sugarcane (*Saccharum* x *officinarum*): A reference study for the regulation of genetically modified cultivars in Brazil. Tropical Plant Biology 4: 62–89.

Clements, H.F. and Awada, M., (1967). Experiments on the artificial induction of flowering in sugarcane. Proc. Int. Soc. Sugar Cane Technol., 12: 795-812.

Coleman, R.E., (1963). Effect of temperature on flowering of sugar cane. Int. Sugar J., 65: 351-353.

Dafni, A. and Firmage, D. (2000). Pollen viability and longevity: Practical, ecological and evolutionary implications. Plant Systematics and Evolution 222, 113-132.

De Calvino, E.M. (1925). The Jeswiet method for the identification of sugarcane varieties. Int. Sugar J., 27: 22-25.

Dutt, N.L., Krishnaswami, M.K. and Rao, K.S.S., (1938). On certain floral characters in sugarcane-l. Proc. Int. Soc. Sugar Cane Technol., 6: 154-170.

Evwin, L. L., Warren, H. L. and Andrews, G. C., (1966). Field plot technique transformation of experimental data. Burgess pub. Comp America, p. 338-349.

Ghonema, M. A., (2017). Flowering Synchronization in Some Sugarcane Genotypes at Various Planting Dates under Natural Environment. Alexandria Science Exchange Journal,.38(2): 238-249.

Gosnell, J.M., (1973). Some factors affecting flowering in sugarcane. Proc. South Afr. Sugar Technol. Assn., 47: 144-147.

Hanna, W.W., and Towill, L.E., (1995). Long-term pollen storage. Plant Breeding Reviews 13: 179–207.

Iovane, M., Cirillo, A., Izzo, L. G., Di Vaio, C., and Aronne, G. (2022). High Temperature and Humidity Affect Pollen Viability and Longevity in Olea europaea L. Agronomy, 12(1), 1.

Kılıç, T., Sinanoğlu, E., Kırbay, E., Kazaz, S., Ercişli, S. (2024). Determining appropriate methods for estimating pollen viability and germination rates in lisianthus. Acta Sci. Pol. Hortorum Cultus, 23(3), 33–42. https://doi.org/10.24326/asphc.2024.5378

King, J.R. (1960). The peroxidase reaction as an indicator of pollen viability. Stain Technology 35: 225–227.

Lisci, M., Tanda, C. and Pacini, E. (1994). Pollination ecophysiology of Mercurialisannua annua L. (Euphorbiaceae) an anemophilous species flowering all year round. Annals of Botany 74: 125–135.

Machado Jr, G.P. (1987). Melhoramento da cana-de-ac¸u´car. In Canade- ac¸u´car: Cultivo e utilizac¸a˜o, ed. S.B. Paranhos, 165–186. Campinas: Fundac¸a˜o Cargill.

Mclntosh, A.E.S., (1930). Pollen shedding in Barbados sugar-cane varieties. Trop. Agric, 7: 296-299.

McIntyre, C.L. and Jackson, P.A. (2001). Low level of selfing found in a sample of crosses in Australian sugarcane breeding programs. Euphytica 117 (3): 245-249.

Melekber Sulusoglu and Aysun Cavusoglu. (2014). *In vitro* Pollen Viability and Pollen Germination in Cherry Laurel (*Prunus laurocerasus* L.). Scientific World Journal Volume 2014, Article ID 657123, 7 pages http://dx.doi.org/10.1155/2014/657123.

Moore, P.H. (1976). Studies on sugarcane pollen. II. Pollen storage. *Phyton, Argentina* 34: 71-80.

Moore, P.H. (1987). Physiology and control of flowering. In Copersucar international sugarcane breeding workshop, 101–127. Piracicaba: Copersucar Technology Center.

Moore PH and Nuss, K.J. (1987). Flowering and flower synchronization. Chapter 7 In: D. J. Heinz ed. Sugarcane improvement through breeding, Elsevier, Amsterdam.

Nayamuth, R., Mangar, M. and Sopaya, R., (2003). Characterization of natural environment for sugarcane flowering ability. AMAS Food and Agricultural Research Council, Reduit, Mauritius. 179-187.

Nepi, M., and Franchi, G.G., (2000). Cytochemistry of mature angiosperm pollen. Plant Systematics and Evolution 222: 45–62.

Nepi, M., and Pacini, E.,(1993). Pollination, pollen viability and pistil receptivity in Cucurbita pepo. Annals of Botany 72: 527–536.

Nuss, K.J., (1979). Factors influencing the numbers of seedlings obtained from sugarcane crosses. Proc. South Afr. Sugar Technol., 53: 167-169.

Olaoye, G., (1996). Studies on flowering in sugarcane in a savanna ecology of Nigeria. I. Relationship between pollen fertility and seed set. Nigerian J Genetics XI: 60-65.

Olaoye, G., Abayomi, Y.A., Akinyemi, S.O., (2010). Contribution of maternal parents to progeny selection in sugarcane (*Saccahrum officinarum* L.). proc. 34th Annual Conf. of Genetics Society of Nigeria. 19th- 24th September, 2010. National Institute of Horticultural Research, Idi-Isin, Ibadan.

Olaoye, G., Olaoye, J.O., Takim, F.O., Idris, A.M. and Bankole, F., (2014). Assessment of Pollen Fertility, Cane Yield and Ethanol Content in Sugarcane Progenies Developed by The Modified Polycross Method. Journal of Sugarcane Research 4 (2): 29 – 38.

Radford, A.E., Dickison, W.C., Massey, J.R. and Bell, C.R., (1974). Vascular plant systematics. New York: Harper & Row Publishers.

Rodriguez-Riano, T., and Dafni, A., (2000). A new procedure to assess pollen viability. Sexual Plant Reproduction 12: 241–244.

Rosenzweig, C., Allen Jr, L.H., Harper, L.A., Hollinger, S.E. and Jones, J.W. (eds.) (1995). Climate change and agricultural analysis of potential international impacts. ASA Apwcial Publication No. 59. American Society of Agronomy. Madison, WI. 382pp.

Sartoris, G.B. (1942). Longevity of sugar cane and corn pollen—A method for long distance shipment of sugar cane pollen by airplane. American Journal of Botany 29: 395–403.

Scarpari, M.S., and de Beauclair, E.G.F., (2008). Anatomia e botaˆnica. In Cana-de-ac¸u´car, ed. L.L. Dinardo-Miranda, A.C.M.

de Vasconcelos, and M.G.A. Landell, 47–56. Campinas: Instituto Agronoˆmico.

Van Breemen, J.F., Liu, L.J., Ellis, T.O. and Arceneaux, G., (1963). Effect of elevation on arrowing and pollen fertility in sugar cane. Proc. Int. Soc. Sugar Cane Technol., 11: 540-545.

Waller, R. A., and Duncan, D. B., (1969). A Bayes rule for the symmetric multiple comparisons problem. J. Am Stat. Assoc. 64:1484~1503.

Table (1): Geographical origins of tested cane materials

No	Genotypes	Origin
1	G.T. 54-9	Giza, Taiwan
2	Ph 8013	Philippines
3	G2006/41	Egypt, Giza
4	F161	Taiwan
5	G2003/47	Egypt, Giza
6	G2004/27	Egypt, Giza
7	EH 78-26-11	Egypt, Hawamdeia

Table (2): Variance analysis of pollen viability in seven sugarcane genotypes at three times (6:30, 8:00 and 9:30 a.m.) using two staining methods (Iodine and Lactophenol blue)

Source of variation	Degree of Freedom	Mean Square	F. Value
Hours	2	2572.66	167.85***
Genotypes	6	702.42	45.83***
Staining method	1	616.06	40.19***
Hours * genotypes	12	121.06	7.90***
Hours * staining method	2	6.07	0.40 ns
Genotype * staining method	6	465.11	30.35***
Hours * genotype * staining method	12	68.12	4.44***
Error	126	15.33	
C. V. (%)			25.43

Table (3): Mean and t-test results for pollen viability of seven sugarcane genotypes at three times (6:30, 8:00 and 9:30 a.m.) using two staining methods (Iodine and Lactophenol blue)

Time	Genotype	Pollen viability Iodine (%)	Pollen viability Lactophenol (%)
6.30	GT54-9	43.2875 ^{def}	58.4725 ^{a}
	Ph8013	38.4000 ^{fghi}	58.2175 ^{a}
	G2004/27	52.1800 ^{b}	51.7075 ^{b}
	EH78-26-11	51.5975 ^{b}	50.6275 ^{bc}
	G2006/41	44.5875 ^{de}	39.5100 ^{efgh}
	F161	41.6300 ^{defg}	38.1625 ^{fghi}
	G2003/47	29.8975 ^{klmn}	36.9825 ^{ghi}
8.00	GT54-9	33.9125 ^{ijkl}	55.1475 ^{ab}
	Ph8013	37.4825 ^{ghi}	53.2550 ^{ab}
	G2004/27	50.4100 ^{bc}	35.3775 ^{hij}
	EH78-26-11	45.6500 ^{cd}	45.5250 ^{cd}
	G2006/41	39.9600 ^{efgh}	34.9450 ^{hijk}
	F161	41.7475 ^{defg}	38.3675 ^{fghi}
	G2003/47	28.9775 ^{lmn}	39.1875 ^{efghi}
9.30	GT54-9	27.4750 ^{mn}	30.3350 ^{jklmn}
	Ph8013	37.6925 ^{ghi}	51.2825 ^{b}
	G2004/27	31.1800 ^{jklm}	25.0375 ^{no}
	EH78-26-11	37.1100 ^{ghi}	40.8575 ^{defg}
	G2006/41	27.9475 ^{mn}	34.5800 ^{hijk}
	F161	30.0550 ^{jklmn}	27.4275 ^{mn}
	G2003/47	21.4000 ^{o}	28.0025 ^{mn}

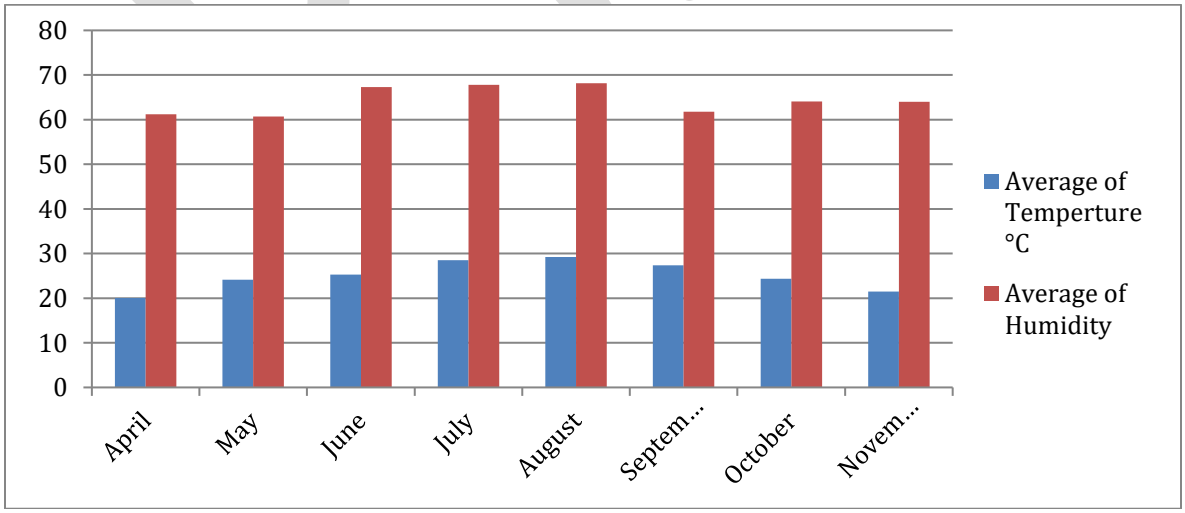

Figure (1): Average of temperature (^{0}C) and Humidity for the evaluation periods.

Figure (2): *Saccharum* spp hyprid inflorescences and pollen harvesting (a), panicle collected (b), panicle with production and exposition of anthers and average portion (c) and harvesting of pollen with brushes (d).

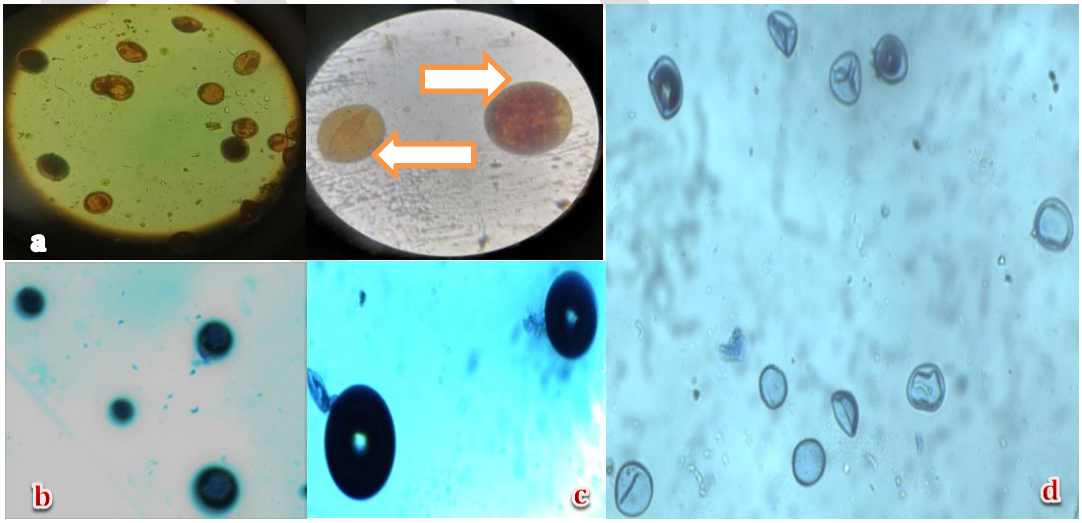

Figure (3): Pollen morphology in sugarcane genotypes: (a) Iodine-stained pollen grains at 40X and 100X, (b) sulcate viable pollen grains stained with lacto-phenol, (c) colpate viable pollen grains, and (d) both viable and unviable pollen grains.

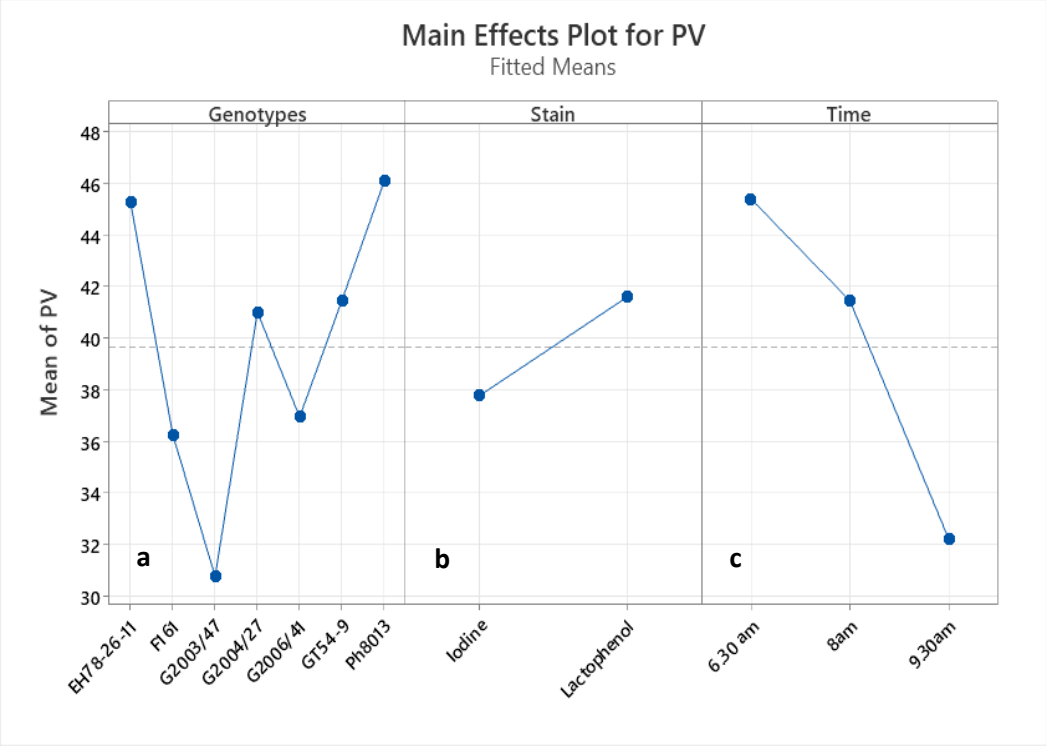

Figure (4): Mean effects for pollen viability within genotypes (a), staining methods (b) and different times (c).

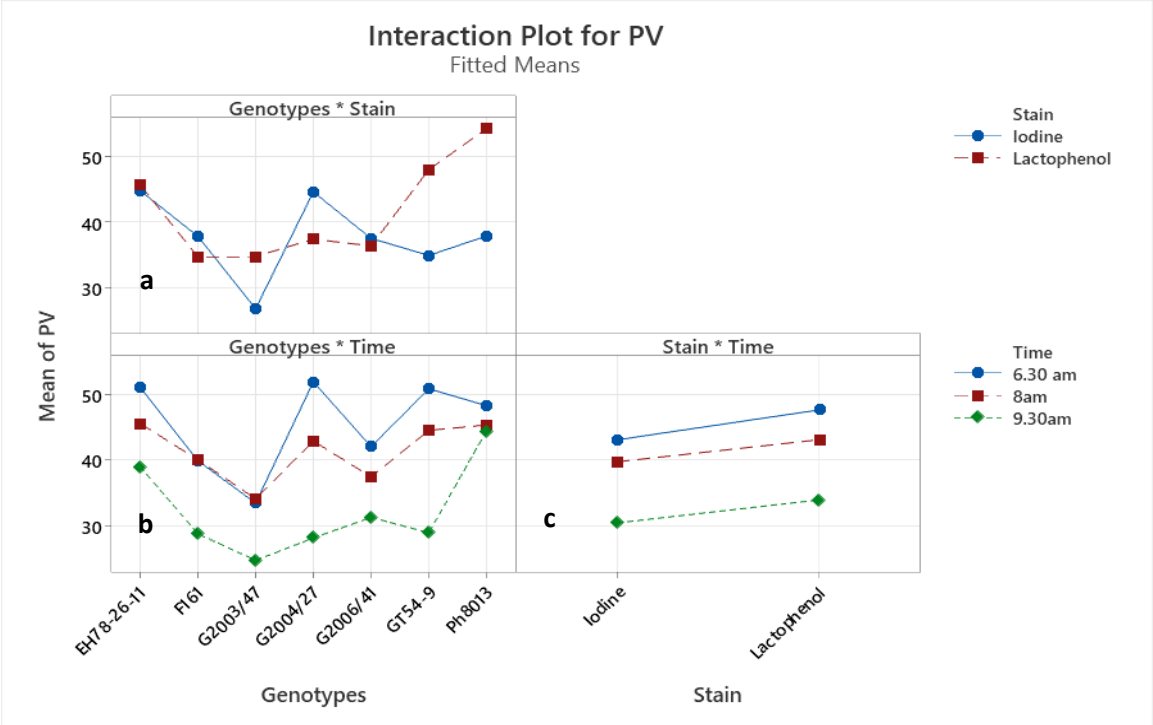

Figure (5): Mean effects for pollen viability of interaction between genotypes & stain method (a), genotypes & Times (b) and stain method & times (c).

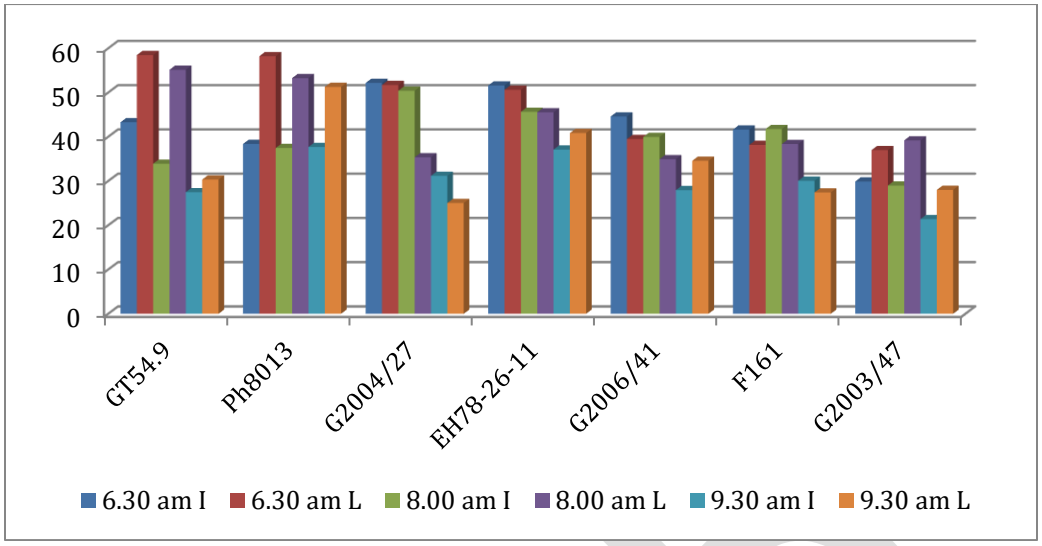

Figure (6): Comparisons between pollen viability of seven sugarcane genotypes at three different times (6.30 a.m., 8.00 a.m. and 9.30 a.m.) obtained by two staining methods (Iodine and Lactophenol blue).

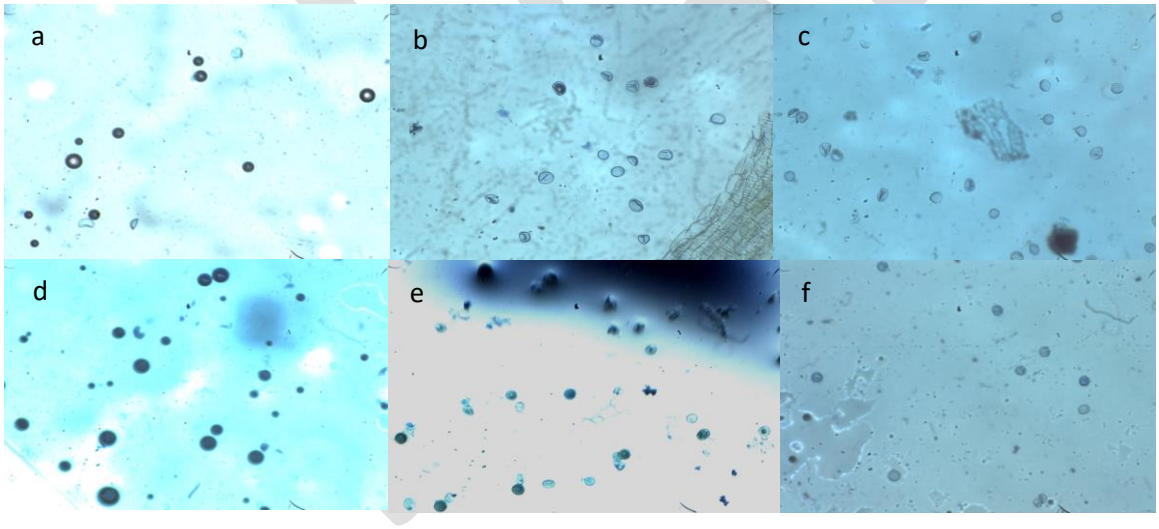

Figure (7): Different absorption of Lactophenol blue during three times intervals for two genotypes G 2004/27 (a: 6.30, b: 8.00, c: 9.30 a.m.) and GT 54 – 9 (d: 6.30, e: 8.00, f: 9.30 a.m.).