IJABR Vol. 5(1): 62 - 71 (2013)

Assessment of pollen production, viability and germinability in three Sesame cultivars.

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Submitted: March 28th 2013; Accepted: December 16, 2013; Published: January 14, 2014.

ABSTRACT

Pollen production, viability and germinability were assessed in three sesame cultivars viz Kenana4, Ex-Sudan and E8. The seeds were collected from the National Cereals Research Institute, Badeggi and were raised to maturity in pots filed with rich loamy soil in a randomised block designed at the experimental garden of CPES, Federal University of Technology, Minna. While the Haemocytometric method was used to determine the pollen production, the IKI (Iodine + Potassium Iodide) stain was used for pollen viability test, and germinability test was carried out using different sucrose concentrations with 1% agar solution. Results showed that all the cultivars had high pollen production and viability percentages were however significantly affected by media concentrations. Although, the viability percentages were higher than the germination percentages, it was an indication that not all viable pollens germinated *in vitro*. This has provided an insight into the reproduction biology of sesame and this can be useful in future breeding experiments. In addition, the highest pollen germination was observed for variety kenana4 and E8 in the 20% sucrose but for variety Ex- Sudan the highest percentage germinability was observed in 30% sucrose solution. From the study, it can be concluded that variety Ex- Sudan, E8 and Kenana4 of sesame are good pollinizers and will be good for breeding experiments.

Key words: Sesame, Production, Viability, Germinability.

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INTRODUCTION

Sesame is a flowering plant belonging to the Family Pedaliaceae and Genus *Sesamum*. The Genus consists of about 36 described species out of which the most commonly cultivated is *Sesamum indicum* L. (Purselglove, 1974; Falusi, 2006). Numerous wild relatives occur in Africa which is its origin and a small number in India (Tribe,

1967). The crop is cultivated for its seeds which contains about 50-52 % oil (Ashri, 1982). The flowers of Sesame are yellow, though they vary in colour with some being purple or blue. It is a herbaceous annual plant of international importance (Raw Materials Update, 2001). It grows up to 50–100cm tall and has opposite leaves that are 4–14cm long with an entire margin. The crop is widely grown in Northern and Central Nigeria within latitudes seven to fourteen degrees North (7–14 ^{0} N) with an annual rainfall requirement of about 1000-1500mm (Iwo *et al.,* 2005).

Pollination and fertilization in the crop are obligatory to obtain fruits, hence the oil rich seeds of sesame, unlike in most parthenocarpic crops. The quality and quantity of pollen grains will determine how effective it can be as a pollinizer. Therefore, the pollen production, viability and germinability ratios of the grown genotypes of Sesame need to be known.

MATERIALS AND METHODS

Three varieties of Sesame were used in this study (kenana4, Ex- Sudan, and E8). The seeds were collected from the National Cereal research Institute Badeggi, Niger State. They were sown in pots filled with rich loamy soil in a randomized block design and nurtured to maturity at the experimental garden of the Centre for Preliminary and Extra-mural Studies, Federal University of Technology Minna, Nigeria. At maturity, Flower buds for pollen production, viability and germinability tests were collected.

Determination of Pollen Production

The anthers of forty flowers from four plants of each sesame variety were counted. Pollen production per flower was determined using haemocytometric method (Mehmet, 2011). Ten flower buds for each plant were used in the study. The flower buds were divided into two groups. Each group contained anthers from five flower buds in small vial bottles. The anthers were thoroughly crushed with a glass rod and then 1ml distilled water was added into each vial bottle.

A drop of the pollen suspension was placed on a two counting area containing Thoma (haemocytometric) slide (0.1mm in depth) to where a special cover slip was replaced. The pollens were placed on randomly chosen four large squares in each counted area with two replicates representing each group of flowers in vials. The average pollen grain amount per flower (P/F) = Pollen count x $1000mm^3$/ $0.1mm^3$/5 flowers. The number of pollens per anther was calculated by dividing the number of pollens per flower by the number of anthers per flower counted, the number of pollens per plant was calculated by multiplying the number of flowers per plant with the number of pollens per flower (Eti & Stösser, 1988).

Pollen Viability Tests

The pollen viability test was carried out after the method described by Eti (1991) and Stosser (1984). Iodine + Potassium Iodide (IKI) stain was used in determining pollen viability. IKI solution was prepared by

dissolving 5g iodine and1g Potassium iodide in 100ml distilled water. One or two drops of the solution was placed on microscope slides and pollen grains of each type were sprinkled on the stain with the aid of a brush. Pollen grains were examined using a light microscope (x 100). The counts were made few minutes after pollen grains were placed on IKI solution.The viability of pollen was scored according to staining level: pollen with dark brown colour as viable, with light red colour as semi-viable and with yellowish-green colour or colourless as non-viable. The study was conducted with a total of eight replicates as two slides and randomly chosen four areas were counted for each slide. About 80 pollens were counted in each field.

Pollen Germinability Test

Sucrose solutions of different concentrations such as 0%, 10%, 20% and 30% were added to basic agar of 1% and used as medium for germinability test. The medium was dropped in Petridishes and pollens were sprinkled onto the medium gently and petridishes closed to prevent water loss of pollens. The Petri dishes were incubated at 30^0c for 24 hours. After germination, pollens in the petri dishes were refrigerated until counted. Two petri dishes were used per sucrose concentration for each variety. Approximately 300 pollens were counted in each petridish. Pollens were considered as germinated if the pollen tube length was at least equal to or greater than the grain diameter. Response to germinability was expressed as percentage.

Data analysis

All data in the experiment were subjected to analysis of variance and Least significant difference was used to determine significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

The mean number of flowers per plant for Kenana4 and Ex- sudan is 29 while E8 had the lowest (20 flowers per plant). Statistical analysis showed that there is no significant difference in the number of flowers produced per plant in all the cultivars used. All the cultivars had 4 anthers per flower.

Fig. 1 Kenana 4 at maturity showing the flower buds

Fig 2. Ex- Sudan at maturity showing the flower buds

Variety Ex- Sudan and E8 had the highest pollen per flower (16,750 pollens) while Kenana4 had the lowest (16,688 pollens). The same trend occurred in the number of pollens per anther. For the number of pollens per plant calculated, Variety Ex Sudan had the highest number of pollens per plant (485,750). This was followed by variety Kenana4 with 483,952 and lastly variety E8 with 335,000 pollens per plant. The differences between these three varieties according to pollen production were low and were not significantly different (Table 1). Among Kenana4 plants, the highest and lowest amount of pollen grains per flower was determined as 23,750 and 10,500 pollens respectively. Among the Ex- Sudan types the amount of pollen grains per flower changed to between 19,000 and 15,250 pollens; in E8 between 17,000 and

16,500 pollens. The plants in cultivar Kenana4 were found statistically different according to the amount of pollen production (Table 1). Mahmoud (2012) reported the presence of many insects in the sesame growing field in his experiment to determine the effect of insect pollinators on Sesame pollination. In this respect, the high amount of pollens found in the Sesame varieties may play an important role in the transfer of pollen by these vectors. The results of this study also shows that these genotypes are closely related with respect to pollen production since there are no significant differences in the pollen production and any of them can be used in both natural and artificial pollination and fertilization studies.

Pollen viability

The highest percentage of pollen viability was found in Ex-sudan (91.78%) which was statistically the same with E8 and kenana 4 (Table 2). All three varieties had good amounts of viable pollens (over 75%). This finding is in line with the findings of Falusi *et al.,* (2001) who discovered high pollen viability percentage in *Sesamum radiatum* and *Sesamum indicum* using Lactophenol cotton blue as stain. This shows that IKI stain is also a good stain that can be used to determine percentage viability in Sesame. The high percentage viability found in Sesame types may also indicate a good pollen germination rate in a suitable in vitro condition.

Pollen Germinability

The result of the pollen germinability test is seen in Table 3. The percentage germinability of the three cultivars used were statistically alike in all the sucrose concentrations except for 10% sucrose concentration where Variety E8 was significantly lower than Kenana4 and Ex-Sudan. Pollens did not germinate at all on a medium without sucrose in all three varieties. Pollen germination increased from 10% to 20% sucrose and then decreased in the 30 % in Kenana4 and E8.

This shows that too low and high concentration of sucrose in medium can affect pollen germination negatively. This is in line with the report of Bolat and Pirlak (1999) and also Ilgin (1995). Stanley and Linskens (1974) also reported that various germination media may affect the germination result of a given cultivar. Pfahler *et al.,* (1997) also studied the germinability ratios of Sesame genotypes and discovered that germinability was affected by some other factors such as temperature and time.

The pollen germinability results were also observed to be generally lower than the percentage viability. This is an indication that not all viable pollens will germinate *In vitro*. Eti *et al.* (1996) found similar results in their experiments emphasizing that pollen grain assessment through the staining method seems to express the germination potential but not its occurrence hence higher percentage viability than percentage germinability. Stanley and Linskens (1974)

reported that the extent of germinability achieved depends on the experimental success in determining the optimal medium for germinability.

CONCLUSION

The present study has established that viability test is a faster and easier method in determining pollen quality, than the germination tests, since the effects of external factors such as temperature, humidity, and germinating media are minimized. IKI stain could be used in determining the pollen viability and indicate germination status in Sesame types. The three genotypes selected appear to have sufficient pollen production, viability and germination to be used in pollination. This can be further tested by *in vivo* pollinations for yield. The high amount of pollen found in the Sesame flowers may play an important role in the transfer of pollens by insects as reported by Mahmoud (2012). Any of these cultivars can be used in both natural and artificial pollination studies.

Table1. Pollen production parameters in Kenana 4, Ex-Sudan and E8

	F/P	A/F	P/F	P/A	P/P
Kenana 4	29a	4a	16688a	4172	483952
Ex-sudan	29a	4a	16750a	4188	485750
E8	20a	4a	16750a	4188	335000
Kenana 4					
1	29a	4a	17250a	4312	500250
2	20a	4a	23750b	5937	475000
3	26a	4a	10500a	2625	273000
4	42a	4a	15250a	3812	640500
Ex-sudan					
1	21a	4a	15500a	3875	325500
2	35b	4a	19000a	4750	665000
3	19a	4a	15250a	3812	289750
4	40b	4a	17250a	4312.5	690000
E8					
1	21a	4a	16500a	4125	346500
2	23a	4a	17000a	4250	391000
3	26a	4a	17000a	4250	442000
4	10b	4a	16500a	3625	165000

Means followed by the same letter(s) within the same column do not statistically differ at 5% level tested by Least significant difference (LSD)

Table 2: Percentage of pollen viability in three sesame cultivars

Cultivar	Viable	Semi-viable	Non-viable
Kenana 4	77.80a	3.40a	18.68a
Ex-sudan	91.78a	2.50a	5.72a
E8	88.88a	4.50a	6.70a
Kenana 4			
1	86.65a	2.25a	11.10a
2	83.05a	3.00a	13.95a
3	80.25a	3.50b	16.25ab
4	61.60a	5.55c	32.85c
Ex-Sudan			
1	99.00b	0.00a	1.00a
2	94.80b	0.54b	4.66b
3	78.30a	3.25c	18.45c
4	94.75ab	0.50b	4.50b
E8			
1	73.55a	4.50c	22.00b
2	93.05b	1.50b	5.45a
3	94.75c	0.00a	5.25a
4	94.15c	1.00b	4.85a

Means followed by the same letter(s) within the same column do not statistically differ at 5% level tested by Least significant difference (LSD)

Table 3: Percentage germinability of pollens of different varieties of sesame in different concentrations of Sucrose

Cultivar	0%	10%	20%	30%
Kenana 4	0a	32.29b	40.13a	28.50a
Ex-sudan	0a	26.75b	45.46a	42.56a
E8	0a	6.00a	59.20a	30.50a
Kenana 4				
1	0a	25.00a	40.50a	25.00a
2	0a	30.00a	35.00a	30.00a
3	0a	35.00a	55.00a	35.00a
4	0a	39.00a	30.00a	24.00a
Ex-Sudan				
1	0a	25.00a	50.50a	45.60a
2	0a	30.00ab	40.60a	35.75a
3	0a	12.00a	30.75a	29.00a
4	0a	40.00c	58.00a	55.50a
E8				
1	0a	10.00a	60.00ab	30.50b
2	0a	2.50a	65.25c	20.00a
3	0a	7.20a	55.60a	45.55ab
4	0a	5.55a	54.60a	25.60b

Means followed by the same letter(s) within the same column do not statistically differ at 5% level tested by Least significant difference (LSD)

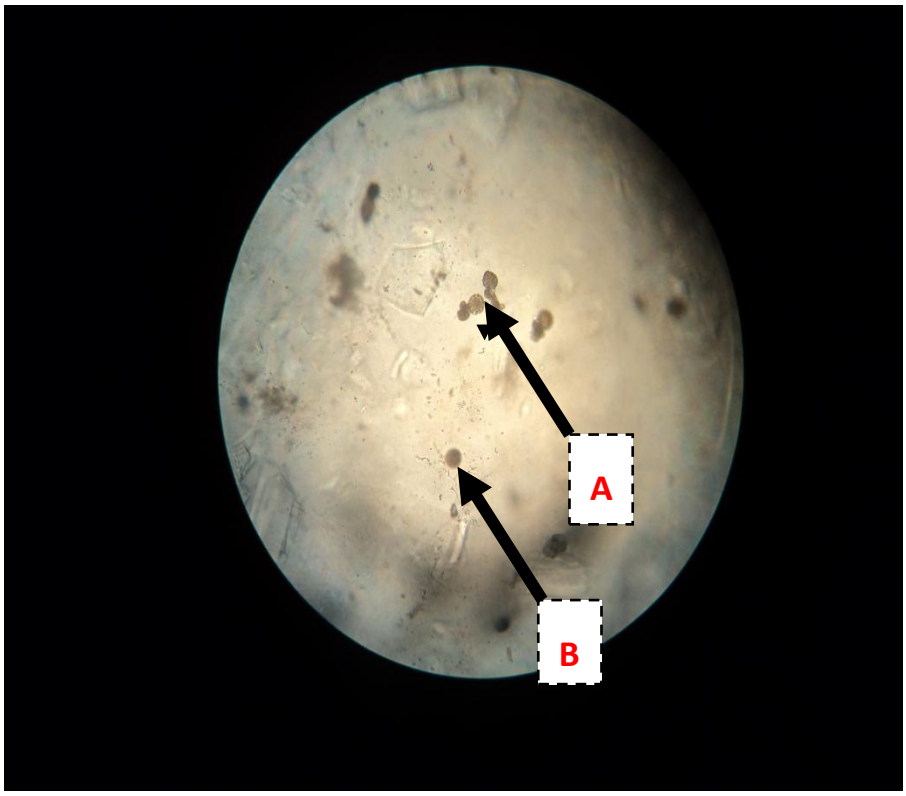

Plate1: Picture of germinated pollen (A) and ungerminated pollen (B) in medium

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