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In-Vivo Pollination; A Better Option for Longevity Test of Pollen Viability of Cowpea (*Vigna unguiculata* L. Walp)

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Abstract: ***A quick and trusted method for evaluating pollen quality and viability is essential in a conventional breeding program. Techniques used in evaluating pollen viability can be classified into in vivo and in vitro tests. Harrison, Heslop, Harrison and Shivanna (2004) categorize the in vitro test into (a) test of germinability, (b) test of stainability, (c) test for enzyme activity and (d) fluorochromatic procedure (FCR). Using pollen of different age crossability can be suitable in testing pollen viability in the field. This research work was conducted in 2007 and 2008 at the screen house of the Institute for Agricultural Research (IAR) of Ahmadu Bello University Samaru Zaria to study pollen viability and its implication for outcrossing in cowpea. Four genotypes, IT86D1010, IT93K452-1, B301 and kanannado were used in the study. Completely Randomized Design (CRD) was used where each cultivar was sown in four labeled pots containing topsoil mixed with manure and sand. This set of planting was repeated three times but staggered to synchronize the flowering of the genotypes. Pollen grains were collected early in the morning (6:00-6:30 am) from a matured pollen and kept in a well-labeled Petri dish in a filter paper. The crossing was done immediately after pollen collection at 0 hours, then after 3 hours of collection and 6 hours. The crossing was done in all forms, including reciprocals. Record of successful and failed crosses was taken when pods started to form. A regression analysis of pollen age on percent successful cross was carried out to determine the crossability, pollen viability, and relationships. The study shows that the three varieties, IT86D1010, IT93K452-1 and B301 were readily crossable both as paternal and maternal parents. Kanannado was crossable as a paternal parent only, but no seed was recorded when used as a maternal parent. Regression analysis shows a significant effect of pollen age on pollen viability and crossability. The experiment suggests that pollen from neighboring plants can threaten breeding materials if transferred by an efficient vector since pollen can be viable under ambient temperature for up to three hours.***

Keywords: In-Vivo, pollen, longevity, cowpea, viability.

I. INTRODUCTION

Pollen viability decline sometimes rapidly with age and exposure to environmental stress. Judy, James and Sara (199) surveyed the hand-pollination experiment in seven major journals from 1980 until mid-1994. Fewer than one-third reported any consideration of pollen freshness or viability, whereas over one-half made some mention of stigmatic receptivity. Increase in cowpea yield through breeding can reduce hunger in developing countries as pointed by Lawal (2015) who asserted that quick maturing cowpea can give consumers food from the current harvest within a shorter time than any other crop (around 55 days after sowing), thereby shortening the "hungry period" that often occur prior to harvest of the current season's crop in a farming community in developing world.

A. Pollen viability

According to Zeng-Yu (2004), "pollen viability is generally considered to indicate the ability of the pollen grain to perform its function of delivering the sperm cells to the embryo sac following compatible pollination" The length of pollen viability after another dehiscence is crucial to successful pollination (Stone *et al*., 1995), particularly for out breeders. It determines the possibility of out-crossing between species, provided that limited specific reproductive barriers exist. With the rapid development of biotechnology, more transgenic crops, including cotton and maize, are becoming available, resulting in biosafety concerns about possible ecological risks. Identifying a reliable method is the key to a more accurate estimation of pollen viability (Song *et al.,* 2004). Moses (2007) stated that a quick and reliable method for evaluating pollen quality is essential inbreeding. Techniques used in evaluating pollen viability can be classified into a field and in vitro test. Harrison *et al.* (2004) categorize the in vitro test into (a) test of germinability, (b) test of stability, (c) test for enzyme activity and (d) fluorochromatic procedure (FCR). Using pollen of different age crossability can be used to test pollen viability in the field.

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B. In-vitro method

Edward, Hauser and John (1964) reported that a comparison was made between Nitro-BT-Stained pollen samples and aniline-blue-lactophenol-stained samples because the latter's validity has been questioned. Nitro-BT can discriminate between pollen capacity for oxidative metabolism and those that do not, suggesting unviable pollen.

C. In-vivo method

The four genotypes used were; IT86D1010, IT93K452-1, B-301 and Kanannado. The two genotypes (IT86D1010 and IT93K-452-1) were released materials from IITA and IAR, Zaria. All the two varieties were early maturing in 65-70 days. B-301 is a landrace from Botswana and is an early genotype, while Kanannado is a local variety and late maturing. All four cultivars are white seeded types.

D. Importance of the crop

Cowpea is an important grain legume grown for its protein-rich grains. It is a cheap source of protein in people's diets in Africa and other world regions. According to IITA (2009) all parts of the cowpea are used as all are rich in nutrients and fiber. The young leaves, immature pods, immature seeds and matured seeds are consumed. The grains are for human consumption cooked solely or in a mixture with rice, yam, millet or other staple food. Cowpea is used to prepare "kose," "alale" ("moimoi") and "danwake," protein-rich local dishes. Green pods and tender leaves are sometimes used as vegetables. Most consumers believe that cowpeas are suitable for their health and growth though, slightly more than 50% also think that cowpea caused digestion problems and discomfort due to flatulence (Ndiaga and Anthony, 2004). The stems, leaves and pod shells are a good source of feed for livestock. The crude protein content of the cowpea haulms ranges from 13% to 17%, with high digestibility and low fibre content as reported by IITA (2002).

Cowpea is used as a pot-herb and fodder plant because it continues producing new leaves if cut back regularly from an early stage. After all, they do not mature in a definite time, as Purseglove (1977) pointed out. According to Adams (2003), cowpea is the second most important pulse crop in Africa, producing over 95% of the world crop Nigeria being the biggest producer. Trading fresh produce (green pods) and processed cowpea foods and snacks provide rural and urban women the opportunity to earn cash income.

E. Objectives of the research

The main objective of this study work is to compare the two methods of pollen viability test, *in vitro* and *in vivo* to find the better means of determining the longevity of pollen viability of cowpea genotypes.

The specific objectives are:

1) Determine the pollen viability of four cowpea lines under ambient conditions using hand pollination
2) Determine the effect of duration of post dehiscence time on crossability between genotypes and
3) Compare the two methods, *in vitro* and *in vivo,* of pollen viability.

F. Experimental design

Two experimental trials were carried out to determine the pollen viability of four cowpea lines.

The research was conducted in a screen house using randomized completely block design (RCBD).

Each cultivar was sown in three pots, making twelve pots completely randomized (pot size was 4litres capacity). Each pot was filled with topsoil mixed with sand and manure. This planting set was replicated three times at weekly intervals to synchronize the flowering of the varieties. The management practices conducted include; watering, weeding and thinning.

G. Pollen Collection and Crossing

Pollen from the genotypes was collected in well-labeled Petri dishes. Forceps were used to gently tear up the selected flower bud to expose the stigma and style for removing anthers with the forceps (emasculation). An adequate amount of pollen was collected from mature flowers of each line early in the morning (6:30 am) and kept under ambient temperature in a filter paper place in a Petri dish. The Petri dishes were labeled with the names of the lines on them. Pollen was selfed and crossed among the genotypes. Pollen in Petri dish was crossed/selfed immediately (0 hrs), after three hours crosses/selfings were repeated and six hours after pollen collection (three times at the interval three hours). Crosses made were tagged with time, date and the name of the pollen donor.

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H. Data Collection

Record of successful and failed crosses were taken the following two days after crossing. Several pods formed from the crosses were recorded. The successful cross can be noticed by continuing the growth and development of the crossed or selfed flower. The nature of the pod was studied to understand seed number per pod.

Percentage viability was calculated as follow;

Viability (%) = [No. of pods formed (in selfings only)/total selfed] x 100. Or

Viability (%) = [(No. of seeds formed (in selfings)]/no. seeds per pod x total no crossed)] x 100

Table of percentage viability from the selfings at 0, 3, and 6 hours of pollen age

Genotypes	%PFS	%SFS	PA (hrs)
V1	78.57	76.46	0
V1	50	50	3
V1	0	0	6
V2	79.07	79.07	0
V2	45.95	45.95	3
V2	15.38	0	6
V3	83.33	83.33	0
V3	35.48	35.48	3
V3	6.67	6.67	6
V4	67.79	67.8	0
V4	5.26	2.51	3
V4	8.7	0	6

PFS= Pods Formed from Selfings, SFS= Seed Formed from Selfings,
PA= pollen age in hours, V_1= IT86D1010, V_2=IT93K452-1, V_3= B301 and V_4= Kanannado.

II. RESULTS AND DISCUSSIONS

The percentage of pollen viability of four genotypes is shown in table 2.This presents the pollen viability result from selfings of the genotypes only at different pollen ages. At zero hours, the pollen viability is highly significant, IT86D1010 indicates 78.57% pod set and 76.46% seed set, IT93K-452-1 recorded 79.07% for both pods and seed set. B301 indicates 83.33% pollen viability for both seeds and pods formed at zero hours. Kanannado recorded 67.8 for both seeds and pods formed at zero hours.

The result indicates that the pollen viability of the four genotypes decreases rapidly, producing weak pollen after 3hours and dead pollen after 6hours. IT86D1010 recorded 50% pollen viability for both pods formed and seeds formed at three hours, IT93K-452-1 indicates 45.95% for both seeds and pods formed, B301 35.48% for both pods and seeds formed. Kanannado recorded the least pollen viability with 5.26 for pods and 2,51 for seeds. All four genotypes showed a significant decrease in pollen viability at 40% - 50%. At 6 hrs, Pollen of IT86D1010 and IT93K-452-1 could not produce seeds.

A. Advantages of in vivo of Pollen Viability Test

The two methods used in testing the longevity of pollen viability are; *In vitro* and *in vivo* methods. *In vitro* methods are carried out in glasses or outside the real plant. It is, therefore, an indirect test under artificial conditions but quick means of pollen viability measurement. Harrison *et al.* (2004) categorize the in vitro test into (a) test of germinability, (b) test of stainability, (c) test for enzyme activity and (d) fluorochromatic procedure (FCR). Using pollen of different ages, crossability can be used to test pollen viability in the field. This method requires much skill and expensive materials to accomplish. Furthermore, pollen viability is mainly determined by chemical methods, which has some shortcomings as pointed by Edward and John (1964), that cytochemical test for pollen viability is unsatisfactory for two reasons (1) it is based on the oxidation of benzidine by peroxidase in the presence of hydrogen peroxide and therefore lack the specificity required in more critical work; (2) the staining reaction is variable for pollen of different taxa. *In vivo* method involves using living plants under ambient conditions to test pollen grain pollination ability at different ages. This method does not require sophisticated procedures or chemicals to carry out. The method requires a screen house and simple agronomic practices to control experimental errors.

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III. SUMMARY AND CONCLUSION

In conclusion, this research work proved pollen viability of cowpea decreases with age. From the findings, pollen viability is weak 3hrs after dehiscence and is lost after 6hrs.

In summary, pollen viability of cowpea genotypes varies with age and variety under ambient temperature when *in vivo methods are used.* The *in vitro,* on the other hand measures the bioproduct or bioprocesses of pollen grain under laboratory conditions.

IV. RECOMMENDATION

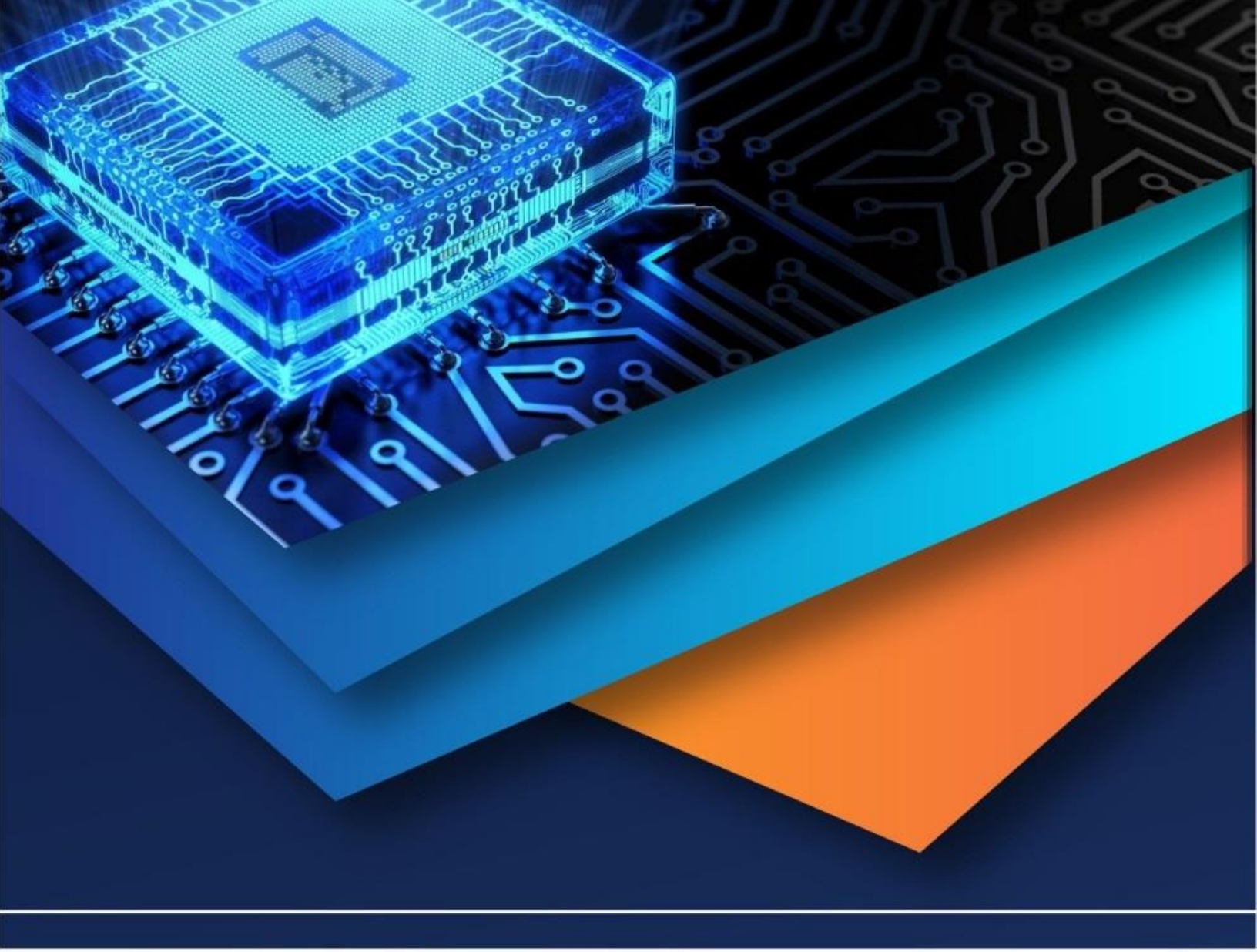
Cowpea breeders and contract growers should avoid contamination of improved seed by observing certain distances in space and time to ensure purity.

Breeder to experiment on the implication of space on crossability of cowpea to avoid possible contaminations of improved seeds of cowpea.

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