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POLLEN VIABILITY STUDIES IN *PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC

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ABSTRACT

Psophocarpus tetragonolobus(L.) Dc. (Winged bean) has immense agricultural possibilities as a potential backyard crop. An outstanding feature of this plant is that, it contains significant amount of protein in all the parts of the plant. Pollen viability studies was conducted using Cotton blue staining and Fluorochromatic Reaction test. The studies showed that maximum viability of the pollen was before the flower opened and lasted for one day. There was good pollen tube growth in the stylar canal and it penetrated the embryo sac successfully.

KEYWORDS: Cotton Blue Staining, Fluorochromatic Reaction Test, Pollen Viability, Winged Bean

INTRODUCTION

The number of chronically undernourished people in the world remains alarmingly high, amounting to 850 million people. Half the world, or nearly three billion people live on less than two dollars a day and around 1.1 billion live in extreme poverty on less than one dollar a day. These conditions have caused over one billion children (more than half of those living in developing countries) to suffer from the severe effects of poverty and 674 million (over a third) are living in conditions of absolute poverty [1]. The underutilized crops are a good boon to the developing countries to overcome this problem.

Legumes include important vegetable crops which are a good source of proteins and oil also. *Psophocarpus tetragonolobus* (L.) DC., commonly known as 'winged bean' is an underutilized leguminous crop. The attractive features of the plant are, the whole plant is edible and all the parts are highly protenaceous when compared to other plants. The tubers contain 20% protein in dry weight. This amount is superior to other tubers such as Yam (2%), Cassava (1%), and Sweet potato (2%). The percentage of crude protein of the seeds (29.8-37.5%) is comparable to that of other legumes [5].

Eventhough, this plant is highly protenaceous and edible, it has certain disadvantages. It requires long stacks for successful flowering and fruiting. It has cliestogamous flowers i.e., the flowers fertilize even before they open, there by leading to less variability in the plant due to lack of cross pollination. The plant is seasonal and flowering occurs during the short day period from November to February and the seeds have a low germination percentage on storage.

A thorough knowledge of the pollen viability will help in understanding the pollination and fertilization of the plant. The present study was carried out with an objective to analyze the pollen viability of winged bean with the help of flurochromatic reaction test and cotton blue staining techniques.

MATERIALS AND METHODS

Flurochromatic Reaction Test and Cotton blue staining method was used to study the viability of pollen.

FLUOROCHROMATIC REACTION TEST (FCR)

FCR test was carried out according to the protocol of Heslop-Harrison and Heslop-Harrison [2]. For this fluorescein diacetate (FDA) was prepared in acetone (2mg/ml). Sucrose solution in sufficient concentration was prepared separately to prevent bursting of pollen grains. Two to five ml of sucrose solution was taken in a glass vial and stock solution of FDA was added drop by drop to it until the resulting mixture showed persistent turbidity. A drop of mixture was taken on a microslide and suspended sufficient pollen ensuring uniform distribution in the preparation. The preparation was incubated in a humidity chamber for 5 – 10 minutes and observed under an Olympus (Japan) fluorescence microscope under UV excitation. The viable pollen grains which fluoresced were counted and the percentage of viability was calculated by the formula given below.

$$\text{Percentage of viability} = \frac{\text{Number of viable pollen} \times 100}{\text{Total number of pollen}}$$

IN VIVO POLLEN GERMINATION - COTTON BLUE STAINING METHOD

In vivo pollen germination and tube growth studies were conducted. For microscopic observation, the pollinated pistils were carefully dissected out from the flower and fixed in Carynoy's fluid. They were transferred to lactophenol solution to which a few drops of 1% cotton blue stain was added and incubated in the oven at 60 ^{0}C for 30 minutes. The stained pistil was mounted in a drop of glycerine on a slide. A cover glass was placed over it and pressed gently for the separation of pistil cells for easy observation of pollen tubes.

RESULTS

FLUROCHROMATIC REACTION (FCR) TEST

The pollen grains were mounted in fluorescein diacetate (FDA) solution and observed under an image analyzer (Figure 1-2). The pollen grains showed maximum, viability at a stage when the flower was about to open. It was also observed that the pollen retained the viability for another day after getting fully opened and later viability tended to lose. About 90% of the pollen grains showed a bright green or yellowish green fluorescence revealing their viability status.

COTTON BLUE STAINING

The cotton blue staining of the stigma and the stylar region revealed that the pollen grains adhering to the stigmatic surface develops pollen tubes and they penetrate through the stylar canal (Fig 3-4). The pollen tube is seen as a single fluorescent strand in the stylar canal.

DISCUSSIONS

Assessment of pollen quality on the basis of pollen viability and its vigour are the prerequisites for pollination studies. In physical method, pollen viability is generally tested by fluorochromatic reaction (FCR) that has been promoted by Heslop-Harrison and Heslop-Harrison [2]. This technique is based on the accumulation of fluorescence only in viable pollen grains, which fluoresce under UV light when mounted in solution of Fluorescein diacetate. Shivanna and Heslop – Harrison [8] showed a good correlation between FCR and *in vitro* germination methods. The FCR test is rapid and simple but destruction (loss of viability) of pollen grains takes place. According to many reports, it has been found satisfactory for a range of pollen species [8, 3, 4]. In *Psophocarpus tetragonolobus* too, the FDA pollen viability test was found to be appropriate for testing the viability at different stages.

However, to get a more accurate assessment on pollen viability and stigma receptivity, *in vitro* tests are more

ideal. Our observation after *in vivo* tests on *Psophocarpus* have shown that stigma is most receptive at stage 2 and the pollen grains germinate on the stigma and pollen tube grows through the intercellular spaces of the stigma and through the single stylar canal to reach the ovule. In *Psophocarpus*, the pollen tube takes 2-3 hrs to grow through the style to reach the ovule.

Cotton blue staining and decolourised aniline blue methods are commonly used to study *in vivo* germination. The fluorescence microscopic method has been used extensively for tracing pollen tube germination in a wide range of species [10, 6, 7]. Many workers have used decolourised aniline blue method for microscopic testing of viability [9]. Cotton blue used for *in vivo* pollen germination experiments revealed pollen germination at the stigmatic region. In the present study, the pollen tubes were observed to penetrate into the stylar region and showed growth towards the ovary.

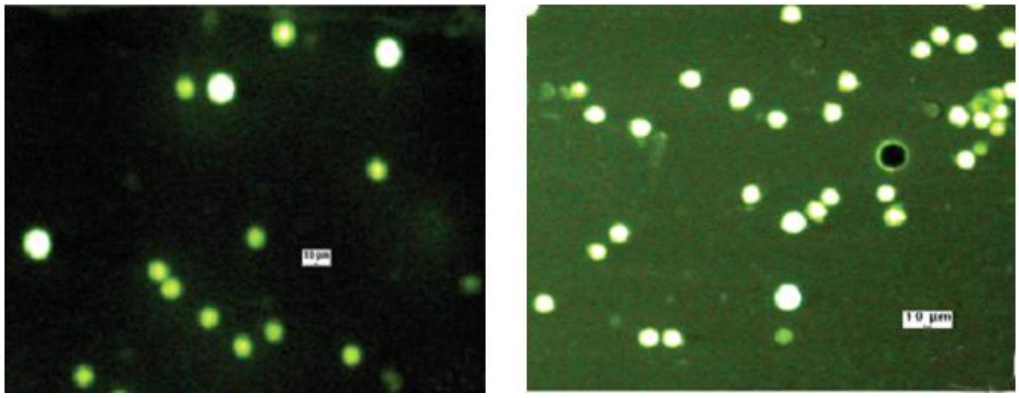
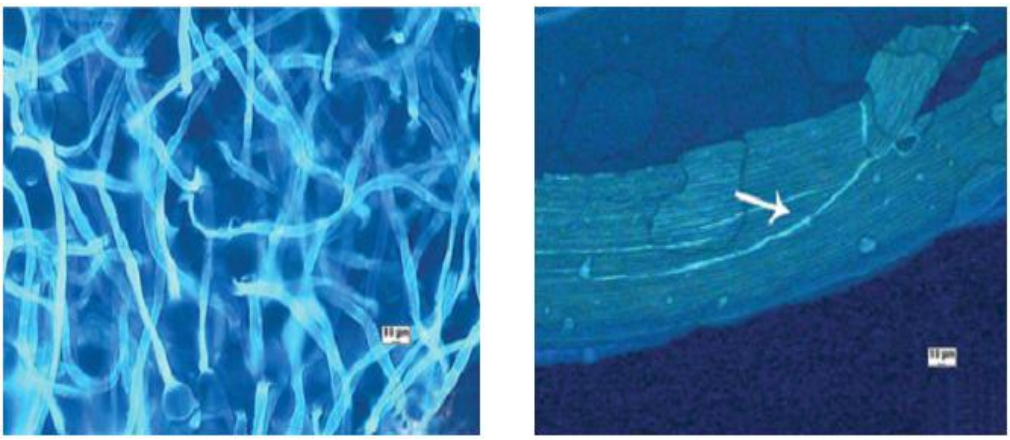


Figure 1, 2: Pollen Viability Test with FDA Staining



Cotton Blue Staining

Figure 3: Pollen Tube Growth on the Surface of the Stigma **Figure 4: Pollen Tube Growth inside the Style**

CONCLUSIONS

Maximum pollen viability of 90% was recorded through FDA staining technique on the day before opening of the flower and it remained only for a day, after which the viability decreased, the ideal character of a cliestogamous plant. The Cotton blue staining test ruled out the possibility of any obstruction in pollen tube development and fertilization. From the study it is concluded that there is no need to adopt any artificial measures to ensure the proper fertilization of the plant.

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REFERENCES

1. Bourgeois, R. and Susila, W. R., 2006. Underutilized Species: an Alternative for Poverty Alleviation? *CGPRT flash.* **4(1):** 1.
2. Heslop – Harrison, J. and Heslop – Harrison, Y., 1970. Evaluation of pollen viability by enzymatically induced fluorescence: intracellular hydrolysis of fluorescein diacetate. *Stain Technology.* **45:** 115 – 120.
3. Heslop – Harrison, J., Heslop – Harrison, Y. and Shivanna, K.R., 1984. The evaluation of pollen quality and further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical & Applied Genetics.* **67:** 367 – 379.
4. Jain, A. and Shivanna, K.R., 1988. Storage of pollen grains in organic solvents. Effects of solvents in pollen viability and membrane integrity. *J. Plant Physiology.* **132:** 499 – 502.
5. Newell, C. A. and Hymowitz, T., 1979. The winged bean as an agricultural crop. In: Ritchie, G. A. (ed.) New Agricultural crops. A.A.A.S. Selected Symp., Westview Press. Boulder, Colorado. pp.21-29.
6. Ramanna, M.S., 1973. Euparol as a mounting medium for preserving fluorescence of aniline blue in plant material. *Stain Technology.* **48:** 103 – 105.
7. Remming, D.W., Heinricks, H.A. and Richardson, P.E., 1978. Sequential staining of callose by aniline blue and lacmoid for fluorescence and regular microscopy on a durable preparation of the same specimen. *Stain Technology.* **48:** 133 - 134.
8. Shivanna, K.R. and Heslop – Harrison, J., 1981. Membrane state and pollen viability. *Annals of Botany.* **47:** 759 – 770.
9. Sniezko, R. and Winiarezyk, K., 1995. Pollen tube growth in pistils of female sterile and fertile plants of *Oenothera* mut. *brevistylis*. *Protoplasma.* **187:** 31 – 38.
10. Stout, A.B., 1972. Cyclic manifestation of sterility in *Brassica pekinensis* and *B. chinensia*. *Botanical Gazette.* **73:** 110 – 132.