



RESEARCH ARTICLE

# Pollen viability and incompatibility in indigenous rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi)

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## Abstract

Pollen viability, germination and compatibility are essential in determining the success of pollination and seed setting of high-valued crops. Rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi) is an underutilized and unexplored indigenous legume with high potential for commercial production. In this study, pollen quality, viability, germination rate and incompatibility among selected six rice bean (*V. umbellata*) accessions from Barili, Cebu, Philippines were evaluated to determine the barriers and effective pollination habit for increased productivity while retaining the important traits, including high tolerance in poor soils, superior climatic resilience and resistance to pest and diseases. Results of acetocarmine calorimetric assay showed that rice beans' (*V. umbellata*) pollens are highly viable, with accessions VU 004 ( $56.33 \pm 4.91\%$ ) and VU 007 ( $54.34 \pm 4.53\%$ ) having the optimum viability rate. Brewbaker and Kwack medium treated with  $0.2 \text{ g.l}^{-1}$  and  $0.3 \text{ g.l}^{-1}$  boric acid ( $\text{H}_3\text{BO}_4$ ) enhanced the germination rate *in vitro* ( $11.56 \pm 5.53\%$  and  $9.47 \pm 6.50\%$  respectively). Bud ( $14.96 \pm 1.53\%$ ) and post-anthesis pollens ( $10.28 \pm 0.94\%$ ) have optimum germination rate in  $0.2 \text{ g.l}^{-1}$  boric acid media, while anthesis pollens are suitable in media supplemented with sucrose and boric acid alone ( $12.20 \pm 1.50\%$ ) and with  $0.1 \text{ g.l}^{-1}$  myo-inositol supplementation ( $8.49 \pm 1.86\%$ ). Pollination test revealed that rice bean accessions have high self-compatibility ( $50.76 + 3.45\%$ ) and low cross-compatibility ( $26.57 + 2.49\%$ ). The findings provide an important background in understanding the pollen quality and intraspecific interaction among indigenous rice bean (*V. umbellata*) accessions in Barili, Cebu to improve production and hybridization.

## Keywords

Pollen grains, indigenous crop, pollen viability, rice bean, self- and cross incompatibility

## Introduction

In plant breeding, pollen grains play a vital role in developing a high-quality variety. Pollen carries the male gametes responsible for successful genetic exchange and ensures the survival of the species (1). Effective pollination is an essential requirement for fruit development and seed setting in plants (2). Therefore, an insight in to the pollen biology are important in order to improve crop productivity (3); such that knowledge in pollen germinability, viability and the compatibility is a convenient and reliable aspect in future breeding programs (4).

Pollination in higher plants enables seed development through pollen-pistil interaction (5). However, compatibility and receptibility barriers are problems in the success of the process. Pollen incompatibility is a pre-fertilization barrier that hinders successful pollination due to: (a) rejection of pollen grains from a male donor by the female receptor flower, (b) failure of pollen grain germination, (c) failure of the pollen tube to reach the ovary and (d) unsuccessful fusion of male and female gametes (6). Incompatibility in plants can be classified as self-incompatibility and cross-incompatibility. Self-incompatibility (SI) occurs when pollen is recognized and rejected by the stigma of plants of the same cultivars (7). On the other hand, cross-incompatibility (CI) is the rejection of pollen by pistil due to lack of recognition of the pollen from the foreign origin (8). Self-incompatibility restricts inbreeding due to self-discrimination of pollen by pistil, while cross-incompatibility (CI) limits hybrid zygote formation between species of different gene pool (9, 10).

Interspecific incompatibility in *Vigna* species is influenced by the parental genotype and meiotic irregularities (6). It was reported that the crossability of interspecific hybrids among *Vigna radiata*, *Vigna mungo* and *Vigna umbellata* and identified fertilization barriers in some crosses, which resulted to embryo degeneration and abnormal seed development (11). They further identified incompatibility barriers among self- and cross pollinated *Vigna* species such as delayed entry of pollen tube to the ovules and delayed and failure in nuclear division of endosperm. Reports are on the low crossability and pollen fertility between *V. radiata* and *V. mungo* crosses due to genotypic and ecological differences and meiotic abnormalities (12). One of the determining factors in successful fertilization and germination is pollen viability (13). Pollen viability is vital in seed setting (14), especially in leguminous and grain crops such as *Vigna* spp. It was reported that over 90% genetically viable pollens of *Vigna unguiculata* however the development in *in vitro* medium resulted to low germination (15). In bambara groundnut (*Vigna subteranea* (L.) Verdc.), rapid loss of pollen viability and germinability was reported due to several environmental factors including temperature, humidity and organic solvents used (16).

Like all other legumes, *Vigna* species is essential in biological nitrogen fixation. In addition, some *Vigna* species, especially the wild and exotic has resistance to pests and diseases (17, 18). Rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi), for instance, has been reported to exhibit complete resistance to bruchids, small beetles attacking vignas (6). The most common and widely spread disease among *Vigna* species, with major economic significance, is the Yellow Mosaic Disease (YMD) caused by Begomoviruses (19). Significant evidences were reported on the high degree of resistance of rice bean (*V. umbellata*) to the disease compared to other *Vigna* species (20, 21). Rice bean (*V. umbellata*) has also superior nutritional value comparable to other legume grains (22). Previous studies reported that the crop is rich in amino acids, vitamins, dietary fiber, min-

erals such as calcium, iron and zinc and antioxidants such as polyphenols and tannins (23, 24).

Although rice bean (*V. umbellata*) has superior traits and nutritional content, the crop is a less known and regarded as underutilized legume (24). The crop is considered as a minor commodity having less commercial value compared to major agricultural crops of the same class. In addition, underutilized crops are species grown extensively in the past or have a potential in the future either for agronomic, genetic or economic reasons but are currently grown in a limited area. Rice bean (*V. umbellata*) is among these crop species that did not receive major attention for improvement and thus scientific knowledge about them is limited (22).

Result of initial fragmented studies on its traits provides insights into its potential as a major agricultural crop and as an important genetic resource for breeding. Information on the mode of transfer and retainment of these essential genes to other *Vigna* sp. (interspecific crossing) or of the same species (intraspecific breeding) is a prerequisite to a crop improvement programme. However, crossability barriers in *Vigna* sp. hinder the development of interspecific hybrids due to the difference in gene pool, resulting in low fertilization and number of seeds (25). Although several attempts have been conducted to overcome these barriers, reproductive and floral biology has yet been fully explored among intraspecific crosses of rice bean (*V. umbellata*).

Understanding the pollination habit and reproductive mechanism among crop varieties are essential to increase the chances of successful fertilization (2, 26). Since pollen grain carries the traits (27), investigating the pollen biology and mechanism provides essential knowledge in the development of inbreds and hybrids with superior quality. Hence, the objective of this study was to evaluate the pollen quality and incompatibility among native accessions of *Vigna umbellata* (Thunb.) Ohwi & Ohashi for improved production.

## Materials and Methods

### *Plant materials, experimental design and crop management*

A total of six rice bean accessions obtained from Barili, Cebu, Philippines and maintained by the Center for Studies in Biotechnology in Cebu Technological University Barili Campus was used in this study. The accessions were assigned with code (VU 001 (Brgy. Balao), VU 002 (Brgy. Kangdampas-San Jose), VU 004 (Brgy. Pangpang), VU 005 (Brgy. Poblacion-market), VU 006 (Brgy. Budbud) and VU 007 (Brgy. Kangdampas-Centro)).

Healthy seeds were used as planting material and propagated in an experimental pot laid out in a Randomized Complete Block Design (RCBD) with three replications and 10 samples per replication.

The experimental setup was maintained following farmer's practice. Soil planting medium, applied with 25 g chicken dung per pot, was prepared seven days before

planting. Cultural practices such as weeding using bolo, watering every 4:00 PM and training of vines to climb in their respective stakes were done manually. The setup was covered with a plastic barrier prior to flowering to avoid pollen contamination from other accessions. Flowers were assessed for pollen viability and germination (*in vitro* and *in vivo*) assays.

### Pollen viability test

Viable pollen count was estimated using acetocarmine calorimetric assay. Around 32,046 pollen samples were collected in three different plants of the same accession (six accessions) and were spread to a glass slide. A drop of 2% acetocarmine solution was added to the slide and was examined using a binocular compound light microscope. Pollen is considered viable if it appeared red color and nonviable if colorless after staining (28). Pollen viability was computed using the standard formula (29):

$$\text{Pollen viability} = (\text{number of viable pollen} / \text{total number of pollen}) \times 100.$$

### In vitro pollen germination test

Pollen collection was done in 3 different flowering stages: pre-anthesis (bud phase), anthesis (flower fully open) and post-anthesis (6 hours after anthesis). Each sample was germinated in a liquid Brewbaker and Kwack (BK) media (30) with different concentrations of boric acid ( $\text{H}_3\text{BO}_3$ ) and varying concentrations of myoinositol (Table 1). Then it was incubated at a controlled temperature (27 °C) for 24 hrs

Flower buds were enclosed in cellophane bags to avoid pollen contamination from other accessions. Pollination was expected to occur after seven hours and the flower was examined under a compound light microscope. Pollen germination was analyzed as described (35) with slight modifications. Pistils were placed in a 1.5 ml microtube and fixed with three ml of ethanol: formalin: glacial acetic acid mixture (8:1:1) at 4 °C overnight. The fixed tissues were rehydrated by subsequently submerging in 70%, 50% and 30% ethanol for 10 min then washed with double distilled water for 10 min instead of submerging overnight. Samples were cleared using 8N NaOH for 30 min, instead of submerging in the solution for 24 hrs and washed three times with sterile double distilled water for another 30 min each. Each tissue was mounted on the slide, then stained with lactophenol cotton blue stain, instead of aniline blue solution, for another 30 min at temperature 60°C. A cover slip was gently pressed onto the slide containing the tissue and was examined using a compound light microscope at 400x total magnification. The setup was conducted in triplicate with a total of around 21,535 pollens for interspecific crossing, and around 4,703 for self-pollination were examined. Pollen grains were considered germinated when pollen tube diameter is equal to or greater than the pollen grain diameter itself. The germination rate was estimated using the formula (29):

$$\text{Pollen germination rate} = (\text{number of germinated pollen} / \text{total number of pollen}) \times 100.$$

**Table 1.** Pollen germination media (PGM) composition.

Treatment	Composition
T <sub>0</sub>	Distilled water (control)
T <sub>1</sub>	100 g.L <sup>-1</sup> sucrose + 0.1 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> + 0.3 g.L <sup>-1</sup> CaNO <sub>3</sub> .4H <sub>2</sub> O + 0.2 g.L <sup>-1</sup> MgSO <sub>4</sub> .7H <sub>2</sub> O + 0.1 g.L <sup>-1</sup> KNO <sub>3</sub> (Standard BK (30) medium)
T <sub>2</sub>	100 g.L <sup>-1</sup> sucrose + 0.2 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> + 0.3 g.L <sup>-1</sup> CaNO <sub>3</sub> .4H <sub>2</sub> O + 0.2 g.L <sup>-1</sup> MgSO <sub>4</sub> .7H <sub>2</sub> O + 0.1 g.L <sup>-1</sup> KNO <sub>3</sub>
T <sub>3</sub>	100 g.L <sup>-1</sup> sucrose + 0.3 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> + 0.3 g.L <sup>-1</sup> CaNO <sub>3</sub> .4H <sub>2</sub> O + 0.2 g.L <sup>-1</sup> MgSO <sub>4</sub> .7H <sub>2</sub> O + 0.1 g.L <sup>-1</sup> KNO <sub>3</sub>
T <sub>4</sub>	100 g.L <sup>-1</sup> sucrose + 0.5 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> (modified Gaaliche <i>et al.</i> 28) medium)
T <sub>5</sub>	100 g.L <sup>-1</sup> sucrose + 0.5 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> + 0.1 g.L <sup>-1</sup> myo-inositol
T <sub>6</sub>	100 g.L <sup>-1</sup> sucrose + 0.5 g.L <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub> + 0.2 g.L <sup>-1</sup> myo-inositol

under dark condition. Around 53,873 pollen samples were examined in a binocular compound light microscope (Olympus CX31, Japan) and were considered germinated if the diameter of the pollen tube is equal to or larger than the pollen diameter (31). All setup was replicated three times and the germination rate was estimated using the formula (29):

$$\text{Pollen germination rate} = (\text{number of germinated pollen} / \text{total number of pollen}) \times 100.$$

### In vivo pollen germination test

Flower buds with light green color were selected as female parent, before anthesis phase and were emasculated in the late afternoon from 4:00 to 6:00 PM. Emasculatation was done during this interval because of the average temperature (20°C) required for anther dehiscence and increased chances for pollen viability (32). Pollination was performed on the next day from 5:00 to 7:00 AM because high receptivity will be observed in this time interval (33, 34).

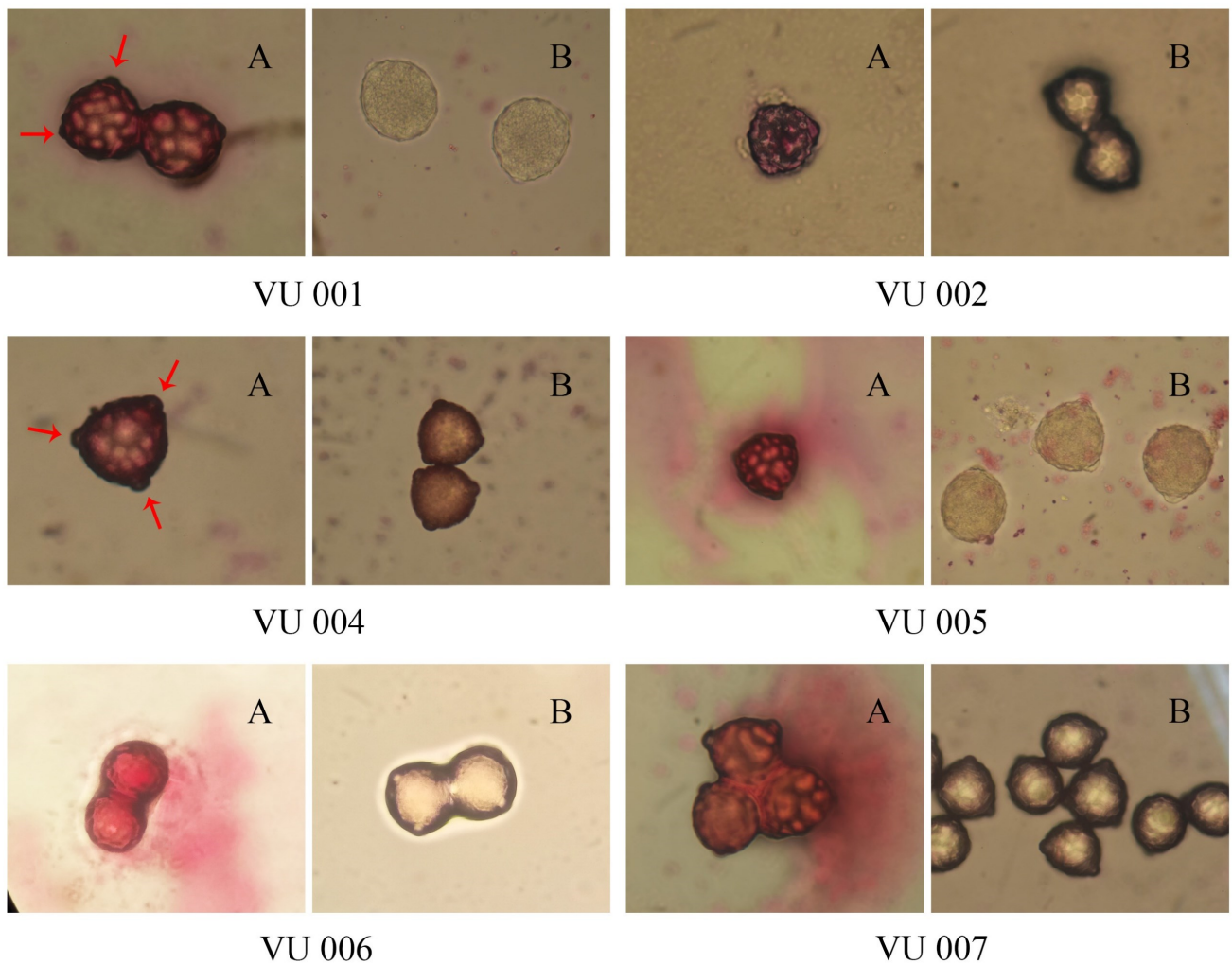
### Data collection and statistical analysis

All data were recorded and photographed. Data were analyzed using IBM SPSS Statistics version 20. For each quantitative variable, data were subjected to analysis of variance ( $p < 0.05$ ). Individual treatment means were analyzed using post-hoc test (Duncan's Multiple Range Test) and expressed with standard error of means (SEM).

## Results and Discussion

### Pollen viability assay

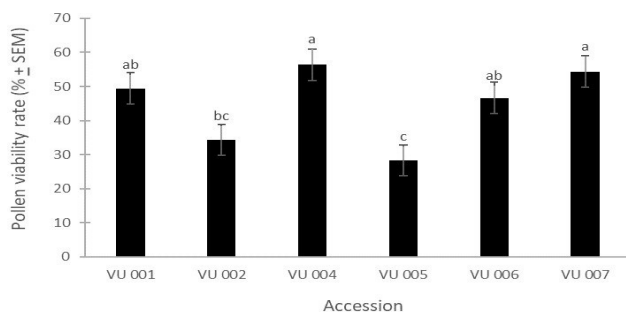
Pollen viability is defined as the ability of the pollen grains to germinate (36). Viability is often associated with chromosome arrangement (37) and meiotic regularities (38, 39). Acetocarmine stains the cytoplasm and the chromatin material in the nuclei of viable pollen (40). Viable pollen retains the stain, which appears pink to deep red, while sterile pollen does not retain any stain and appears transparent (Fig. 1). The sterility of pollen has been observed in dicots



**Fig. 1.** Acetocarmine calorimetric assay of pollen grains in different rice bean (*V. umbellata*) showing the germinal pore (arrow): (A) Viable and (B) nonviable pollen grains using a compound light microscope at 400x total magnification.

having irregular chromosome segregation, abnormal sporad formation and unequal-sized fertile pollen grains (41). Cytomixis on meiotic behavior also affects the viability of pollen as observed in wild Himalayan poppy (*Meconopsis aculeata* Royle) (42). The current study revealed that *V. umbellata* pollen has a tricolporate pollen – having three distinct pores, which is common to all members of Papilionoideae (43-45).

The acetocarmine calorimetric assay revealed that accessions VU 004 ( $56.33 \pm 4.91\%$ ) and VU 007 ( $54.34 \pm 4.53\%$ ) had the highest pollen viability rate while VU 005 ( $28.33 \pm 4.03\%$ ) obtained the lowest rate (Fig. 2). The aver-



**Fig. 2.** Pollen viability rate in different rice bean (*V. umbellata*) accessions. (The same letters indicate no significant difference among accessions using Duncan's multiple range test (DMRT) at 5% level of significance;  $P = 0.023$ )

age pollen viability rate for all accessions is  $44.90 \pm 3.14\%$ . The overall rate is 49.82% lower compared to Yard Long Bean (*Vigna unguiculata* subsp. *sesquipedalis*) (46).

Pollen viability is directly associated with pollen quality and is connected with reproductive biology, determining the effective seed set after pollination (47). The significantly high pollen viability rate in accessions VU 004 and VU 007 increases the chance of effective pollination and higher fertilization rate, indicating strong sexual reproduction and successful seed formation. It was reported that stronger pollen viability is an effective measure for seed setting enhancement (48). Whereas, low pollen viability increases the failure in pollination, which further results in low seed set and reduced breeding efficacy (49).

#### Optimization of pollen germination medium (PGM) in rice bean (*V. umbellata*)

*In vitro* pollen germination test is another method of testing the viability of pollen in artificial media. The medium is generally composed of sugar and ionic salts, such as calcium nitrate, magnesium sulfate and boric acid (1, 28, 30, 50). Organic supplements such as polyethylene glycol, vitamins, amino acids and plant growth hormones were also incorporated to increase the rate of pollen germination (51-53). BK (30) medium is the most widely used pollen germination

media (PGM) which was tested and was found to be suitable in 86 plant species (30, 54).

In the current study, the response of pollen grains of different rice bean (*V.umbellata*) accessions to boron (T<sub>1</sub> – T<sub>3</sub>) and myo-inositol (T<sub>4</sub> – T<sub>6</sub>) were assessed. A relatively lower overall germination rate (6.89 ± 7.09%) was observed among accessions in the artificial media (Table 2).

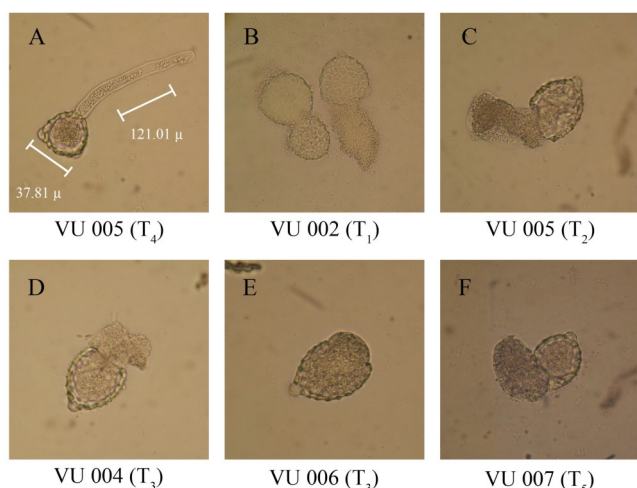
**Table 2.** Pollen germination rate among rice bean (*V. umbellata*) accessions in different medium compositions.

TR EA TM EN T	ACCESSION (± SEM)						Average Germination Rate (± SEM)
	VU 001	VU 002	VU 004	VU 005	VU 006	VU 007	
T <sub>0</sub>	1.18 ± 1.8 <sup>bc</sup>	1.55 ± 0.69 <sup>c</sup>	8.17 ± 2.60 <sup>a</sup>	1.48 ± 1.03 <sup>c</sup>	5.19 ± 1.40 <sup>bc</sup>	8.18 ± 1.44 <sup>b</sup>	4.29 ± 5.30 <sup>b</sup>
T <sub>1</sub>	9.84 ± 1.30 <sup>a</sup>	7.18 ± 1.12 <sup>ab</sup>	9.59 ± 2.60 <sup>a</sup>	13.65 ± 1.02 <sup>a</sup>	9.76 ± 2.34 <sup>ab</sup>	11.63 ± 1.99 <sup>ab</sup>	10.38 ± 5.54 <sup>a</sup>
T <sub>2</sub>	9.27 ± 1.90 <sup>a</sup>	11.59 ± 1.31 <sup>a</sup>	12.18 ± 1.76 <sup>a</sup>	8.66 ± 0.61 <sup>b</sup>	11.71 ± 1.45 <sup>a</sup>	15.96 ± 2.72 <sup>a</sup>	11.56 ± 5.53 <sup>a</sup>
T <sub>3</sub>	4.90 ± 1.68 <sup>abc</sup>	6.94 ± 1.52 <sup>ab</sup>	10.34 ± 2.22 <sup>a</sup>	7.00 ± 2.35 <sup>b</sup>	14.17 ± 1.91 <sup>a</sup>	13.48 ± 1.68 <sup>ab</sup>	9.47 ± 6.50 <sup>a</sup>
T <sub>4</sub>	5.19 ± 2.57 <sup>ab</sup>	7.27 ± 3.13 <sup>ab</sup>	7.56 ± 2.36 <sup>a</sup>	6.85 ± 1.64 <sup>b</sup>	15.05 ± 2.28 <sup>a</sup>	9.33 ± 2.47 <sup>ab</sup>	8.54 ± 7.68 <sup>a</sup>
T <sub>5</sub>	2.68 ± 1.52 <sup>c</sup>	3.68 ± 1.94 <sup>bc</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	4.79 ± 2.67 <sup>bc</sup>	12.06 ± 4.25 <sup>ab</sup>	3.87 ± 7.70 <sup>b</sup>
T <sub>6</sub>	0.00 <sup>c</sup>	0.64 ± 0.64 <sup>c</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.11 ± 0.79 <sup>c</sup>

The T<sub>2</sub> showed promising results with 11.56 ± 5.53% average germination rate for all accessions, with VU 007 having the highest rate (15.96 ± 2.72%). Accessions VU 001, VU 002, VU 005, and VU 007 showed promising pollen germination response in BK (30) media. On the other hand, VU 004 and VU 006 both have optimum pollen germination response in BK (30) media supplemented with 0.2 g.l<sup>-1</sup> (T<sub>2</sub>) and 0.3 g.l<sup>-1</sup> (T<sub>3</sub>) boric acid and PGM containing sucrose and boric acid alone (T<sub>4</sub>). The effectiveness of BK (30) media has been reported by previous study on black gram (*Vigna mungo* L. VAR. DPU-88-31) with 73.00% average pollen germination (55). Whereas around 44.25 to 55.00% pollen germination was observed among *Momordica* sp. germinated media containing sucrose and boric acid alone (3).

#### Effects of boron and myo-inositol on pollen development in pollen germination media (PGM)

Bursting and lysis of pollen tube and pollen grains were also observed in different media (Fig. 3). In addition, T<sub>1</sub>, T<sub>2</sub>



**Fig. 3.** Normal (A) and burst/lysed (B-F) pollen tube and pollen grains as a response to varying media formulation in rice bean (*V. umbellata*) using a compound light microscope at 400x total magnification.

and T<sub>3</sub>, where bursting were observed, has significantly higher pollen germination rate. Similar results were observed in pollen germination in mango, where bursting was observed in medium treated with 1.0 ml.l<sup>-1</sup> boric acid (56). The treatment also resulted in 37.34% higher germination rate compared to medium without boric acid. In comparison, medium treated with high concentrations of boron

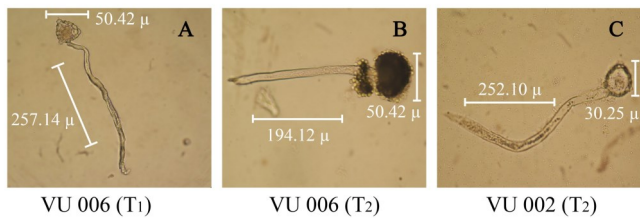
resulted in shorter, swelled and increase in diameter of pollen tubes in *Malus domestica* trees (57). In the present study, 50% increase in boron, in the form of boric acid (T<sub>2</sub>), also increases the pollen germination rate at around 10.21%. However, an 80% (T<sub>4</sub>) increase of the salt caused 17.73% decrease in germination rate. Similar findings were reported in *Picea meyeri* PGM, treated with 0.001 to 0.01% boric acid, where germination rates ranged from 38 to 61% (58).

The role of myo-inositol in pollen tube development has been reported in previous studies (59, 60). Higher plants utilize uronosyl and pentosyl, a derivative of myo-inositol present in the cell wall, for polysaccharide formation and synthesis of exudates found on the stigma and style (61-63). While in this study, myo-inositol inhibits germination by 54.68 to 98.71% (T<sub>5</sub>-T<sub>6</sub>), in addition to no germination observed in some rice bean (*V. umbellata*) accessions. The lower germination result can be attributed to the regulating capacity of inositol polyphosphate kinase reported in *Arabidopsis* (*AtIPK2a*). Previous study revealed that the reduction in *AtIPK2a* transcript levels resulted in enhanced pollen germination and pollen tube growth of transgenic *Arabidopsis thaliana*, under nonoptimal low Ca<sup>2+</sup> concentrations culture medium (64).

#### Effects of stages in flowering in in vitro pollen germination

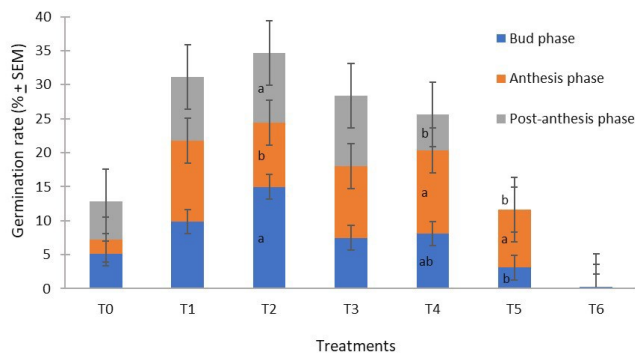
To determine the most efficient phase of rice bean for pollen germination, pollen grains from different flowering stages (bud phase, anthesis and post-anthesis) were germinated in the artificial media. Germinated pollen grains developed longer pollen tube compare to the diameter of the ungerminated pollen itself (Fig. 4).

Individual treatment composition showed variable effects of pollen germination at different flowering stages. BK (30) medium with 0.2 g.l<sup>-1</sup> boric acid (T<sub>2</sub>) exhibited a relatively higher germination rate with 34.68%, combining the



**Fig. 4.** Germinated pollen (pollen tube length is greater than pollen diameter) in different media during bud phase (A), anthesis (B), and post-anthesis (C) using a compound light microscope at 400x total magnification.

germination rate in all phases (Fig. 5). The same treatment was also observed to have a higher germination rate in bud pollens ( $14.96 \pm 1.53\%$ ) and post-anthesis pollens ( $10.28 \pm 0.94\%$ ). Boron, in the form of boric acid, plays an essential



**Fig. 5.** Pollen germination rate among rice bean (*V. umbellata*) accessions in different stages of flowering.

role in *in vitro* pollen germination by regulating the pollen tube growth through its ability to stimulate the plasma membrane  $H^+ATPase$  (65). Contrasting results were obtained in *in vitro* pollen germination in Chinese fir (*Cunninghamia lanceolata* L.) using the same concentration of boric acid, which completely inhibited pollen germination (66). They further stated that not using the optimal concentration of boric acid hinders pollen germination. Based on the current findings and the previous results, the optimum boron requirement varied in every species (57).

On the other hand, anthesis pollens have the highest germination rate in modified Gaaliche *et al.* (28) media alone ( $T_4$ ) ( $12.20 \pm 1.50\%$ ) and with  $0.1 \text{ g.l}^{-1}$  myo-inositol ( $T_5$ ) supplementation ( $8.49 \pm 1.86\%$ ). Myo-inositol has been incorporated in several growth media and has the ability to stimulate pollen tube growth in some plant species and cell wall biosynthesis (60, 67, 68). The effects of PGM composition in pollen germination at different flowering stages has also been reported in passion fruit (*Passiflora* spp.), having 24.08 to 54.87% higher in anthesis compared to post and pre-anthesis (69).

### Incompatibility in rice bean (*V. umbellata*)

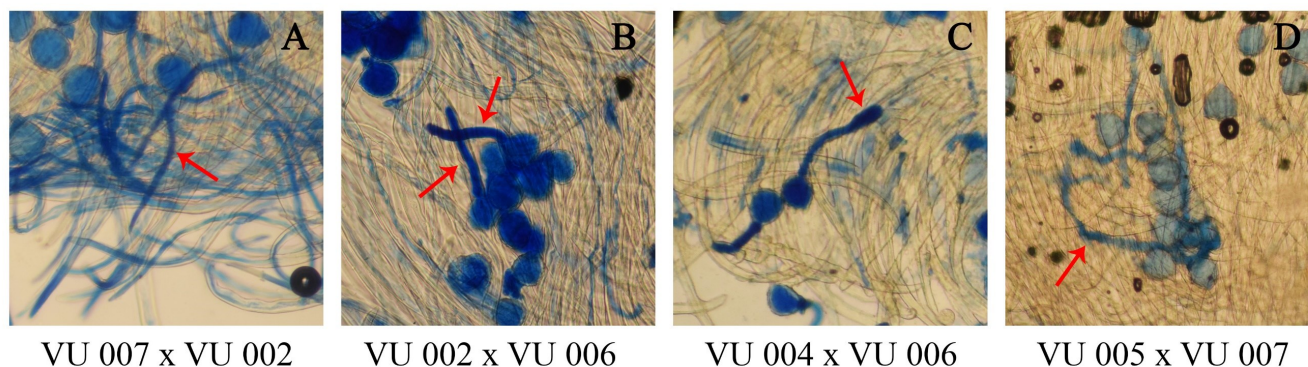
Compatibility refers to the ability of the stigma to recognize and accepts the pollen grains. The process is highly selective and a factor determining the quantitative and qualitative productivity aspects of agronomic crops (70). In the current study, each accession was cross-pollinated *in vivo*, and germination rate and pollen penetration in style were determined. The result showed  $26.57 \pm 2.49\%$  germination rate among accession crosses. Highest and significant

mean rate ( $p \leq 0.05$ ) was observed in three cross-pairs (male x female) namely VU 002 x VU 004 ( $55.12 \pm 11.35\%$ ), VU 004 x VU 007 ( $54.53 \pm 6.79\%$ ), and VU 002 x VU 005 ( $51.83 \pm 5.49\%$ ) (Table 3). In addition, VU 004 x VU 007 has the

**Table 3.** Cross-compatible pollen-stigma interaction and style penetration in rice bean (*V. umbellata*) accessions.

PARENT COMBINATION		GERMINATION RATE ( $\pm$ SEM) IN STIGMA	POLLEN PENETRATION IN A SINGLE STYLE
Male (Pollen Source)	Female (Pollen Receptor)		
VU 001	VU 002	$46.24 \pm 11.53^{ab}$	+
VU 001	VU 004	0.00 <sup>c</sup>	-
VU 001	VU 005	$6.30 \pm 6.30^c$	$\pm$
VU 001	VU 006	$2.29 \pm 2.29^c$	-
VU 001	VU 007	$3.99 \pm 3.99^c$	-
VU 002	VU 001	$3.62 \pm 3.62^c$	-
VU 002	VU 004	$55.12 \pm 11.35^a$	$\pm$
VU 002	VU 005	$51.83 \pm 5.49^a$	++
VU 002	VU 006	$43.49 \pm 2.37^{ab}$	++
VU 002	VU 007	$41.75 \pm 3.27^{ab}$	++
VU 004	VU 001	$47.58 \pm 1.12^{ab}$	++
VU 004	VU 002	$42.63 \pm 9.68^{ab}$	+
VU 004	VU 005	$42.56 \pm 6.41^{ab}$	$\pm$
VU 004	VU 006	$34.39 \pm 6.20^{ab}$	$\pm$
VU 004	VU 007	$54.53 \pm 6.79^a$	+++
VU 005	VU 001	$48.39 \pm 7.97^{ab}$	++
VU 005	VU 002	0.00 <sup>c</sup>	-
VU 005	VU 004	$6.67 \pm 6.67^c$	$\pm$
VU 005	VU 006	$8.23 \pm 8.23^c$	++
VU 005	VU 007	$49.60 \pm 5.45^{ab}$	$\pm$
VU 006	VU 001	$34.18 \pm 18.77^{ab}$	$\pm$
VU 006	VU 002	$3.24 \pm 3.24^c$	$\pm$
VU 006	VU 004	0.00 <sup>c</sup>	-
VU 006	VU 005	0.00 <sup>c</sup>	-
VU 006	VU 007	$48.87 \pm 10.10^{ab}$	$\pm$
VU 007	VU 001	$37.98 \pm 10.04^{ab}$	+
VU 007	VU 002	$27.81 \pm 1.11^b$	$\pm$
VU 007	VU 004	$50.00 \pm 1.31^{ab}$	+
VU 007	VU 005	$5.65 \pm 5.65^c$	$\pm$
VU 007	VU 006	0.00 <sup>c</sup>	-
Average		$26.57 \pm 2.49$	$\pm$

highest pollen penetration observed in style. The table also showed no germination observed in some crosses (VU 001 x VU 004, VU 005 x VU 002, VU 006 x VU 004, VU 006 x VU 005, and VU 007 x VU 008). Previous reports mentioned that failure germination could be caused by pollen tubes that are unable to penetrate the stigma and style, embryo abortion during embryogenesis, and the most common in legumes, embryo degeneration (33). The intra-specific incompatibility observed in some rice bean (*V. umbellata*) accession is due to swelling of pollen tubes (Fig. 6). Swollen tube was also observed in wild and cultivated sesame (*Sesamum indicum*) (71). Delayed pollen tube growth along with other structural abnormalities like twisting, swelling, high branching, bi-furcated tip and variation in callose form were also noticed in the interspecific crosses of *Abelmoschus* spp. (72).



**Fig. 6.** Normal (A) and swollen (B-D) pollen tube (pointed by an arrow) in incompatible inter-specific crosses (male x female) of rice bean (*V. umbellata*) using a compound light microscope at 400x total magnification.

No significant difference was observed among self-pollinated accessions (Table 4); however, the highest germination rate was observed in VU 001 ( $64.01 \pm 1.83\%$ ). The

**Table 4.** Self-compatible pollen-stigma interaction and style penetration in rice bean (*V. umbellata*) accessions.

PARENT COMBINATION		GERMINATION RATE ( $\pm$ SEM) IN STIGMA	POLLEN PENETRATION IN A SINGLE STYLE
Male (Pollen Source)	Female (Pollen Receptor)		
VU 001	VU 001	$64.01 \pm 1.83$	$\pm$
VU 002	VU 002	$47.44 \pm 4.92$	$\pm$
VU 004	VU 004	$45.33 \pm 13.32$	$\pm$
VU 005	VU 005	$43.94 \pm 7.96$	-
VU 006	VU 006	$54.85 \pm 10.98$	$\pm$
VU 007	VU 007	$48.98 \pm 8.67$	$\pm$
Average		$50.76 \pm 3.45$	$\pm$

average germination rate of self-pollinated accessions is 47.66% higher than the cross-pollinated accessions. Like cowpea (*Vigna unguiculata* (L.) Walp.), rice bean (*V. umbellata*) is self-pollinated with somatic chromosome number of 22 (73, 74), resulting in higher self-compatibility and pollen tube growth. Similar results were found out in the preceding studies on *in vivo* pollen tube growth of self-pollinated *Vigna* spp. with normal and high pollen grain germination (75).

## Conclusion

Based on the acetocarmine calorimetric assay findings, the pollen grains of indigenous accessions of rice beans (*V. umbellata*) are highly viable, with accessions VU 004 and VU 007 having the optimum viability rate. In general, BK medium treated with  $0.2 \text{ g.l}^{-1}$  and  $0.3 \text{ g.l}^{-1}$  boric acid enhances the germination rate *in vitro*. Bud and post-anthesis pollens have optimum germination rate when supplemented at  $0.2 \text{ g.l}^{-1}$  boric acid media, while anthesis pollens are suitable in myo-inositol supplemented media. Rice bean (*V. umbellata*) accessions have lower interspecific compatibility but have higher self-compatibility.

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## Authors contributions

MLDP, JAP and MCN designed the study, led and supervised the experimental setups, performed data analysis and checked the manuscript. JHR assisted the experiments, collected data and drafted the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

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