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Research Article

SELECTION AND OPTIMIZATION OF *IN VITRO* POLLEN GERMINATION MEDIUM IN MAIZE (*Zea mays* L.)

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Abstract: The present study was undertaken with the aim of optimising a suitable pollen germination medium for *in vitro* germination of maize pollen grains. During *kharif* 2016, of the four pollen germination media evaluated, highest pollen germination percentage of 90.58 percent and a mean tube length of 915 µm was observed in the liquid medium PGM3. However, pollen grains did not germinate in both the solid media evaluated. When the experiment was repeated in *kharif* 2017 with the inclusion of two additional solid germination media, pollen grains failed to germinate on any of the solid media as well as in the liquid medium PGM3 which supported maximum pollen germination during *kharif* 2016. These results indicate the inconsistencies in reproducibility of *in vitro* maize pollen germination. The study needs to be continued with an attempt to understand the various factors that affect germination of maize pollen grains under *in vitro* conditions.

Keywords: *in vitro pollen germination, maize*

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Introduction

Owing to the structural simplicity, ability to grow in controlled conditions and responsiveness to different external factors, pollen grains have been extensively used in various morphological, biochemical, molecular, biotechnological and evolutional research [1]. With the emergence of the promising technique of 'gametophytic selection', gametes, more specifically 'pollen grains' are subjected to selection pressures and subsequently used in breeding crop plants for improved biotic and abiotic stress tolerance [2]. Assessing the viability of pollen grains is a necessary criterion for utilizing them in various crop improvement programmes [3]. Pollen viability can be tested either through staining with chemical dyes or through *in vitro* or in vivo germination tests. Besides being the most reliable method of assessing pollen viability, *in vitro* pollen germination has also been used to screen genotypes for biotic and abiotic stress tolerance in a number of crop species. This technique involves exposing the pollen grains to stress conditions and assessing their ability to germinate and produce long and stable pollen tubes. Pollen germination and tube growth variability was reported in different genotypes of sorghum, maize, rice, soybean, chickpea, cotton, groundnut, canola *etc*. for abiotic stresses like water deficit, high and low temperature, salinity *etc*., [4-12]. The germination requirements of pollen (*viz*., media composition, temperature, humidity, plant nutrition status, nature of pollen grains *etc*.,) vary with crop species and also among different varieties of a crop which necessitates the need to standardize media composition for *in vitro* pollen germination. Maize, the third most important staple cereal, is severely affected by drought stress particularly at pollen formation stage resulting in substantial yield loss [13]. Hence, to breed maize for drought stress tolerance, identification of tolerant genotypes is a prerequisite. The genotypic variation can be identified at pollen grain level under *in vitro* conditions by evaluating their ability to respond to external cues. Extremities and inconsistencies in maize *in vitro* pollen germination has been reported from previous studies [14]. Therefore, an attempt was made in the present study to select and standardize a suitable pollen germination medium in maize having high reproducibility to assess the viability of pollen grains and further carry out *in vitro* screening of genotypes to drought stress tolerance.

Material and methods

Plant material and growth conditions

Maize inbred line BTM14 was used to standardize *in vitro* pollen germination medium. The inbred line BTM14 was grown in the experimental fields of Department of Plant Biotechnology, UAS, GKVK, Bangalore during *kharif* 2016 and *kharif* 2017 following standard agronomic practices.

Pollen germination media

The *in vitro* germination of pollen grains of inbred line BTM14 was studied using six media reported earlier by various research groups *viz*., PGM1: 0.03 % $CaCl_2 \cdot 2H_2O$, 0.01 % H_3BO_3 and 22.25 % sucrose [15]; PGM2: 0.03 % $CaCl_2$, 0.01 % H_3BO_3, 12 % sucrose and 0.7 % special agar [16]; PGM3: 0.03 % $CaCl_2$, 0.01 % H_3BO_3 and 12 % sucrose [17]; PGM4: 0.03 % $Ca(NO_3)_2$, 0.01 % H_3BO_3, 15 % sucrose and 0.6 % bacto-agar [18]; PGM5: 0.1 % $CaCl_2$, 0.005 % H_3BO_3, 10 % sucrose, 0.0006 % KH_2PO_4, 6 % PEG 4000 and 0.3 % agar [19] and PGM6: 0.03 % $CaCl_2$, 0.01 % H_3BO_3, 17.5 % sucrose and 0.7 % special agar [20]. Among the six media, PGM1 and PGM3 were liquid media and PGM2, PGM4, PGM5 and PGM6 were solid media. During *kharif* 2016, only PGM1, PGM2, PGM3 and PGM4 were used to assess *in vitro* pollen germination of BTM14.

In vitro pollen germination

Fresh pollen grains of the inbred line BTM14 dehisced in the morning hours (8.30-9.30 AM) were collected in petri dishes from the field grown plants and brought immediately to the laboratory. Stock solutions of the media components were prepared separately and fresh medium was prepared each time from the stock solution before conducting the experiment. *In vitro* pollen germination of BTM14 was assessed in both liquid and solid germination media following the sitting drop method in cavity slide. Two cavity slides with two cavities each were used for each medium. One hundred microliter of germination medium was poured into each cavity of the cavity slides and the freshly collected pollen grains of the inbred line BTM14 were dusted uniformly onto the germination medium. The cavity slides were then transferred to petri dish containing moist filter paper to maintain a

relative humidity of ~70-80 percent and incubated for one hour at room temperature (Burke et al., 2004). After the incubation period, pollen germination was observed under a projection microscope (EUROMEX-HOLLAND model CMEX DC. 1300x). From each cavity, five microscopic fields were selected to count the number of germinated pollen grains. Pollen was considered germinated when the pollen tube length was equal to or more than the diameter of the pollen grains. The total number of pollen grains and the number of germinated pollen grains per field of view were counted and germination percentage was calculated [Germination percentage=(number of germinated pollen grains/ total number of pollen grains)*100]. The length of pollen tube of five randomly selected pollen grains per field was measured using Image Focus v3.0 software and averaged. The experiment was repeated during *kharif* 2017 using all six media to select the medium which supported consistent pollen germination.

Results and discussion

In vitro pollen germination is an important and reliable method of assessing pollen viability. This aids in screening genotypes for various biotic and abiotic stresses by subjecting the pollen grains to different selection pressures, thus necessitating the need for an optimised pollen germination medium. Therefore, an attempt was made in the present study to select and optimize a suitable *in vitro* pollen germination medium to further evaluate maize genotypes for drought tolerance. In the present study, *in vitro* pollen germination studies conducted in *kharif* 2016 revealed that neither of the solid medium used *viz*., PGM2 and PGM4 supported any pollen germination. Both the liquid media PGM1 and PGM3 showed germination and tube growth within 30 minutes of incubation. However, after 30 minutes, pollen tube burst was observed in these media. Among the two liquid germination media, PGM3 showed the highest pollen germination percentage of 90.58 percent and a mean tube length of 915 µm [Table-1] and [Fig-1]. Previous studies have reported constraints in reproducibility of *in vitro* pollen germination in maize. To test the consistency of pollen germination on the liquid media, PGM1 and PGM 3, the experiment was repeated in *kharif* 2017 with the inclusion of two additional solid media. It was found that during *kharif* 2017, pollen grains failed to germinate and grow a tube in all the six media studied including the liquid medium which supported highest germination and tube growth during *kharif* 2016. Thus, from the present study it was observed that *in vitro* germination of maize pollen grains was not consistent and reproducible across seasons and that agar in the solid medium seemed to inhibit pollen germination.

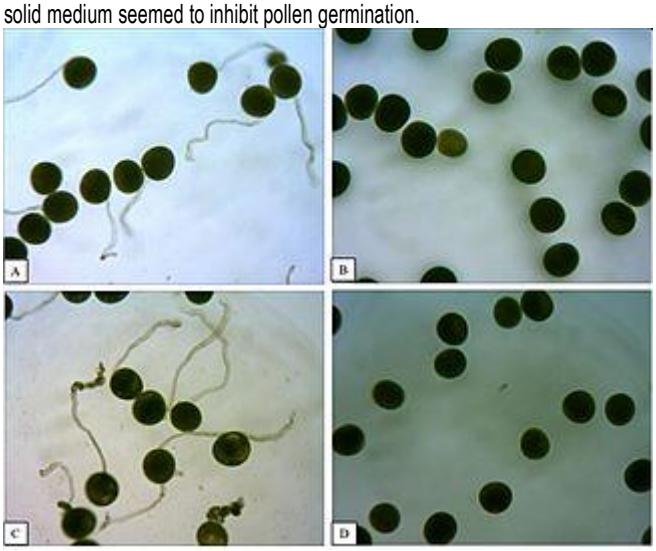

Fig-1 *In vitro* pollen germination of maize inbred line BTM14 on different pollen germination media (A: PGM1, B: PGM2, C: PGM3 and D: PGM4)

Table-1 *Percent pollen germination and pollen tube length of the maize inbred line BTM14 on different media during kharif 2016*

Media	Germination %	Pollen tube length (µm)
PGM-1	28.18±2.42	916.76±23.54
PGM-2	0	0
PGM-3	90.58±1.25	1269.2±37.08
PGM-4	0	0

The earlier work in maize also showed that the medium previously reported to have supported pollen germination in maize did not give stable and reproducible results [19]. Upon repetition of the experiment, fluctuations in *in-vitro* pollen germination was also observed in the optimized media of *Arabidopsis thaliana* and *Brassica rapa* [21-22]. Non-reproducibility of maize pollen germination on various media could be attributed to the influence of different external (photoperiod, temperature, plant nutritional status, time and method of collection of pollen grains) and internal factors (quality and quantity of nutrient reserves of pollen and metabolization of these reserves) and to the tricellular nature of maize pollen grains [23-24]. It has been reported that the germination percentage of tri-cellular pollen grains is reduced on germination medium or under *in vitro* conditions compared to bi-cellular pollen grains. This problem could be mainly due to the recalcitrant nature of tri-cellular pollen grains which are released in a highly hydrated state having a water content of 30-40 percent as against 1-5 percent with bi-cellular pollen grains [25-26]. The present study could not identify a reliable media which supported pollen germination consistently. Hence, the study needs to be continued to obtain a suitable pollen germination medium yielding reproducible results across seasons. This could be further used to screen maize genotypes for different stresses based on the germinating ability of their pollen grains.

Application of research: Selection of suitable pollen germination medium will help in screening genotypes of maize for various biotic and abiotic stresses. This will further aid in identifying contrasting genotypes which can be used in breeding maize for tolerances to various stresses.

Research Category: Gametophytic selection

Abbreviations: PGM: Pollen germination medium, PEG: Polyethylene glycol

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Study area / Sample Collection: Department of Plant Biotechnology, University of Agricultural Sciences-Bangalore, GKVK, Bengaluru.

Cultivar / Variety / Breed name: BTM14 inbred line

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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