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Research Article

Microstructural Observation of Meristematic Tissues in *Macrotyloma uniflorum* Seed Germination Using Foldscope

Soumitra Banerjee*, Meghashree HM, Swarrna Haldar and Nagananda GS

Centre for Incubation, Innovation, Research and Consultancy (CIIRC), Jyothi Institute of Technology, Bengaluru, India

***Corresponding Author:** Soumitra Banerjee, Centre for Incubation, Innovation, Research and Consultancy (CIIRC), Jyothi Institute of Technology, Bengaluru, India.

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Abstract

Horse gram is grown in southern part of India, i.e. Karnataka, Andhra Pradesh etc. and can be cultivated in low-fertile soil and adverse climatic conditions. This legume is known for its medicinal and nutritional properties. Horse gram is a rich source of protein, carbohydrate, essential amino acids and a good source of energy. Germinated horse gram is considered better than raw one because of better digestibility, reduction of anti-nutritional factors. Studies have reported about the compositional changes occurring in germination although no much study was found reporting about the changes in microstructure during germination. This study aimed to report the changes in microstructure using foldscope, which is basically an inexpensive microscope. Microstructure changes happening during germination of horse gram, i.e., meristematic cell development in both the shoot and the roots, was observed using a foldscope and the same was reported, in the paper.

Keywords: Horse Gram; Foldscope; Microstructure; Germination

Introduction

Horse gram (*Macrotyloma uniflorum*) is an underutilized legume, which are cultivated particularly in southern part of India, i.e. Karnataka, Andhra Pradesh etc. The plants are fit for cultivation in low-fertile soil and adverse climatic conditions. Horse gram have been referred in ancient Ayurveda as a medicinal bean with curing properties. Recent studies have revealed nutritional composition of horse gram. Horse gram is rich in protein, carbohydrate, essential amino acids and a good source of energy. Beside macronutrients, horse gram contains many essential micronutrients like iron, molybdenum, phosphorus, vitamins like carotene, riboflavin etc. Recent research findings have indicated presence of beneficial antioxidants and polyphenolic compounds in horse gram [1-3].

Beside nutritional factors, horse gram also contains anti-nutritional factors, which hindered mineral absorption, protein digestibility, and associated with "hard-to-cook phenomenon" of legumes. One method to reduce the effects of anti-nutritional factors is by germination. Germinated horse gram was found safer for human consumption without any anti-nutritional factors. It has been reported that effects of anti-nutritional factors like trypsin inhibitor, hemagglutinin activities, phytates and tannin, could be reduced by post-harvest operations like dehiscing, germination, cooking, and roasting. Germinated horse gram seeds are known for better digestibility and improved nutritional profile [4,5]. Although number of studies have reported about the effects of germination on the nutritional composition of horse gram seeds, although very few studies were found on the changes in microstructure of horse gram during germination, while no one have used foldscope for studying the changes of microstructure of seeds/legumes using foldscope. The present study is focused on Microstructural Observation of Meristematic tissues in *Macrotyloma uniflorum* seed germination using frugal innovation, i.e. foldscope.

Materials and Methods

Surface sterilization and sample preparation

Horse gram was procured from local market of Bengaluru, in the state of Karnataka (India) where it is cultivated in abundance. The good seeds were separated by hand picking, then the seeds were surface sterilized by using 4% salon solution with 6 drops of tween 20. The seeds were rinsed thoroughly by using running tap water for several times to remove the surface contaminants and dirt. Finally, the cleaned seeds were rinsed once again with sterile distilled water.

Germination of the seeds

The seeds were soaked in distilled water for overnight, i.e. 12 hours at room temperature. After soaking period, the water was decanted, and semi-wet seeds were kept for germination in a well ventilated space for 3 days. On each day, seed samples were collected and dried using dry tissue paper. Special care was taken while removing the excess moisture to prevent damages of the germinated seeds and used for further macrostructural and microstructural studies.

Microstructural observation of meristematic tissues in *Macrotyloma uniflorum* seed using foldscope

Foldscope, the inexpensive microscope, was received from Department of Biotechnology, under Government of India (India) as a part of a project titled as, "Wide use of the Foldscope as a research and education tool". The received foldscope had 140X magnification and was capable to view 1.9 µm sized objects. The germinated seeds were observed under foldscope to check its microstructural changes in meristematic tissues with regular intervals.

Results and Discussion

Before moving further into the result and discussion, the basic morphological structure [6,7], of the germinated horse gram seed is shown in figure 1 and different parts are labeled.

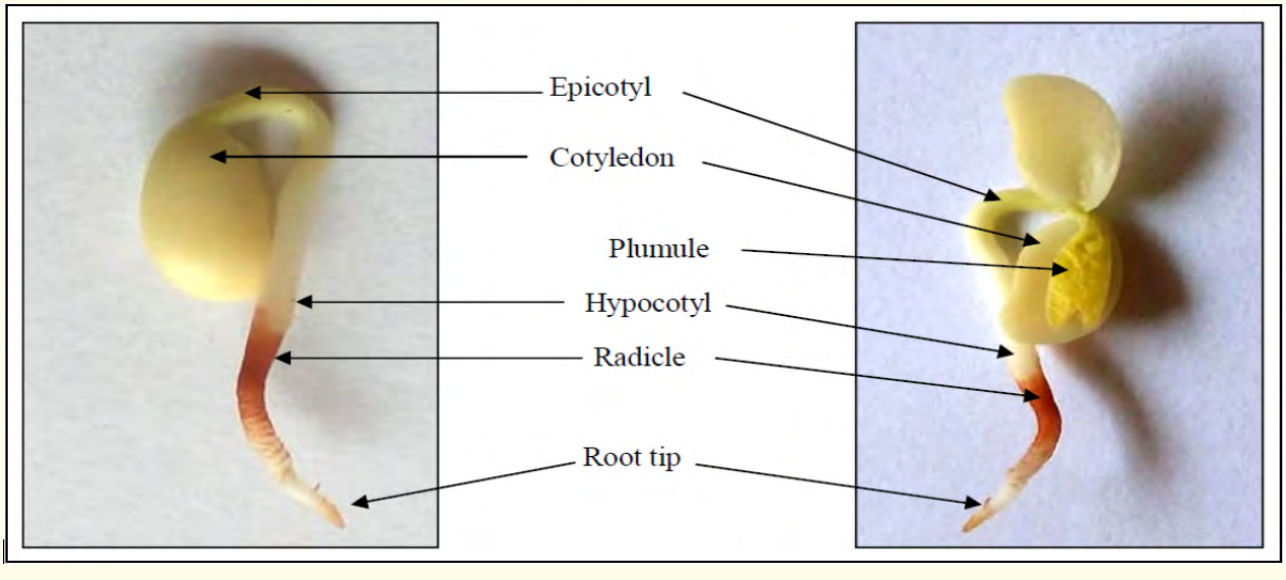

Figure 1: Labeled diagram of Germinated horse gram: whole seed without seed coat (Left) and dissected seed (Right).

Macro structural visualization of germinated Macrotyloma uniflorum seeds

Procured horse gram seed was cut open and shown in plate 1 along with dissected horse gram seeds which were water soaked and germinated for 1 - 3 days. One can see with time how the plumule and radical are getting developed during the process of germination with time. The visuals were captured using a digital camera, allows viewing the macrostructure level only. The limitation of these views is, the changes occurring in the microstructure could not be seen during germination.

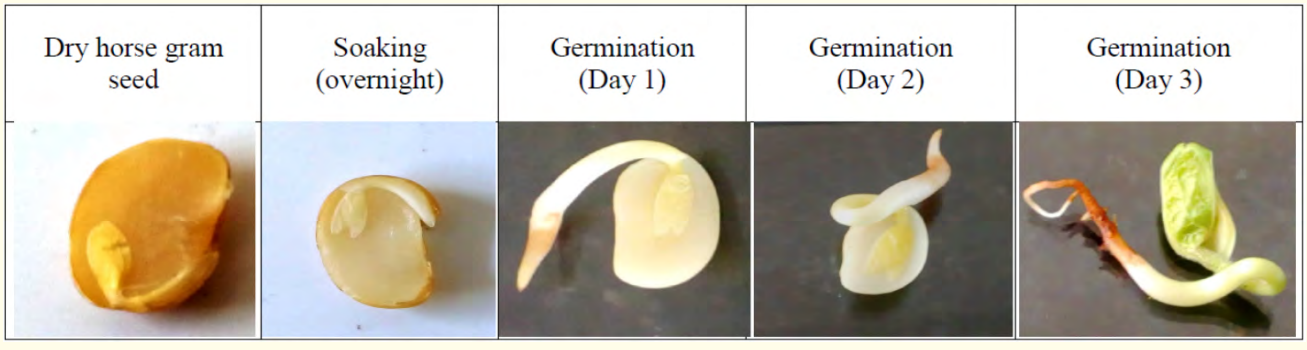

Plate1: Visual changes occurring in horse gram after soaking and germination.

Microstructural observation of meristematic tissues in *Macrotyloma uniflorum* seed using foldscope

Effects of germination on plumule can be seen from plate 2, where change in color from gray to greenish could be observed. This happens because enzymatic activity sets in the developing embryo and accumulation of chlorophyll pigment occurs which is needed for further growth and development. Change in plumule color from white/yellow to green was also reported by Knott [8], while describing the germination of pea (*Pisum sativum*).

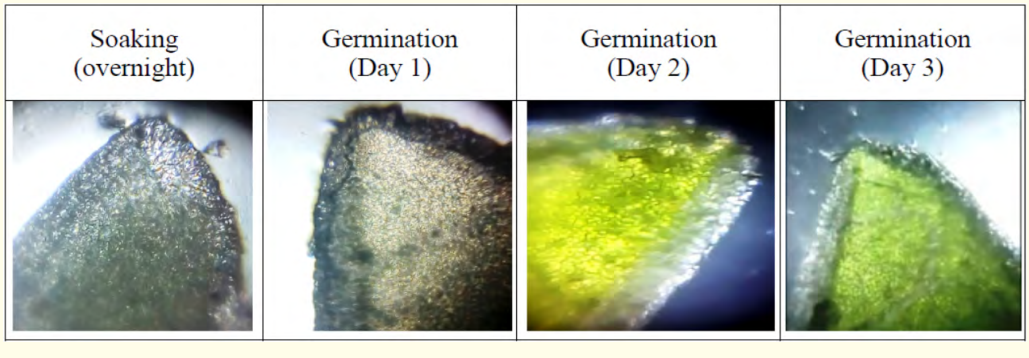

Plate 2: Effects of germination on plumule.

A closer view of the plumule, revealed the meristematic cells arrangement and their changes occurring with germination. Change in color from gray to greenish could be more prominently observed from plate 3. It can be found that in Day 2, the embryo has started attaining chlorophyll pigment with the regeneration of the meristematic cells. In Day 3, the meristematic cells with chlorophyll pigment have started regenerating at a greater pace. In Day 3, embryonic meristematic cells have started developing its first leaf structures.

Development of radicle with actively dividing root meristematic tip is visible from the microstructure as presented in plate 4.

Plate 5 is the continuation of Plate 4 where the magnified form of developed radicle surface is presented, with lateral roots. Carberry and Abrecht [9], reported increase in radical and hypocotyls length, for germination of kenaf seed under constant temperature. For horse gram seeds, development of hairy structure was observed on the radical surface after 2nd and 3rd day of germination,

Plate 3: Effects of germination on Plumule meristematic cells of horse gram.

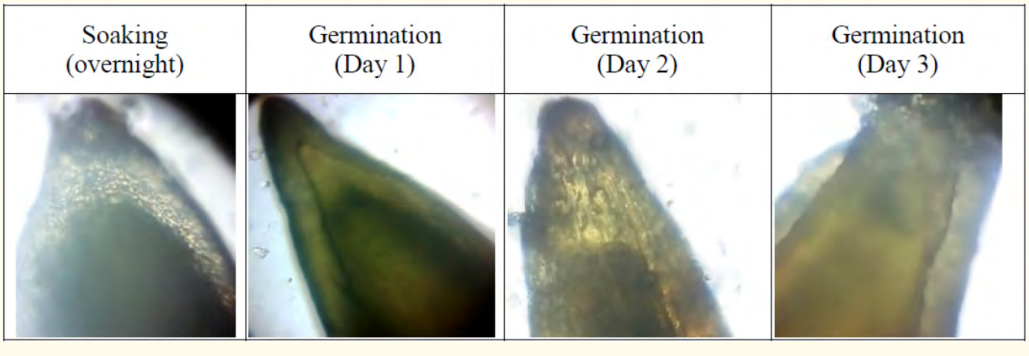

Plate 4: Effects of germination on Radicle of horse gram.

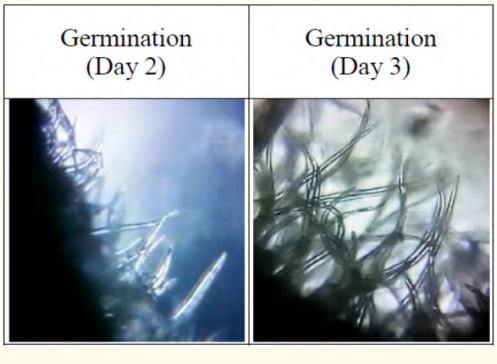

Plate 5: Effects of germination on the growth of hairy structures on radicle of horse gram.

as shown in plate 5. Hairy structures were denser and longer on Day 3 as compared to Day 2 of germination.

Changes in cotyledon surface were observed during the germination of horse gram as shown in plate 6. During the initial stages of soaking, stored nutrients in cotyledon, gets utilized with time along with the process of germination. A change in the nutritional content, during germination of horse gram seeds, was reported by the work of Pal., *et al* [10]. In another separate study conducted by Harmuth-Hoene., *et al.* [11], changes of nutritional composition were reported for wheat, mung beans and chickpeas. Like horse gram, Canola seed cotyledon to change its color, when exposed to favorable germination condition [12].

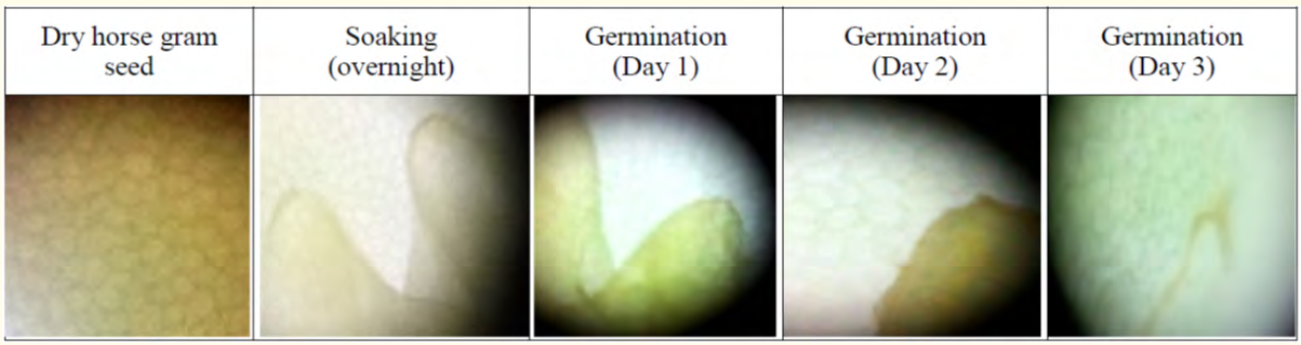

Plate 6: Effects of germination on cotyledon of horse gram.

Conclusion

Through foldscope, it is possible to study the microstructure changes, i.e. meristematic cell development in both the shoot and the roots, occurring during germination. Foldscope being an inexpensive lightweight microscope would be a great tool for the researchers to explore more about the changes in microstructure during germination of other seeds as well.

Acknowledgement

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