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ORCHID SEED GERMINATION AND CARBOHYDRATE MOBILIZATION PATTERN

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The seed germination in orchid is a complex process. A single capsule (fruit) produces several millions of seeds. But the percentage of germination and the number of plants developed are very low due to lack of functional endosperm. The process of germination proceeds symbiotically in nature with the association of some mot fungus and asymbiotically in aseptic conditions. The significance of fungus and its importance was well established by Bernard (1899). He found that the fungal infection is necessary for orchid seed germination. These fungi were chiefly responsible for breaking down complex starch into simple sugars for the germinating seeds. The work of Knudson (1922) showed for the first time that the germination of orchid seeds would be possible in-vitro without the association of fungi. The development of seedling from a seed is not like in any other angiosperms. The rudimentary embryo enclosed in the seed coat develops like a dormant bud. In the process of development the seeds swell in size and burst out of the seed coat. A cone shaped spherical seedling is formed and this is called protocorm stage (Bernad, 1909). The first leaf primordium is formed as a bulge and the protocorm increases in size and subsequently rhizoids and leaf primordia are formed (Arditti and Bill, (1965). The further sequential development in the protocorm in respect of carbohydrate accumulation and mobilization is unknown. The study was undertaken to find out the seed germination and carbohydrate mobilization pattern in protocorm at the Indian Institute of Horticultural Research, Bangalore.

Materials and Methods

The seeds of *Bletilla hyacinthina* were sown in an artificial medium for germination in an aseptic condition. Five culture media were tested for its performance. The seeds took only seven days for greening and germination in Vacin and Went medium. The seeds prior to germination inbibed water, swelled and formed into a protocorm like body (PLB), which later developed into shoot, root and pseudobulb. The small pseudobulbs as and when they appeared were taken out and fixed in FAA. The standard procedure of plant micro-techniques were followed for making slides. The sections were subjected to periodic acid Schiff's reagent method of staining to locate insoluble polysaccharides and toludine blue method for locating DNA and RNA. The slides were observed under binocular microscope and the photomicrographs were taken. The observations made in the slides and photographs revealed a clear pattern of carbohydrate mobilisation in protocorm like bodies and pseudobulbs.

Results and Discussion

An account of the changes in the macro-molecular substances such as insoluble polysaccharides, RNA and DNA during the differentiation of plantlets from orchid seeds is given below.

In the protocorm like body, tha lower parenchymatous cells contain rich accumulation of large sized PAS+ granules which were confirmed as starch grains by iodine potassium iodide test (Fig. 1). The basal cells of the rhizoids contained the starch grains while terminal cells were devoid of them. Towards the apex of the PLB, in the parenchymatous cells the starch gradually diminished in size and they were completely absent in the cells near the apical end of the PLB (Fig. 2). A gradient is created from base to the apex in the distribution of insoluble poly-sacharides in the protocorm like bodies. The cells of the shoot apex contain PAS + cytoplasm free from such grains. The cells at the basal end of the PLB are large while those at its apex are very small. Thus a decreasing gradient is created in the PLB from base to the apex in the size of cells, size of nuclei and in the size and quantity of the stored starch grains. An increasing gradient is observed in the RNA and protein content from base to the apex in the PLB. The enlarged cells near the basal end of the PLB appear to be concerned with the active starch accumulation and subsequent degradation into simple carbohydrates to be transported to the shoot apex which is a region of active growth and differentiation.

The narrow group of cells in the upper middle part of the germinating seed connecting the apical meristematic zone with the basal storage region aid in translocation of nutrients from the base to the apex. These group of cells lack definite zylem and phloem differentiation, hence can aptly be called as provascular strands. The provascular strands seem to be not merely a passive structure of translocation but their cells are also metabolically active. The increased activity of this group of cells may be perhaps concerned with the prevention of reversible reaction and/or with the transformation of some raw metabolites into other forms as needed by the meristematic cells of the shoot apex.

The pattern of starch accumulation in pseudobulb [is different from that observed in PLB. The pseudobulb is made up of two kinds of cells (Fig. 3). The small cells contain a very rich accumulation of densly stained starch grains, while the large cells contain relatively less densly stained starch grains. The relatively less number of large starch grains present in the large cells are distributed around the nuclei and they appear to be at various stages of disintegration. The cells of pseudobulb surrounding the vascular strands have rich accumulation of starch. The cells in the crown of leaves do not contain any starch, however, their cytoplasm is PAS+. Further at the base of the pseudobulb adjacent to the tip of the PLB the cells are small and they do not contain any PAS+ bodies or starch. The roots, lateral shoots and their primordia are free from such accumulation but their cells contain PAS+ cytoplasm.

With the differentiation of leaves the plantlet becomes photosynthetically more active. The surplus photosynthates flow back to the leaf base move to the base of the shoot and get converted into storage starch. The cells at the base of the shoot multiply and enlarge to form a bulbous structure known as pseudobulb. When the concentration of storage substance in the pseudobulb is optimum, some of the cells at the base of the pseudobuib probably differentiate into lateral shoot primordia. This may form into vegetative or reproductive shoots. Mean while, some of the cells at the base of the pseudobulb differentiate into root primordia. At this stage the plant becomes autotrophic.

Carbohydrate mobilization pattern

The carbohydrates absorbed from the medium through the rhizoids diffuse to the basal cells of the PLB from where they flow into other cells. The absorbed carbohydrates probably get converted into storage starch and later the stored starch grains degrade into specific type of sugars which move to the tip of the PLB to help the differentiation of shoot apex. The mobilization of sugars in the PLB is through diffusion from cell to cell in its basal part, while it is through provascular strands in its upper part. All these indicate that the mobilization of carbohydrate is acropetal in PLB.

In the autotrophic plantlet the leaves are photosynthetically active. After utilization of some of the photosynthates for its own differentiation, the leaf allows the excess to flow into the base of the pseudobulb. The photosynthates move downward in the vascular strands and accumulate as starch grains, Later, after attaining an optimum level of storage, the starch near the base of the pseudobulb gets degraded into specific kinds of sugars which flow into differentiating and growing lateral shoots and roots. The mobilization pattern of carbohydrate in *Bletilla hyacinthina* seedling is represented diagramatically in Fig. 4.

Summary

The mobilization pattern of carbohydrate is quite specific in orchid seedlings. Its movement in the PLB is acropetal and in the pseudobulb is basipetal, A neutral zone is formed in between the two which lacks mobilization.

സംഗ്രഹം

ബ്ലറ്റില്ല ഹയാസിന്തിന എന്ന ഓർക്കിഡിന്റെ വിത്ത് പരീക്ഷണശാലയിൽ രോഗാണുവിമുക്തമായ കൃത്രിമ ffloa0u>^a)r!Jro>1<!54 വളർത്തി തൈകൾ ഉൽപാദിപ്പിച്ചു. ഈ തൈകളിൽ അന്നജത്തിന്റെ സംഭരണവും നീക്കവും പഠിക്കുകയുണ്ടായി. ഓർക്കിഡ് ചെടിയുടെ വിത്തിലുള്ള ഭ്രൂണം വളർന്ന് പ്രത്യേക രൂപമില്ലാത്ത ഒരവയവം ഉണ്ടാകുന്നു (PLB). raraaj വീണ്ടും ra^njonraxoo സംഭവിച്ച് ഉള്ളിയുടെ ആകൃതിയിലുള്ള തണ്ടും ഇലകളും ഉണ്ടാകുന്നു. ഭ്രൂണത്തിൽനിന്നും ഉണ്ടായ അവയവത്തിൽ വേരുകൾ മാതിരിയുള്ള നാരുകൾ ഉണ്ടാകുന്നു. അവ മാദ്ധ്യമത്തിൽനിന്നും അന്നജം (പഞ്ചസാര) ആഗിരണം ചെയ്ത് മുകളിലേക്ക് നയിക്കുന്നു. എന്നാൽ ഉള്ളിമാതിരിയുള്ള roiene'lroft കാണുന്ന ഇലകൾ അന്നജം നിർമ്മിച്ച് ചുവട്ടിലേക്കു നയിക്കുകയും, ചുവട്ടിൽ സംഭരിക്കുകയും ചെയ്യുന്നു. ഇവ രണ്ടിനും മദ്ധ്യേ ഒരു കൂട്ടം കോശങ്ങൾ ഉണ്ട്. അവയിൽ കൂടി raracms സഞ്ചരിക്കുന്നില്ല. ഈ കോശസമൂഹത്തിനു മുകളിലായി യഥാർത്ഥ വേരുകൾ ഉണ്ടാകുന്നു.

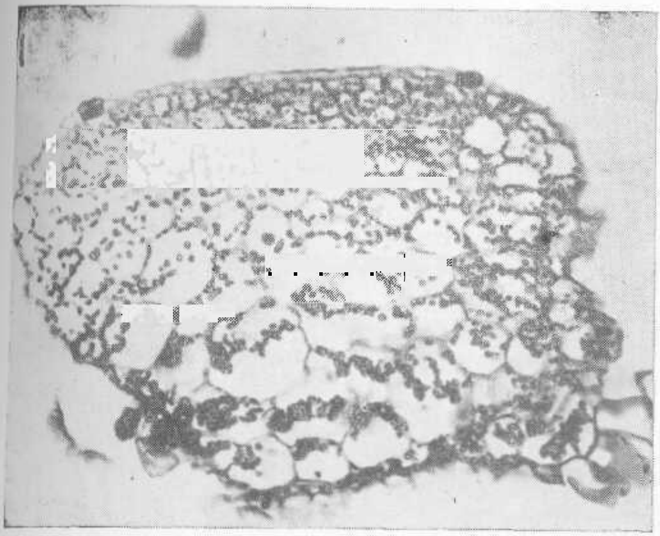

Fig 1. A fully grown PLB showing the distribution of carbohydrates (X40)

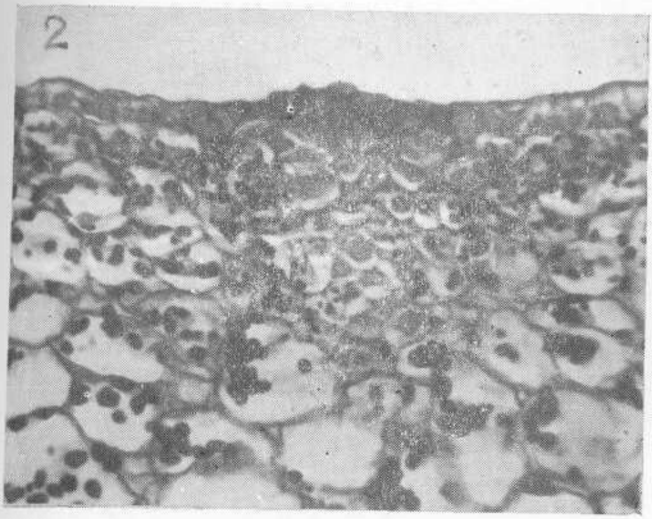

Fig 2. PLB showing signs of shoot differentiation and carbohydrate distribution (X40)

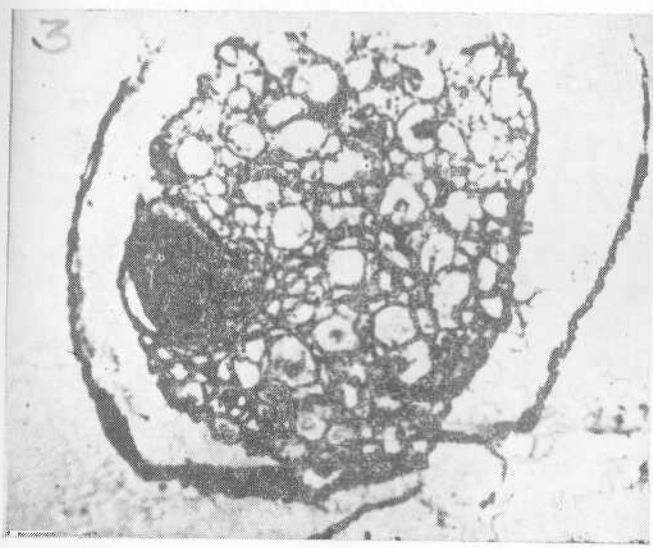

Fig 3. A pseudobulb in which lateral shoots develop (X 40)

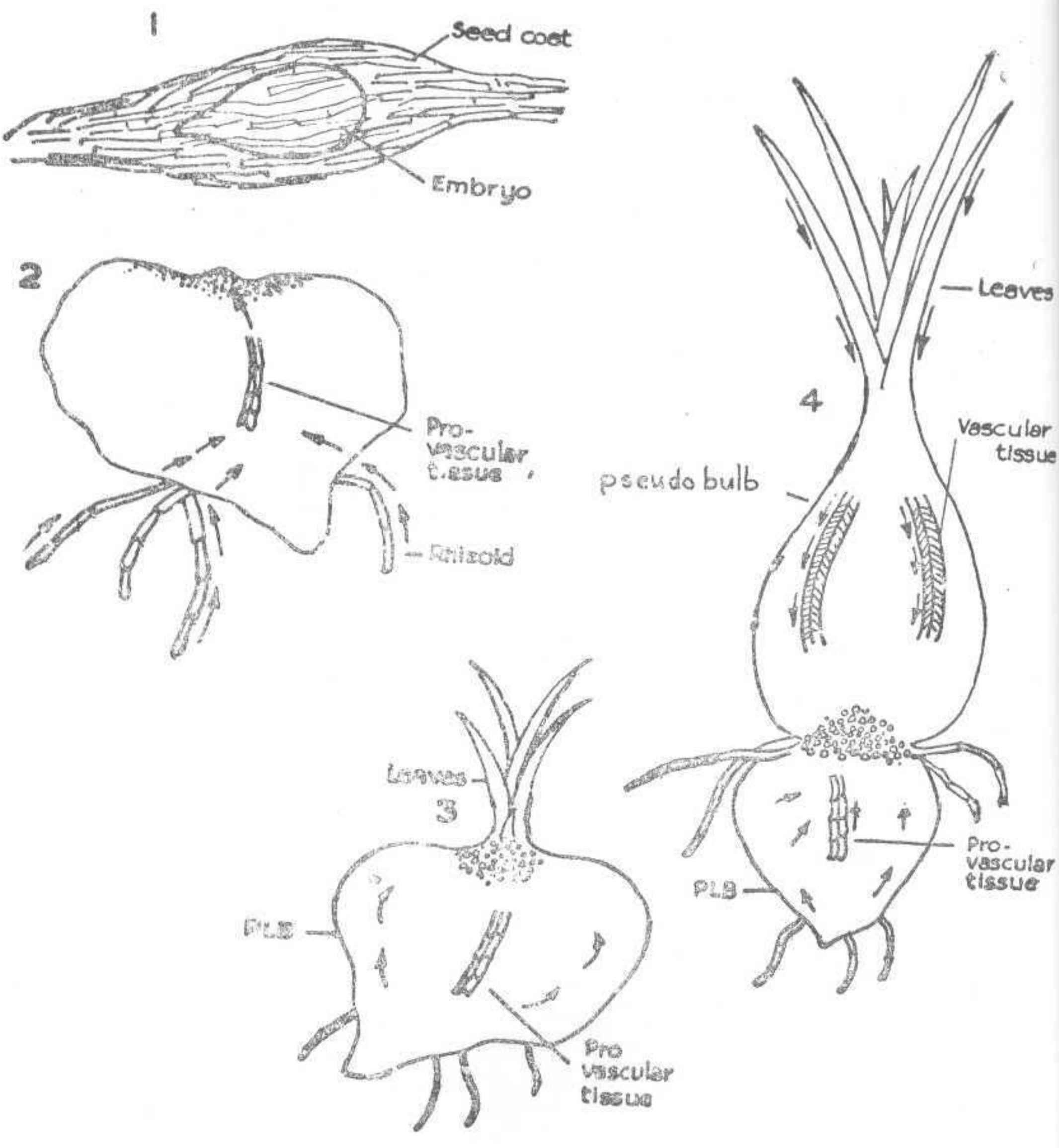

Fig 4. Nutrient mobilization pattern in developing orchid seedling (diagramatic)

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