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Impact of Sucrose Concentrations on *in vitro* Pollen Germination of Okra, *Hibiscus esculentus*

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Abstact: Exogenous sugars, especially sucrose is very essential for providing osmotic environment and nutrition to *in vitro* pollen grain germination. Pollen bursting is frequently observed in artificial medium lacking suitable sucrose concentration. Four sucrose concentrations 10, 20, 30 and 40% were tried for okra pollen germination. At 10% sucrose, majority of pollens brusted; however, at 20% sucrose, 80% of pollen grains germinated by producing tube lengths in the range of 3000 to 4000 μm. At 30%, the pollen germination% and tube length declined by about more than twice against 20% sucrose level. At 40% concentrations, pollen grains' germination and tube length reduced remarkably by giving only 500 to 700 μm pollen tubes. Not only tube length retarded at these high concentrations but pollen tubes also changed to more thickened and shortened structures. It was therefore observed that 20% sucrose is an appropriate concentration for *in vitro* okra pollen germination.

Key words: Sucrose concentrations, *in vitro* pollen germination, okra

Introduction

The role of exogenous sugars in pollen germination is two fold; one is osmotic regulation and another is nutritional source. A number of earlier workers believed that exogenous sugars are required for osmotic purpose and not for nutritional (Visser, 1955). Others supported the view apart from having osmotic role that externally supplied sugars either *in vivo* or *in vitro* serve as important source of nutrition (Malik *et al*., 1982). Visser (1955) defended that sugars are only essential for generating favorable osmotic condition for pollen germination because he noted that many pollens germinate readily in pure water and attend considerable pollen tube length without sugars or substrate. However, after lot of work done supported exogenous sugars as nutritional source and Visser (1955) eventually believed in that.

More evidence became available that utilization of exogenous sugars by pollen is both direct and indirect. Vasil (1964) noted that pollen tube size varied with changed concentrations of sucrose. Direct experimental evidence of pollen grain using exogenous sugars, however was first demonstrated by O' Kelley (1957) when supplied, [C^{14}] labeled fructose and glucose to the pollen tubes. Whether sucrose serves as only osmotic control or nutrition source has now been further clarified with the studies of pollen bursting. Frequent pollen grains and pollen tubes bursting are a common source of annoyance and perplexity in the work of *in vitro* pollen germination. This pollen bursting is believed to be controlled with changing osmotic equilibrium of pollen grain by changing sucrose concentration. The present study is intended to figure-out the optimum sucrose requirement of *in vitro* okra pollen germination.

Materials and Methods

Four concentrations of sucrose i.e., 10, 20, 30 and 40% were added in Taylor's (1972) basal medium with 300 mg [Ca $NO_3]_2$ 140 mg $MgSO_4$, and 50 mg H_3BO_3 as a medium for okra pollen grain germination. A drop or two of freshly prepared medium was placed on clean microscope glass slides in circular form with camel hair brush. Five slides of each sucrose concentration were prepared. When the flowers dehisced and anthers bursted at about 10:00am, the pollen grains from healthy looking flowers were shed onto the sitting droplets of each medium, spread with brush so as to assure the complete saturation of pollen grains in the medium. Water moistened filter papers were placed in the bottom of petri plates. Additional drop or two of water was also left in the petri plates so as to generate the required humidity of 70%. The pollen grain inoculated slides were then carefully kept on moistened filter papers in Petri plates, covered half a way with the tops. After this preparation, the Petri plates were kept at room temperature of 30± ^{o}C. After 3 hours of incubation, the slides were observed under microscope on 10x objective. A drop of 0.5% acetocarmine was added to germinated pollen grains for taking photographs. The pollen tube measurements were taken from negative films in millimeters and then converted into micrometers (μm).

Results and Discussion

Frequent bursting of pollen grains and pollen tubes is major difficulty in the work of pollen culture. This is due to uptake of large quantities of water thus can be controlled by adjusting osmotic concentration of the medium. It is therefore, now believed that exogenous sugars are involved not only in the nutrition of germinating pollen but also important in providing and maintaining proper osmotic environment for pollen germination and continued growth of the pollen tubes.

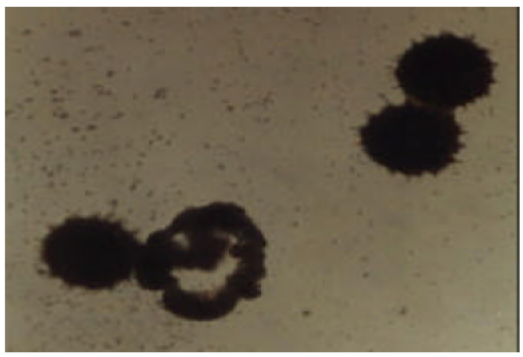

Fig. 1a: Pollen grains germinated at 10% sucrose levels

The impact of various sucrose concentrations on *in vitro* okra pollen grain germination was very obvious, especially in tube length and its structure. At 10% sucrose level, the pollen grains germinated into pollen bursts as shown in Fig.1A. In this small sucrose percent, it appeared that osmotic control of pollen grains was not properly maintained, thus pollen grains brusted rather than germinated in to the real pollen tubes. In 20% sucrose, the pollens gave about 80% germination with pollen tubes measuring 3000 to 4000 μm. These pollen tubes were normal in shape and structure (Fig.1B). No pollen bursts

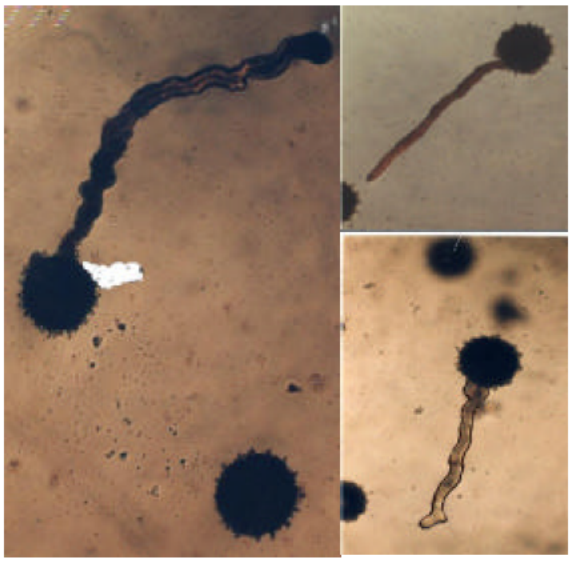

Fig. 1b: Pollen grains germinated at 20% sucrose levels

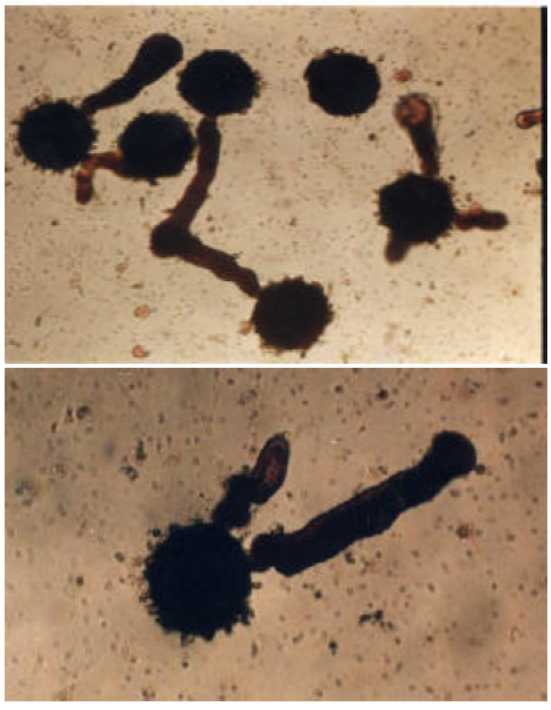

Fig. 1c: Pollen grains germinated at 30% sucrose levels

were observed as shown in Fig. 1A. At 30% sucrose, the pollen germination was less compared to 20% sucrose level, the tube size however was considerably smaller ranging from 1500 to 1800 µm. The pollen tubes were thicker than the normal as shown in Fig. 1C. In 40% sucrose concentration, the pollen germination percent and tube length reduced drastically (Fig. 1D). Though there was no big difference in tube length between 30 and 40% sucrose concentrations; however, differences in tube length were still striking. The

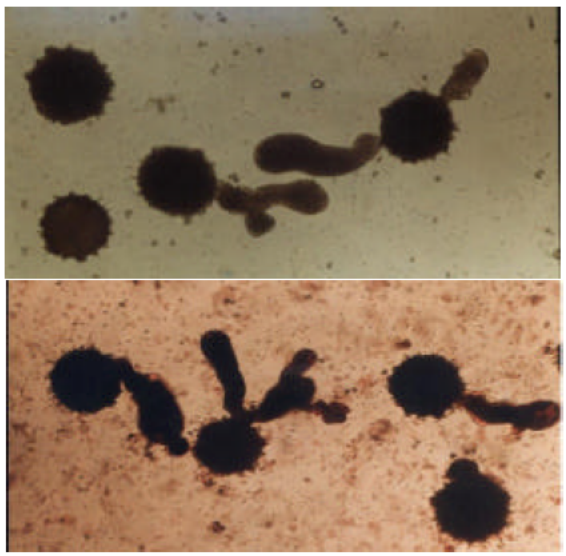

Fig. 1d: Pollen grains germinated at 40 per cent sucrose levels

30% gave slightly longer (800 to 1000 µm) and narrower tubes as compared to smaller and thicker tubes in 40% sucrose (500 to 700 µm). Thus, the prominent feature of pollen tubes in higher concentrations of sucrose were smaller and thicker tubes. Barrow (1980) also reported that as sucrose concentration increased, the cotton pollen tubes became shorter and ejected at slower rates. This pollen tube feature could be attributable to higher outer osmotic pressure, exerted on pollen grains as compare to inner pressure of pollen grains or lower absorption of pollen grains due to higher sucrose concentrations. These results thus suggest that sucrose is required more for osmotic regulation than nutritional source. Philomena and David (1984) also reported 90% germination of cotton as well okra pollen grains in 25% sucrose. Baloch *et al.* (2000) however do not agree 25% sucrose as an appropriate concentration for cotton pollen germination but considered 40% sucrose suitable for cotton pollens. Contrary to Baloch *et al.* (2000), Choudhry and Akhmedova (1982) successfully germinated cotton pollen grains in 25% sucrose. Vasil (1960) observed that bursting of *Cucumis melo* and *Memordica charantia* pollen grains in a sucrose medium can be decreased and largely eliminated by increasing the osmotic concentration of medium by the addition of exogenous sugars.

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