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A Technique for Demonstrating Meristematic Sites in Bulbs and Corms

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Abstract. **This paper describes a fast, accurate method of demonstrating the sites of high metabolic activity in bulbs and corms. The use of 2,3,5-triphenyl tetrazolium chloride (TTC) is outlined as a site-specific stain for meristematic activity in these specialized plant structures.**

Demonstrating the morphological and anatomical features of bulbs, corms, and other specialized reproductive structures to a plant propagation class always has been perplexing. The sites of the meristematic zones on the bulb stem plate, as compared to the location of meristematice buds on the surface of the corm, are difficult for many students to visualize without time-consuming histological preparation and associated delays.

The use of 2,3,5-triphenyl tetrazolium chloride (TTC) as a seed testing agent has been well documemted (1, 2, 4, 5). The TTC test is effective in pinpointing areas of high metabolic activity (i.e., meristematic sites), or lack thereof, in imbibed seeds, regardless of potential dormant conditions or seed viability. There is little evidence of research using TTC staining to observe active metabolic areas in specialized plant structures. Roistacher et al. (6, 7) and Simchom et al. (8) used TTC to study dormant cormels and corms, respectively. Klingman (3) investigated the viability of wild garlic (*Allium vineale* L.) with TTC. This rapid staining method for demonstrating meristematic activity in seeds was investigated as a possible method for pinpointing similar sites of meristematic activity in bulbs, corms, etc.

Laboratory exercise. A freshly prepared 1% aqueous solution of TTC should be adjusted to a pH of 6.0 to 7.0. This solution can be used immediately or stored in an amber container for several months at 5°C until used as recommended for seed testing (1). It is important to bring the TTC solution to room temperature (20° to 25°) before treating the plant material so that the reaction time within the tissue sample will be shortened.

Clean, healthy material representative of the bulbs or corms to be tested are selected for this exercise. The dry, membranous tunic is removed to expose fleshy scales (leaf bases) of the bulb, or the swollen stem tissue of the corm. These specialized structures are prepared for treatment by bisecting them longitudinally through the center with a thin bladed sharp knife. It is more illustrative of the meristematic zones being studied if the section can be made through a primary bud on the bulb or corm, thus exposing tissue to treatment in the growth center region.

Once the structures have been sectioned, they should be placed, cut, surface immersed, in a petri dish containing the TTC solution. Reaction times vary depending on the type of bulb or corm tested and the temperatures involved, but about 30 min usually is sufficient. The development of an insoluble red compound (formazan) in the meristematically active regions is easily followed by holding up the petri dish to observe the reaction in the cut tissue immersed in the TTC solution (Fig. 1).

After sufficient time elapses for the TTC to react in the tissue, the zones of meristematic activity become very evident when contrasted with the surrounding tissue mass not as metabolically active (Fig. 1). When sufficient staining intensity is reached in the tissue, the TTC solution can be drained and replaced with clean water to hold the stained tissue for class observation without risk of overstaining. It is possible in most cases to store the stained tissue in clear water at 5°C for several days without significant loss in quality if there is a need to use the samples for multiple observations.

This simple exercise can be effective in helping students locate the potential growth sites on these storage organs, thus affording a better understanding of propagation methods such as scaling, twin scaling, and division.

Literature Cited

1. Hartmann, H.T. and D.E. Kester. 1983. Plant propagation: principles and practices. Prentice-Hall, Englewood, N.J.
2. Grabe, D.F. (ed). 1970. Tetrazolium testing handbook. Assn. Offic. Seed Anal.
3. Klingman, G.C. 1959. Wild garlic bulblet viability determined by 2,3,5-triphenyl tetrazolium chloride. Weeds 7:474–480.
4. Lakon, G. 1949. The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol. 24:389–394.
5. Lindbein, W. 1965. Tetrazolium testing. Proc. Intl. Seed Test. Assn. 30:89–97.
6. Roistacher, C.N., J.G. Bald, and K.F. Baker. 1953. The tetrazolium test for dormancy and germinability of gladiolus cormels. Science 118:186–187.
7. Roistacher, C.N., K.F. Baker, and J.G. Bald. 1957. 2,3,5-triphenyl tetrazolium chloride as an indicator of germinability and dormancy of gladiolus cormels. Hilgardia 26:685–704.
8. Simchon, S., R. Shilo, and A.H. Halevy. 1972. Studies on the dormancy of gladiolus corms: refinement of the triphenyl tetrazolium chloride method for estimating the dormancy of gladiolus corms. J. Hort. Sci. 47:505–508.

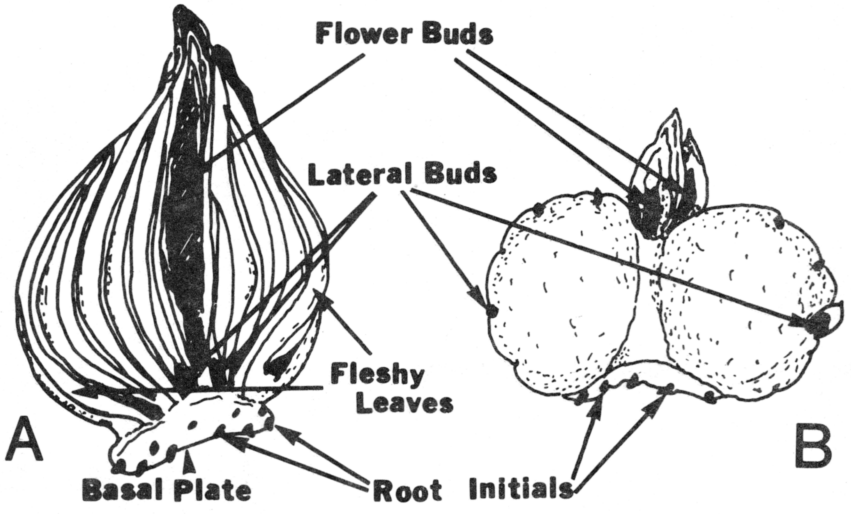

Fig. 1. Diagram of tulip bulb longitudinal section (**A**) and crocus corm longitudinal section (**B**) stained (dark areas) with TTC. Note that stain concentrations (**A**), defining sites of high metabolic activity, demonstrate origin of root initials, lateral and flower buds all from the basal plate. In contrast, stain concentrations (**B**) indicate that origins of root initials, lateral and flower buds are limited to the corm surface. Fleshy leaf tissue (**A**) and interior corm tissue (**B**), representing areas of low metabolic activity, do not stain.

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