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Dependence of thermal responses of seeds on membrane transitions

(seed germination/plasmalemma leakage/fluorescent probes)

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ABSTRACT **Transitions at 28–32°C in membranes of many kinds of seeds influence their germination and subsequent growth. Changes at 28–32°C in the rates of loss of endogenous amino acids from imbibed *Barbarea verna* and *Lactuca sativa* seeds indicate permeation of the plasma membrane. A transition in the same temperature range is shown by the change in fluorescence with temperature of substituted naphthalenesulfonate probe molecules associated with membrane fragments isolated from the seeds. Some other plant responses that appear to be causally connected with the membrane(s) transition(s) in the 28–32°C region are growth of maize seedlings and tomato roots, flowering of some bulbs, and control of seed and fern spore germinations by light. Generality of the control is indicated.**

Plants and seeds grow best in rather narrow temperature ranges that are well within ultimately limiting high or low values. Thus, germination of many kinds of seeds is best in the 15–30°C range and is greatly reduced above 32°C. Sometimes temperature variation is required for optimum response. Seeds commonly show increases in germination when held for short periods at the higher temperatures before being returned to 15–30°C. Plant growth also is often favored by alternation of day and night temperatures. The generality of the seed responses and the narrow range of critical temperatures encourages a search for cause.

Correlations between germination and other properties of seeds with critical ranges of temperature change suggest an approach to the subject. Thus, many kinds of seeds imbibed in water have enhanced leakage of sugars (1) and amino acids (2) with increased temperature. The maximum rate of change of these losses often occurs in the 30–32°C range (2). Membrane fragments separated from responsive seeds show changes at 28–32°C in the rate of change of fluorescence with temperature of anilinonaphthalenesulfonate probe molecules added to suspensions of the fragments (3). The phenomenon is further examined here.

MATERIALS AND METHODS

Germination of *Barbarea verna* (Miller) Aschers (early wintercress, collected locally) and *Lactuca sativa* Linnaeus cv. Grand Rapids (lettuce, purchased) seeds were measured on lots of 100 seeds at 1°C intervals on a thermogradient plate. Other seeds, including those of *Amaranthus retroflexus* Linnaeus, were collected locally and held in dry storage at −20°C. Amino acid leakage to ambient solutions was measured as described (2) on seeds held for 4 hr at the various temperatures after imbibition for 16–18 hr at 20°C.

Membrane fragments were separated by grinding 0.5 g of imbibed seeds with silica in a mortar at room temperature with three successive 3-ml lots of 2 mM potassium phosphate buffer at pH 7.2. The pellets from centrifuging for 45 min between $3000 \times g$ and $25,000 \times g$ were resuspended in buffer. Narrow fractionation and density gradients were used in one case but not systematically because of the drastic grinding procedure required to disrupt the seed tissues. A solution of a probe compound was added to the suspension to final 10–100 μM. A cuvette containing the mixture was placed in the jacketed holder of an Aminco fluoromicrophotometer. Temperature was varied by flowing water in the jacket with convection in the sample effecting heat distribution. The temperature was measured by a thermistor placed in the sample just outside the excitation beam. Heating rates were varied between 0.1°C and 2.0°C/min.

The probe molecule was 2-(*N*-methylanilino)naphthalene-6-sulfonate (MANS) obtained from Molecular Probes, Roseville, MN. Several other substituted naphthalenesulfonates gave similar results that are not reported here. Fluorescence excitation was with a mercury arc, using a limiting 390-nm filter with a 410-nm short wavelength cut-off filter in the fluorescence radiation. An Aminco ratio spectrofluorometer with a xenon arc source was used in establishing the best conditions for measurements of fluorescence and verification of purity and characteristics of probe responses.

RESULTS

Seed Germination and Amino Acid Leakage. Imbibed seeds of the lots of early wintercress and lettuce used exceeded 75% germination at 20–28°C after short exposure to red light to transform phytochrome. The values rapidly decreased to zero in the region of 30–35°C (Figs. 1 and 2). The inflection point of the germination against temperature curve is near 30°C for early wintercress and 32.5°C for lettuce. Germination of the early wintercress seeds in darkness reaches a sharp maximum at 29°C and decreases similarly to that in the lighted seeds at 29–33°C. Lettuce seeds in darkness show an inflection point of germination against temperature near 27°C. Amino acid leakage from lettuce seeds, although low, changes by about 1.6-fold between 26°C and 30°C, with a median value near 28°C. The leakage increases rapidly at temperatures above 34°C (Fig. 2). The amino acid leakage from early wintercress seeds, which was measured at 5°C intervals in previous work (2), increases sharply above 30°C.

Fluorescent Measurements. A graph of the relative values of the fluorescence (F) against wavelength for 100 μM MANS has a maximum value near 508 nm (Fig. 3). This is enhanced about 3-fold in intensity and shifted to a 439-nm maximum in the presence of a $3000–25,000 \times g$ membrane sample from *A. retroflexus* seeds (Fig. 3). Scattering by the sample of radiation in the 410- to 540-nm region (curve c, Fig. 3) transmitted by the 390-nm limiting filter (xenon arc excitation) is also shown. Similar results were obtained from lettuce and early wintercress

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Abbreviation: MANS, 2-(*N*-methylanilino)naphthalene-6-sulfonate.

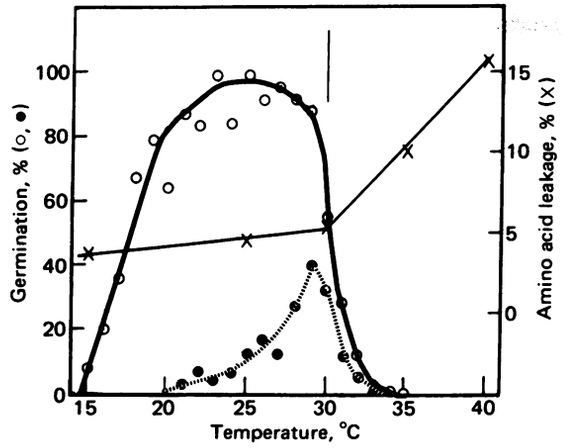

FIG. 1. Changes with temperature in responses of imbibed *B. verna* seeds: germination in darkness (●) or after transformation of phytochrome by red light (○) and leakage of amino acids in 4 hr after 17-hr imbibition at 20°C (×).

but with lower enhancement of F by the membrane preparations.

The values of F depend on a number of instrumental features and on the sample sizes, the content of responsive tissues, and the degree of penetration and scatter of the exciting radiation in the suspension. These values are accordingly expressed on an arbitrary scale for each preparation. Semilogarithmic graphs of the arbitrary F values against $10^3/K$ in the region of 15°C to 40°C for membrane fragments in the presence of 100 μM MANS show the highest values near 30°C and below 22°C in the rates of change of log F against $10^3/K$ (Fig. 4). The arbitrary ordinate scales are selected to facilitate intercomparisons of the several graphs. At 30°C the log F value for a 100 μM MANS solution at 450 nm is about 0.15 that for a membrane preparation of lettuce in 100 μM MANS (Fig. 3). The values of log F for MANS solutions decreased with decreased temperatures in contrast to the increase in the presence of membrane fragments (Fig. 4).

Semilogarithmic values of intensities of scattered radiation from membrane preparations plotted against $10^3/K$ also have their highest rates of change in the region of 30°C (Fig. 5). The

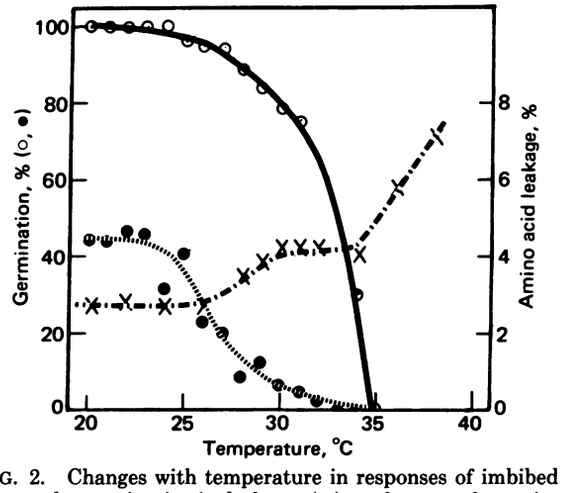

FIG. 2. Changes with temperature in responses of imbibed *L. sativa* seeds: germination in darkness (●) or after transformation of phytochrome by red light (○) and leakage of amino acids in 4 hr after 17-hr imbibition at 20°C (×).

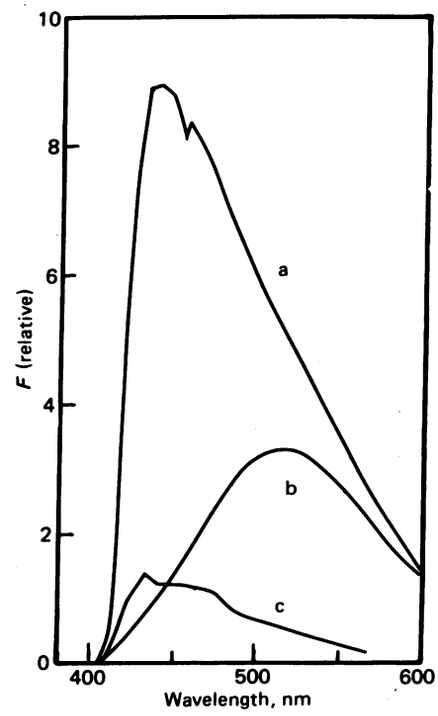

FIG. 3. Relative intensities of fluorescent radiation at various wavelengths excited by 360-nm radiation absorbed by a 100 μM MANS solution in the presence (a) or absence (b) of a membrane sample separated from *A. retroflexus* seeds. Scattering of extraneous radiation by the sample in the absence of MANS is also shown (c).

graphs are resolved into linear segments (Figs. 4 and 5) over at least 8°C intervals except for preparations from early watercress (curve c of Fig. 4).

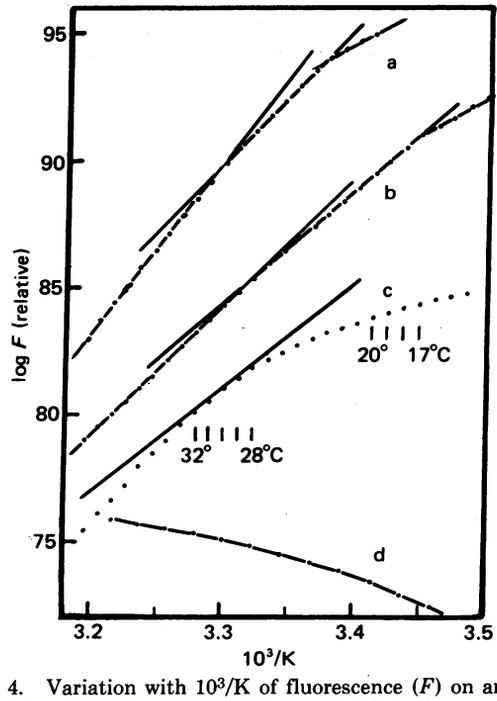

FIG. 4. Variation with $10^3/K$ of fluorescence (F) on arbitrary logarithmic ordinate scales of 100 μM MANS in the presence of resuspended membrane samples separated at >3000 × g and <25,000 × g: (a) *A. retroflexus*, (b) *L. sativa*, and (c) *B. verna*. Curve d is for log F against $10^3/K$ given by 100 μM MANS in the absence of membrane material.

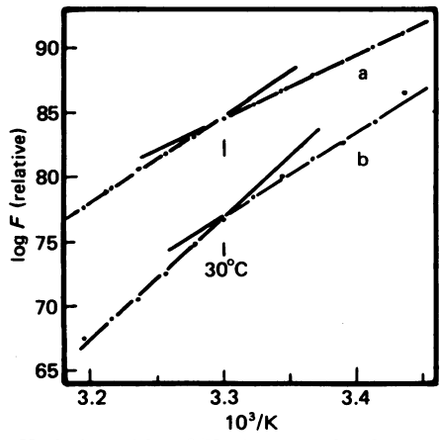

FIG. 5. Variation with 10^3/K of scattered radiations from suspensions of membrane materials of *A. retroflexus* (a) and *B. verna* (b) seeds in the absence of a fluorescent probe.

DISCUSSION

Several physiological responses of the seeds are associated with transitions in one or more of their cellular or organelle membranes. This is clearly the case for leakage of endogenous amino acids through the plasma membrane. The highest rates of change of this leakage with temperature are at 30–32°C not only for early wintercress seeds (Fig. 1) but also for five other species (2). Seeds of two previously studied species, *Amaranthus albus* and *Abutilon theophrasti*, did not show the enhanced rate of leakage (2).

Germination percentages of early wintercress seeds in darkness (Fig. 6) are instructive. The values at constant temperatures in the 20–35°C region reach a sharp maximum at 29°C. Factors favorable to germination apparently increase at constant temperatures above 20°C but encounter a limiting factor that becomes dominant if the seeds are held above 29°C for long periods. The favorable factors, however, continue to increase if the temperature is raised above 29°C for short periods. Thus, a period of 64 min at 35–40°C before return to 20°C enhances the germination from 7 to 48% (Fig. 6). Similar results have been reported for lettuce seeds (4, 5). Loss of endogenous constitutents or leakage from organelles to the cytoplasm could be one of the factors limiting germination at constant temperatures above 29°C.

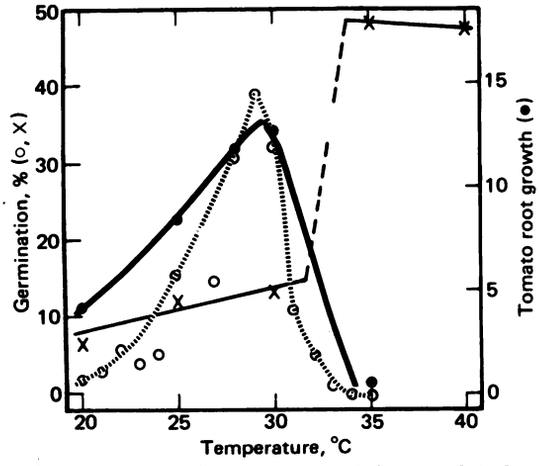

FIG. 6. Variation with temperature of the growth in length in units of 2.5 mm per culture per day of tomato roots in axenic culture (●) after White (7). This growth is compared with germination of *B. verna* seeds in darkness (○) and at 20°C after shifts of 64 min to indicated temperatures (×).

Germinations of six of the nine kinds of seeds considered here and in previous work (2) are similar to those of early wintercress. In several kinds of seeds, however, a deleterious effect of constant temperature is evident below 30°C. For lettuce seeds in darkness, as well as after phytochrome change, the effect is evident above 25°C. The leakage response is at least biphasic, with a second increase above 34°C. The two regions possibly involve transitions in more than one type of membrane—e.g., mitochondrial as well as plasma membranes.

The number of seeds showing a 28–32°C transition for both physiological and physical (fluorescent) responses is large enough to indicate some generalization. Seeds such as *Amaranthus retroflexus* that do not show amino acid leakage, or evidence in dark-germination of a transition, display a change in fluorescence near 30°C of the membrane probe system (Fig. 4). These seeds show a striking change at 30–32°C of germination under influence of the membrane-associated protein phytochrome in its far-red absorbing form (3). A further example of this type is evident in germination of spores of the fern *Onoclea sensibilis* Linneaus (6).

A physiological change of very different type with a transition at 29°C is shown by tomato root growth in axenic culture [reported in 1937 (7)]. The graph of relative growth against temperature is closely similar to that for germination of early wintercress seeds (Fig. 6). Similar displays are evident for both roots and shoots of maize seedlings growing in soil. Walker (8) followed these at 1°C intervals over the 12–35°C region. Pronounced decreases in the rates of growth were evident at 30–35°C. Plants at these temperatures developed straplike leaves that failed to unroll. This disorder was first evident at a temperature as low as 27°C. It is typical of calcium deficiency in maize, as Walker pointed out. The plants, however, were growing under high-calcium conditions. Calcium, as a required element for plant growth, primarily affects membrane organization and function (9). Disruption of the organization by high temperatures or low levels of calcium apparently leads to the same type of display with the disparate affecters.

The production of tulip, hyacinth, and other bulbs for growth and forcing was firmly established in The Netherlands in the decade of 1920–1930. A prominent factor in this industry was the control of the development of the flower buds in the bulbs by the use of appropriate temperature regimens (10). An example is the prevention of flowering in fully developed bulbs by holding them at temperatures above 30°C. Flowering after such a period can be attained simply by lowering the temperature. In this way bulbs produced and developed in The Netherlands could be distributed in both the northern and southern hemispheres.

Several published studies report findings similar to the ones under discussion (3, 5, 11). Chilling-sensitive mung bean seedlings were observed by Raison and Chapman (11) to have a marked change in rate of growth near 28°C. The succinate oxidase activity of mitochondria isolated from the seedlings also had a transition in the region of 28°C. The partitioning of an electron spin label probe molecule in a suspension of mitochondrial or chloroplast membrane fragments also had a transition region at 28°C.

Raison and Chapman (11) directed attention particularly to transitions in the region of 15°C in the mitochondrial and chloroplast materials from the mung bean seedlings. Membrane preparations from the several kinds of seeds used here also show transitions below 20°C. Consideration of these transitions is deferred, however, until the correlated physiological responses are better recognized and examined and better-specified membrane fractions can be prepared.

The naphthalenesulfonate probe molecules are considered to associate with proteins (12), including the protein components of membranes. The association leads to the enhancement of the fluorescence and the shift in the wavelength of the fluorescence maximum (Fig. 3). The transition in the membrane(s), however, displayed by the change in F against temperature is considered to arise chiefly from the lipid moiety as shown by work with lipid vesicles. The purpose here was to examine correlations of fluorescent and physiological displays rather than details of the molecular processes involved.

The several findings indicate that membrane transitions can be a primary cause for changes in growth and other physiological responses of plants in the region of 30°C. While the transitions probably involve the degree of order of components in the membrane lipids, detailed knowledge of such a change in order is meager (12), particularly for lipids in multicomponent membranes in contrast to lipid/H_2O micelles. The transition observed here, however, is in keeping with increase in some specific type of order within the lipid as it is cooled (13). The ordering process on cooling continues as the temperature is decreased below its onset at 30°C to <20°C. This temperature region of changing organization of lipids is the one most favorable for seed or spore germination, as well as growth, flowering, and several other aspects of development of many plants.

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