AN ABSTRACT OF THESIS OF

_____Shun Luo_____for the degree of ___Master of Science___

___in Crop Science___presented on _____MAY. 28, 1987_____

Title: THE EFFECT OF TEMPERATURE ON BIOCHEMICAL ACTIVITIES OF EMBRYO

AND SEEDLING OF GERMINATING RICE (Oryza Sativa L.) SEED

Abstract approved: _____Redacted for Privacy___

Dr. Te May Ching

Some biochemical activities in embryo or seedling of

germinating rice (Oryza Sativa L.) seed were studied to explain the

effects of suboptimum (20 C), optimum (30 C), and supraoptimum (40 C)

temperatures on seed germination and seedling growth. It was observed

that 40 C slightly speeded up seed germination but severely inhibited

the growth of young seedlings, 30 C delayed germination compared to 40

C, but resulted in the highest seedling growth rate, and 20 C delayed

seed germination the most and reduced seedling growth rate to the

lowest. Biochemical activities, DNA, RNA and protein synthesis were

traced by the incorporation of H-thymidine, H-uridine and

S-methionine, respectively, and metabolic activities as quantified by

enzymatic activities were determined at 16, 40, and 64 hours of

germination.

DNA synthesis was low in imbibed seeds but inversely

proportional to temperature; then increased two-fold in growing

seedling at the optimal temperature, slightly increased at 40 and

declined at 20 C at later stages. The synthesis of RNA was 2- to

declined at 20 C at later stages. The synthesis of RNA was 2- to 3-fold higher at 30 C than 40 and 20 C, at the three sampling times. Protein synthesis increased with germination time, but highest rate was observed in growing seedlings at 30 C reaching 5-fold of that at 40 and 20 C at 64 hours of germination.

Acid phosphatase activity was higher in embryos grown at 30 and 40 C in imbibed seed, then a highest activity was found in growing seedlings at 30 C. The activity of sucrose synthetase increased with germination time, the highest at 30 C, lowest at 20 C, and intermediate at 40 C. The activity of glutamine synthetase was proportional to the temperature in imbibed embryos, then the seedling grown at 40 C exhibited lowest activity, highest at 30 C and intermediate at 20 C.

The known optimal temperature resulted in a timely germination of the rice seeds and a highest growth rate of the germinated seedlings. This normal pattern of germination and growth may be attributed to a coordinated synthetic ability of a suitable amount of DNA and adequate genetically specified kinds and quantity of RNA and proteins through a long term's adaptation of rice seeds at the tropical or subtropical environment.

The Effect of Temperature on Biochemical Activities of
Embryo and Seedling of
Germinating Rice (<u>Oryza Sativa</u> L.) Seed

by

Shun Luo

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed May. 28, 1987
Commencement June 1988

APPROVED:

Redacted for Privacy

Professor of Seed Physiology in charge of major

Redacted for Privacy

Professor of Biochemistry and biophysics in charge of minor

Redacted for Privacy

Head of Department of Crop Science

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Dean of Graduate School

Date thesis presented _____ May. 28, 1987 _____

Type by Shun Luo for _____ Shun Luo _____

ACKNOWLEGEMENT

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INTRODUCTION

The regulatory effect of temperature on seed germination and

metabolic changes have been studied in crimson clover (Ching, 1975),

maize (Riley, 1981a, 1981b), and soybean (Seyedin et al., 1982,

Vertucci and Leopold, 1983). Although the specific regulatory

mechanisms of germination and subsequent embryo growth are not clear,

varied energy metabolism (Ching, 1975), different enzyme activities

(Ching, 1975), and different rates and kinds of protein synthesized

(Ching, 1975) were reported. The uptake of water (Vertucci and

Leopold, 1983), the mobilization of the reserves (Heydecker, 1977),

and the activation of synthetic systems (Payne, 1976) were affected by

different temperatures during the early stage of germination.

The optimal temperature for germination and seedling growth

vary among species, and it is known that neither suboptimal nor

supraoptimal temperatures are suitable for maximal seed germination

and seedling growth (Ching, 1975). The morphological changes caused

by the different temperatures have been attributed to the temperature

effect on the various enzyme activities (Ching, 1975) and synthetic

activities (Riley, 1981ab). During seed germination, the metabolic

activities are amphilibolic; that is both catabolic in the sense of

degrading reserve compounds to provide energy and raw materials for

early growth of the seedling, and anabolic in the sense of producing

machinery for protein synthesis and biogenesis of various organelles

needed for the catabolic activity as well as the true anabolic

synthesis of new cells and tissues (Ching, 1972). The anabolic and

catabolic activities are usually not coordinately altered at sub- or

supra-optimal temperatures (Ching, 1975). At the germination stage

(20-24 hours) of optimal temperature (20 C) for crimson clover, ATP

utilization was evident and the subsequent synthesis was rapid,

resulting in a highest EC and largest ATP pool size in the seedling

than that at the other temperatures (Ching, 1975).

Temperature does affect the activity of various enzymes, such

as protease (reserves catabolism), RNase (macromolecular degradation),

alpha-amylase (carbohydrate degradation), ATPase (ATP hydrolysis),

acid phosphatase (phosphorous metabolism), glutamine synthetase

(nucleic acids and amino acid biosynthesis) and fumarase (respiration)

in crimson clover (Ching, 1975). Temperature regulates the activities

of enzymes in many aspects. Some enzyme activities increased with

increasing temperature while others are resistant. Different enzyme

activities at various temperatures may be attributed to the kinetic

and catalytic properties of specific enzymes, i.e. 1) Keq, the

activation energy; 2) substrate-binding affinity, specific activation;

or 3) inactivation temperature of hydrophobic or hydrogen bonds, the

changes in patterns of isozymes etc (Weinder and Ziemens, 1975).

Among the weak interactions the higher order of protein structure,

hydrophobic bonds and hydrogen bonds are most essential. Hydrophobic

bonds are predominantly important for cold inactivation of enzyme

molecules because "free energy of denaturation" becomes negative at

low temperature. Hydrogen bonding, on the other hand, becomes weakened at high temperature (Tappel, 1966), for example, the free energy contribution to the stabilizing of native chymotrypsinogen from hydrophobic bonding increases as temperature increases up to 75 C, while below the 30 C, the free energy contribution is mainly from hydrogen bonding (Brandts, 1967).

The rate of specific protein synthesis and degradation varys at different temperatures which affect the activities of various emzymes by total quantity in a biological unit. It has been known that the replication of DNA, transcription of RNA, and translation of peptide, and the processing of these macromolecules are all influenced by temperatures that lead to different size and growth rate of young seedlings.

Temperature was reported to have an effect on seed radicle emergence different from that on seedling growth (Mathews, 1982). What is the mechanism of temperature variation affecting the radicle emergence and the seedling growth is not clear. This study was conducted to give a background on temperature effects on rice seed germination and seedling growth, and to quantify the specific changes in soluble metabolites, some essential metabolic enzyme activities, and the synthetic abilities of DNA, RNA and protein at suboptimal (20 C), optimal (30 C), and supraoptimal (40 C) temperatures. The experimental data may provide leads for further detailed studies on the mechanisms of temperature effects on seed germination and seedling growth.

LITERATURE   REVIEW

I: DEFINITION OF SEED GERMINATION AND SEEDLING GROWTH

The physiological concept of germination becomes the process
which starts with supply of water to air-dried seed and ends where the
growth of the seedling starts, most commonly by protrution of the
embryonic radicle through the seed coat. Essentially, germination
is the bringing of the embryonic axis into an active state which was
temporarily suspended during dessication and dormancy, and the
initiation of a specific genetic program of a new plant life cycle.

Growth is generally defined as an increase in cell number
and/or in cell content. Seedling growth, therefore, means a
continuous translocation of hydrolyzed reserves from storage site to
the germinated embryo. Through anabolic activities the germinant
enlarges and increases in cell numbers; upon further development and
differentiation, an autotrophic seedling emerges (Ching, 1972).

II: METABOLIC CHANGES IN GERMINATING CEREAL SEEDS

A) Water Uptake-----The rate of water imbibed by seed vary with the temperature. The diffusion speed of water molecules is related to temperatures (Vertucci and Leopold, 1983). The water uptake by seed has been studied using many crops, e.g. wheat, peas, rape and rice at early stage of germination (Allerup, 1958; Takahashi, 1984). The sensitivity of plant seed to temperature at early stage of germination depends on the genetic background, the specific maturation climate, and the environment during the imbibition. It's been known that imbibition proceeds more slowly at lower temperature and faster at optimal temperature. Temperature is also a factor which can break or intensify the seed dormancy after the maturation of seeds (Dalianis, 1980; Therios, 1982). The temperature effect on the imbibition of water is dependent on the molecular diffusion speed of the water molecule (Vertucci and Leopold, 1983).

B) Catabolic Degradation-----At the onset of germination, reserve starch stored in the endospermic tissues of the cereal seed, e.g., rice, barley, and wheat is mainly degraded by amylases, and the glucose produced is transported to the embryo and eventually metabolized either as a metabolic fuel for producing ATP and reducing power (NADH) or as carbon backbones of various structural macromolecules. The detailed route of glucose utilization was traced and it was found the glucose was converted to sucrose by sucrose synthetase in the scutellum of wheat, barley (Edelman et al., 1966), rice, then transported to growing embryo through vacuolar tissue in the scutellum. Immediately following the first phase of mobilization

in the scutellar and aleurone tissues, a second phase of activity

takes place in which the starchy endosperm reserves are mobilized.

This is mainly acheived by amylases which are de novo synthesized

in the aleurone layer and secreted into the starchy endosperm. Both

the synthesis and the secretion are induced by the gibberellin, and

both the dose response to the hormone and the time course of release

precisely parallel to those of alpha-amylase (Wyse, 1983).

C: Anabolic Synthesis----The protein and RNA synthesis occur

at a substantial rate at the earliest stage of embryo imbibition. The

importance of these processes to early embryo growth is uncertain.

The protein synthesis is the macromolecular process most closely

linked to early germination (Datta and Ketaki, 1983). The best

indication of in vivo protein synthesis can be obtained by following

the incorporation of radioactive amino acids into protein over a

defined and usually brief time period. Exposure of germinating

embryos to a number of different inhibitors of protein synthesis

prevents the normally occuring elongation of the embryonic axis

(Walton and Soofi, 1969). This obervation has led to the conclusion

that protein synthesis is required for axis (both root and shoot) cell

extension.

The recognition that a substantial increase in the rate of

protein synthesis occurs early in embryo germination, together with

the apparent obligatory role for protein synthesis in subsequent

embryo growth, prompted attempts to study the synthesis of new mRNAs.

Experiments with an mRNA synthesis inhibitor in which protein

synthesis (Waters and Dure, 1966) and axis elongation (Klein et al., 1971) were essentially unaffected suggested that the newly transcribed mRNAs were not obligatory to the early phases of germination and that the protein synthesis occuring during this period was probably catalyzed by long-lived, or stored mRNA, i.e. mRNAs transcribed during the embryogenesis. More recent experiments with embryos of both wheat and rye have indicated, however, that at least in these two systems, mRNA synthesis is occuring at a substantial rate in the earliest time period after onset of germination (Sen et al., 1975; Spiegel et al., 1975). It is concluded that the long-lived mRNAs, may insure the success of seed germination but they are not the exclusive message for early germination (Sanchaz-Martinez, et al. 1986)

III: TEMPERATURE EFFECTS ON MORPHOLOGICAL
AND METABOLIC CHANGES

A) Germination Characteristics-----under certain condition of temperature and the availability of oxygenand water, the quiescent seed imbibes water and commences active metabolism. Many of the necessary enzymes of intermediary metabolism are present in the quiescent seed (Mayer and Shain, 1974) and need only to be hydrated to resume activity. The metabolism of germinating seed is determined in many aspects: genetic background, such as the characteristic of dormancy, and the seed maturing environment. The existence for every

plant species of upper and lower temperature limits outside of which

germination of its seed can't take place, was first formulated earlier

than a century ago by Sachs in 1860 (Koller 1972), who was the first

to study the effects of temperatures on seed germination of several

cultivated plants. The term "optimum" temperature was used for

describing that intermediate temperature at which best germination was

obtained. With increased insight into the properties of enzymes and

the nature of enzymatic reactions, it became evident that any

physiological process in which an enzyme mediated step, such as the

replication, transcription, translation and processing of DNA, RNA,

and proteins, was an essential component, can take place only between

certain temperature limits. Its performance have been shown to

improve as temperature was increased from lower limit until a certain

temperature was reached above which any further increase in

temperature would result in reducing performance, reaching zero at the

upper limit.

B) Temperature Effects on Germination-----The growth of rice

seedling, coleoptile, mesocotyl, and radicle is influenced by light,

oxygen, moisture, nutrition and temperature (Takahashi, 1984). A lot

of studies have been carried out about the effect of temperature on

the growth of coleoptyle and mesocotyl.It is certain that the

variation of temperature alter the course of normal germination

process. A study of temperature effect on the germination of 22

Italian varieties of wheat over a wide range of temperature was

conducted (Macchia, 1986). The maximal germination within four and

half days was observed at 36 C, but the optimal temperature for

viability and germination is at 24-28.5 C. In cultivation, the most

desirable germination temperature is usually the one where viable

population achieves its highest germination (Harrington, 1962).

Whereas, at temperatures higher than this the seedling growth usually

becomes restricted, though the germination rate is higher. In fact, a

usual correlation may exist (Gulliver and Heydecker, 1973) between the

rise in germination rate and the restriction of seedling growth: high

temperature are likely to increase the rate of many componental

processes of germination; in seeds in which they can function and are

well co-ordinated at the higher temperature, the germination rate

increases; in young seedlings in which they have changed.

C) Temperature Effects on Energy Supply---Once the seed start

to imbibe, enzyme and organelle activity, and RNA and protein

synthesis are activated to exert their functions in the catabolic and

anabolic activities in relevant tissue of the seed. Further metabolic

activities have to be sustained to acheive germination and the young

seedling growth. Various metabolic aspects of germinating seed have

been studied extensively in the past hundred years.

The energy metabolism at different temperatures has been

studied in germinating crimson clover seed(Ching, 1975). Because the

biological energy in the form of ATP is limited in dry seed, de novo

synthesis of ATP commences very early in seed whenever it is hydrated.

The synthesis and the utilization of biological energy,

$$ATP \ + 1/2 \ ADP$$
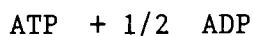
and the energy charge (EC = ----------------------) are

$$ATP \;+\; ADP \;+\; AMP$$

modulated by the temperature. At supraoptimal temperature of 30 C, germinating crimson clover seeds have a higher ATP content during first 24 hours not only due to higher ATP synthesis but also to lack of ATP utilization. At 30 C, an extremely rapid increase of energy charge (EC) resulted in a broad peak of 0.74 at two hours of germination; a peak of 0.88 at 6 hours, then a continuous decline to 0.6. This steady decline may limit endergonic enzyme activities and reduce biosynthesis. The germination percent was low at 30 C.

The change in EC of crimson clover seeds grown at 10 C reached a peak of 0.92 at 12 hours of germination and then declined to 0.75 at 24 hours. The high EC probably was due to the limited biosynthetic activity at the low temperature that reduced ATP utilization. Regardless of the higher EC after hydration in seeds grown at 10 C, germination was not observed at this temperature until much later at 48 hours.

At 20 C the EC increased rapidly to a peak of 0.87 at 6 hours of germination, the high level was maintained until 16 hours of germination, then a slight elevation was observed. A prominant decrease of EC was seen after 20 hours of germination at 20 C when the radicles of most seeds were emerging. Radicle emergence is an energy-requiring process (Ching 1982). Based on these observations, it is clear that energy metabolism, ATP pool size, and EC are not the modulators through which temperature exerts its effect on germination or growth.

D) Temperature effect on Synthetic Abilities--- The effect of temperature on the protein synthesis has been recently concentrated on the changes in the quantity and quality of the macromolecules (Luis et al., 1986). The kinds of peptide induced at 0 C in young rapeseed (Brassica napus) seedlings were different from those at optimal temperature. Some specific polypeptides were preferentially accumulated, several others are repressed while many are insensitive to the cold temperature. Only one enzyme, the small subunit of ribulose 1,6-bisphosphate carboxylase was identified as repressed by 0 C and others were not identified yet. Responding to the elevation of incubation temperature (18 C for 48 hr),rapeseeds changed the kinds of proteins being synthesized. General protein synthesis is repressed and a set of more complex small polypeptides is produced (at 18 C). These peptides are probably related to the thermostability (at 18 C) of the plant.

The inhibition of high temperature on maize germination (Riley, 1981a) was due to lack of polysome quantity from embryos imbibed for 3 days at 41 C indicating that the low rate of protein synthesis was due to the non-availability of active mRNA (Riley, 1981b). In another word, at supraoptimal temperature of 41 C a reduction in RNA transcription in a maize embryo resulted in low protein synthetic ability.

It is therefore clear that various temperatures not only changes the rate or quantity of the synthesis in macromolecules but also may alter the kinds or quality of the macromolecules to be synthesized. These alternations may result in abnormal growth or no growth of the germinating seedlings.

E) Temperature Effects on Catabolic Activities and Metabolites-----There is little information regarding the temperature effects on catabolic activities of seed reserves but the site and products of catabolic activities are known.

During the first day of germination, reserve protein in the scutellum are metabolized by proteinase and pepetidase which are present in the queiscent grain (Mikola and Kolehmainen, 1972) and which are presumably activated upon the hydration of the tissue. Part of the reserve in the aleurone layer is similarly hydrolyzed and the content of free amino acids in this tissue increase sharply during the first day of germination. Subsequent hydrolysis of protein bodies in endosperm provides a sustained supply of amino acids as substrates for nucleotides biosynthesis as well as for the synthesis of new proteins in the growing seedlings.

During the early stage of Douglas fir seed germination, the increase in soluble sugar was found in the embryo, while at later stages, a rapid increase was observed in the seedling (Ching, 1966). These sequential changes agreed with the finding in angiosperm and verified that the lipid reserves were transferred into forms of sugars

during Douglas fir seed germination. In the germinating cereal seeds, the starch reserves are catabolyzed in the endosperm into soluble sugars to provide the growing needs of the embryo.

IV: TEMPERATURE EFFECTS ON ENZYME ACTIVITIES

The enzyme activity is a fundamental cellular event intimately involved in the process of germination and the proper metabolic activity for the subsequent seedling growth. Enzyme activity is known to change in response to the environmental shift, i.e. temperature alternation. Among the many enzymes participating in rice seed germination and seedling growth processes, acid phosphatase, glutamine synthetase, sucrose synthetase were selected to estimate the temperature effects on phosphate metabolism, purine biosynthesis and the supply of carbohydrates from the endosperm, respectively.

Acid Phosphatase (E.C. 3.1.3.2)----Non-specific acid phosphatase activity is widely distributed throughout the living world. The major degradative acid phosphatase occurs in the lysosome of animal tissues, and in vacuole of plant tissues. Acid phosphatase catalyzes the hydrolysis of many phosphate esters to release the phosphate (Pi) and other moieties for various needs in synthesis and metabolic regulation. The isozyme pattern of acid phosphatase in germinating crimson clover is different when grown at 10, 20, and 30 C though their functions were not specified (Ching, 1975).

Glutamine Synthetase (L-glutamate:ammonia

ligase(ADP) EC 6.3.1.2.)-----Glutamine synthetase (GS)

plays a key role in the assimilation and control of nitrogen

metabolism (Stewart and Rhodes 1977). GS is responsible for

converting catabolically hydrolyzed reserve protein amino acids to

some required kinds for new protein synthesis for cell expansion and

cell division during seed germination and providing the substrate for

purine biosynthesis. The major transporting form of amino acids from

the storage sites into and throughout the growing seedlings are the

amides, i.e. asparagine (Asn) and glutamine (Gln). Hence, the amino

acids liberated from storage proteins must be further metabolized,

included the conversion of amino nitrogen to amido nitrogen by Asn

synthetase and GS. The enzyme has been characterized in pea, barley,

alfalfa (Francisco, 1986), and rice (McNally and Hirel, 1983). GS has

been observed to have two isoforms localized in chloroplast and

cytosol. The chloroplast activity is responsible for primary nitrogen

assimilation while that in cytosol plays a role in the re-assimilation

of ammonia released by the photorespiratory nitrogen cycle (Keys et

al. 1978). In higher plants, GS catalyzes the following reaction:

$$\text{L-glutamate} + \text{ATP} + \text{NH4}^{+} \xrightarrow[\text{Mn}^{++}]{\text{Mg}^{++}} \text{L-glutamine} + \text{ADP} + \text{Pi}$$
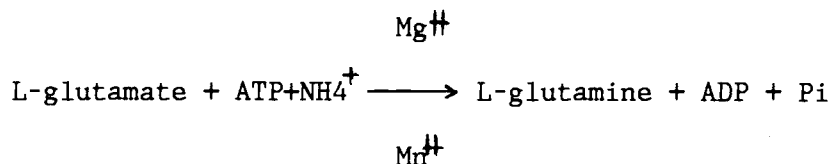
The resulting glutamine is a fundamental metabolite acting as an amine

donor during the synthesis of most amino acids and nucleotides

(Stadtman,1973).

Sucrose synthetase (EC 2.4.1.13)-----Sucrose Synthetase (SS)
catalyzes the following reaction:

$$SS$$

UDP-glucose + Fructose $\rightleftharpoons$ Sucrose + UDP

(ADP)   (ADP)
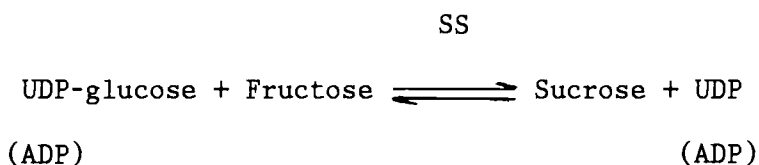
The activity of this enzyme is interesting because of its highly
reversible nature (Keq = 1.6). The enzyme is widespread in plants and
its activity is found in seeds, stem, tubes, roots and leaves, but it
is most important in the non-green tissues (Pontis, 1977). The
products of starch degradation, mainly glucose is converted to
sucrose and is transported into the root and shoot of developing
seedling. A very small amount of G-1-P formed by phosphorylase from
starch can also be used indirectly to form sucrose by SS. The enzyme
is specific for fructose and sucrose but interestingly enough lacks
specificity for nucleotide sugar. Sucrose synthetase is active with
adenosine diphosphate glucose (ADP-glucose) as well as UDP-glucose,
less active with thymidine diphosphate glucose and only slightly
active with guanidine diphosphate-glucose in the synthesis of
sucrose. The factors controlling the relative degree of synthesis or
hydrolysis of sucrose by SS are not well understood. However, Mg^{++} and
other divalent cations (Mn^{++}, Ca^{++}, Ba^{++}) stimulate the reaction in the
direction of sucrose synthesis and inhibit the reverse reaction.
Sucrose synthetase is inhibited by UDP and ADP (Fekete, 1964) and
sucrose cleavage is inhibited by fructose (Wolosiuk, and Pontis.
1974).

MATERIALS AND METHODS

I: MATERIALS

1985 crop of rice, <u>Oryza Sativa</u> L. var. S-201, was used for this study. S-201 came from the University of California, Davis, one of the leading varieties in California. Seed were screened twice using number 6*1/2 and 8*1/2 sceens, and blown once by a rice blower to get rid of lighter and inert components. The average 100-seed weight was 2.83±0.83 grams and the moisture content of the seeds are 14.1 %. Four replications of 5 grams of seeds were germinated at constant 20, 30, and 40 C with 16 hours per day light at 100 uE $m^{-2}s^{-1}$ for three to seven days in 12x12 cm^{-2} plastic boxes with 2 layers of filter paper and 10 ml of distilled water. Additional water was added whenever needed.

II: METHODS

A) MOISTURE CONTENT

The fresh weight and dry weight of seeds or seedlings were determined by oven-drying four replications of 10 seed each at 105 C for 24 hr at designated times of germination. The percentage of moisture content of seeds or seedling were calculated as:

moisture content(%) $=$

(fresh weight-dry weight)/fresh weight X 100

B) SEED GERMINATION AND SEEDLING GROWTH

After the onset of imbibition at different temperatures, the germination percentage was recorded at intervals of eight hours. Seeds with embryo radicle extended 2 mm in length were considered as germinated. The shoot length of 4 replications of 10 seedlings for each treatment was measured every day after radicle emergence. The mean and standard deviation was calculated for each sample.

C) SYNTHESIS OF PROTEIN, RNA, AND DNA

1) Protein Synthesis----Four replications for each sampling at 16, 40 and 64 hours of germination were studied. Twenty, sixteen, or ten embryos or seedlings of each replication from three germination stages were incubated with 1 uCi of ^{35}S-methionine (specific activity 1120 mCi mM^{-1}) in phosphate buffer (pH 6.5, 0.02 M) at each temperature for two hours. Embryos or seedlings were washed 4 times using cold phosphate buffer and surface-dried, then ground in 5 ml 10% TCA with pestle and mortar on ice, followed by centrifuging the slurry at 30,000 xg for 20 minutes. Discarded the supernatant, and washed the pellet twice with 10% TCA. The washing was discard after each centrifugation. The residue was hydrolyzed in 1 ml of 1N NaOH at 70 C for one hour, and the insoluble residue was removed by centrifugation at 10,000 xg for 10 minutes. Two aliquot (25 ul) of the hydrolysate were counted by a liquid scintillation counter (Beckman modal LS-7000) in a counting cocktail (Biofluor from Du Pont), and another two aliquots (100 ul) were analyzed for amino acid content by a modified

Moor-Stein method using leucine as the standard (Ching, 1966).

2) Nucleic Acids Synthesis----Twenty, sixteen or ten embryos or seedlings of the three germination stages were incubated with 1 uCi ^{3}H-thymidine (specific activity 2.0 Ci mM^{-1}) or ^{3}H-Uridine (specific activity 47.1 Ci mM^{-1}) in phosphphate buffer (pH 6.5, 0.01 M) for DNA or RNA synthesis, respectively, for 2 hours at the three temperatures. After incubation, embryos or seedlings were washed 4 times with the cold phosphate buffer and surface-dried. The washed embryos or seedlings were homogenized in 5 ml of 0.25 N perchloric acid (PCA) and centrifuged at 30,000 xg for 10 minutes. The supernatant containing nucleotides was discarded and the PCA pellet was washed twice with 5 ml 0.25 N PCA each. The pellet from H-uridine incubated embryos or seedlings was hydrolyzed in 1 ml 0.3 N KOH at 37 C with shaking. The hydrolysate was cooled on ice and 0.6 ml 1.1 M PCA was added to the hydrolysate. After 10 minutes, the mixture was centrifuged at 10,000 xg for 10 minutes and the supernatant was collected as the RNA hydrolysate.

The PCA pellet from ^{3}H-Thymidine incubated materials was hydrolyzed in 1 ml 0.5 N PCA at 70 C for 20 minutes; the reaction mixture was centrifuged at 10,000 xg for 20 minutes; the supernatant collected as the DNA hydrolysate. Two aliquotes of 25 microliter DNA or RNA hydrolysate were counted by L.S.C.

D) ENZYME ASSAYS

The enzyme activities were determined at 3 stages, namely, 16, 40,and 64 hours after the onset of imbibition. A sample size of 20, 15, 10 embryos or seedling was used for the 3 stages, respectively. The embryos or seedlings were ground in a mortar with pestle to extract enzymes in 2 ml Tris-HCl buffer (100 mM, pH 7.5) containing 5 mM MgAc and 10 mM mercapto-ethanol (grinding buffer, GB), 25 mg of GB saturated PVPP (polyvinylpolypyrrolindone) and 25 mg XAD-4 Amberite non-ionic absorbant were added to each sample to remove polyphenols. The slurry was transfered to a polyethylene tube, and the mortar and pestle were washed twice with 1 then 2 ml of GB. The washings were combined with the original slurry, and the tube was centrifuged at 20,000 xg for 10 minutes. The supernatant was used to determine soluble protein content by the Coomassie blue method and for assays of different enzyme activities shown below. All the procedures were conducted at 0-4 C except as specially noted. All temperature and time treatmentswere replicated four times. They were compared by ANOVA.

1) Acid phosphatase-----Acid phosphatase hydrolyzes at phosphate ester bond under acidic condition (pH 4.0):

$$R-o-\overset{oH}{\underset{o}{p}}-oH + H2O \longrightarrow ROH + Pi$$
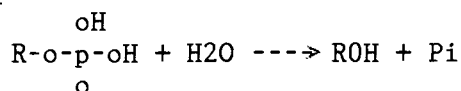
acid phosphatase was assyed by the hydrolysis of synthetic substrate, p-nitrophenyl-phosphate (pNpp), by incubating 100 ul of enzyme preparation in 0.9 ml of 0.1 M acetate buffer (pH 4.0) and 100 ul of 18 mM pNpp at 30 C for 10 minutes, and 1 ml of 0.5N NaOH was added to stop the enzyme reaction followed by adding 1 ml of water. The p-nitrophenol (pNp) produced was read at 400 nm against a reagent

blank (Ching, 1975). The results was calculated as total activity

(TA) and specific activity (SA) by following formula:

TA = n moles of pNp produced min embryo or seedling

$$= \frac{A400}{0.019^*} \times \frac{3.1}{10} \times \frac{0.25 \text{ or } 0.33 \text{ or } 0.5}{0.1}$$

* millimolar extinction coefficient of pNp is 19

SA = n moles of pNp min mg protein

$$= \frac{TA}{\text{mg soluble protein/embryo or seedling}}$$

2) Glutamine synthetase activities in the embryos or seedlings

was determined by the method of O'Neal and Joy (1973). The assay

reaction is:

$$\text{Glutamate + Hydroxylamine + ATP} \xrightarrow{Mg^{++}} \text{Glutamyl hydroxymate + ADP + Pi}$$
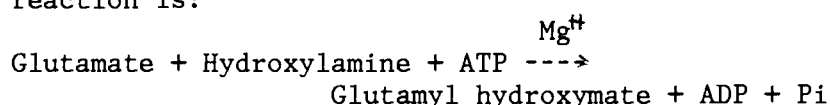
The reaction product, glutamyl hydroxymate, yields a characteristic

brown color with ferric chloride and was read spectrophotometrically

at 540 nm. An aliquot (0.4 ml) of enzyme preparation was added into

0.5 ml reaction mixture consisting of 0.1 M HEPES (pH 7.5), 0.04 M

MgAc, 0.16 M glutamic acid, 0.16 % mercaptoethanol, and with or

without 0.02 M ATP. The reaction mixture without ATP was the blank.

After preincubation of the reaction mixture at 37 C for 2 minutes,

0.1 ml of 0.2 M hydroxyamine HCl was added and the mixture was

incubated at 37 C for another 10 minutes. The reaction was stopped by

adding 1 ml of color reagent consisting of 0.18 M FeCl3, 0.67 M HCl

and 5% Trichloroacetic acid. The colored mixture was diluted by 1 ml

of H_2O, and centrifuged at 5,000 xg for 1 minute to remove any

turbidity. Finally, the amount of reaction product (glutamyl

hydroxymate) was read at 540 nm against the reaction mixture without added ATP. The total activity was defined as micromoles of product per minute per embryo or seedling. The results are calculated as following formula:

TA = uMoles glutamyl hydroxmate/min/embryo

$$= \frac{A540}{0.262*} \times \frac{1}{0.4} \times \frac{0.25 \text{ or } 0.33 \text{ or } 0.5}{10}$$

* absorbance of 1 uMole glutamyl hydroxymate in 3 ml reaction mixture at 540 nm

SA = uMoles glutamyl hydroxmate min mg protein

$$= \frac{TA}{\text{mg soluble protein/embryo or seedling}}$$

3) Sucrose Synthetase (Sucrose-ADP or UDP Glacosyl Transferase) (Wyse, 1983)----The enzyme preparation was dialyzed for 6 hours in the GB to remove soluble sugars in the preparation. An aliquote of the enzyme preparation (0.2 ml), 0.55 ml of reaction buffer consisting of 0.3 M MES buffer (pH 6.5), 0.02 M sucrose and 5mM ADP was incubated at 35 C for 5 and 15 minutes. After each time, 0.2 ml of the reaction mixture was taken and boiled in 0.2 ml of Nelsons reagent (Potasium-sodium tartrate, 6g; anhydrous Na_2CO, 12g; $CuSO4$, 2g; $NaHCO$, 8g; Na_2SO_4, 90g in 500 ml solution) for 10 minutes. Then 0.2 ml arsenomolybdate consisting of ammonia molybdate 25g, 21 ml 96% $H SO$, 3g $NaHAsO$ in 500 ml solution, was added to dissolve the precipitate from boiling. Finally, 2 ml of H2O was added, allowed to stand for a few minutes, and then read at 500 nm against a reagent

blank. The total and specific activities were calculated by following

formula:

TA = nMole sucrose formed min embryo or seedling

$$= \frac{A500}{0.0032^*} \ x \ \frac{0.75}{0.2} \ x \ \frac{0.25 \ or \ 0.33 \ or \ 0.5}{0.2 \ x \ 5 \ or \ 10}$$

* absorbance of 1 nMole sucrose in 2.4 ml

reaction mixture at 500 nm

SA = nMole sucrose formed min mg protein

$$= \frac{TA}{mg \ soluble \ protein/embryo \ or \ seedling}$$

E) DETERMINATION OF SOLUBLE SUGAR AND AMINO ACIDS

1) SOLUBLE SUGARS----The soluble sugar content of each sample

was determined by the Anthrone method (Yemne and Willis. 1954). For

each temperature, four replications of 20, 15, or 10 embryos and

seedlings at 16, 40, 64 hours after onset of imbibition respectively,

were ground in 5 ml of 85% ethanol, and centrifuged at 10,000 xg for

10 minutes. The supernatant was diluted 5 times by water; 0.2 ml of

the diluted extract was taken and 0.55 ml water, 0.25 ml anthrone (2%

in ethyl acetate) reagent, and 3 ml concentrated sulphuric acid was

added. The tube was swirled gently at first and then mixed

vigorously. The tube was heated in boiling water for 3 minutes,

cooled gradually in the air, and 20 minutes later, the assay mixture

were read against a reagent blank at 620 nm. The standard curve was

made by different concentrations of glucose solution for the

calculation of assay results by following formula:

Soluble sugar (ug/embryo or seedling)

$$== \frac{A620}{0.0139*} \times \frac{5 \times 5}{0.2 \times 20 \ (15, \ or \ 10)}$$

* absorbance of 1 ug glucose in 4 ml reaction mixture at 620 nm

2) FREE AMINO ACID----Free amino acids were extracted as for soluble sugars, except that the dilution was 20 times and 1 ml diluted extract was used for each assay by the modified Moor-Stein method (Ching, 1966). To 1 ml sample, 0.5 ml of buffer reagent consisting of 2.15 M of sodium acetate buffer (pH5.5), 0.0004 M potassium cyanide, and 53% (W/V) methyl cellosolve (2-methoxyl-ethanol), and 0.5 ml ninhydrine (3.75% in ethanol) was added. The reaction mixture was heated in boiling water for 20 minutes and then cooled. After 5 ml of 60% ethanol was added, the mixture was shaked for 5 minutes. The mixture was read against the reagent blank at 570 nm. A standard curve was made by different quantities of leucine and it was used to calculate the amino acid content by following formula:

$$amino \ acid \ (ug) == \frac{A570}{0.019*} \times 20 \ \frac{5}{20 \ (15, \ or \ 10)}$$

* absorbance of 1 ug leucine in 7 ml reaction mixture at 570 nm

RESULTS AND DISCUSSIONS

I. CHANGES IN WATER UPTAKE

The uptake of water by seed is an essential, initial step
toward germination. Takahashi (1961) reported that during the
germination process the water uptake of rice seeds is in a
physiological triphasical pattern. Similarly, in this study a
triphasic pattern of water uptake by germinating rice seeds at optimum
temperature of 30 C was observed within 7 days germination
(figure-1). The water uptake by the rice seeds at 20, 30, and 40 C
was proportional to the temperature until two and half days later in
germination, when seeds began to germinate at 30 C. The water content
in emerging seedling was about 40%, regardless of the germination
temperature. The time required to reach that water content was 96, 52
and 46 hours at 20, 30 and 40 C, respectively. The water uptake at
the early stage of germination is dependent upon many factors: a)
water availability in seed or germination medium; b) water potential
of seed matrix which is genetic controlled by seed chemical
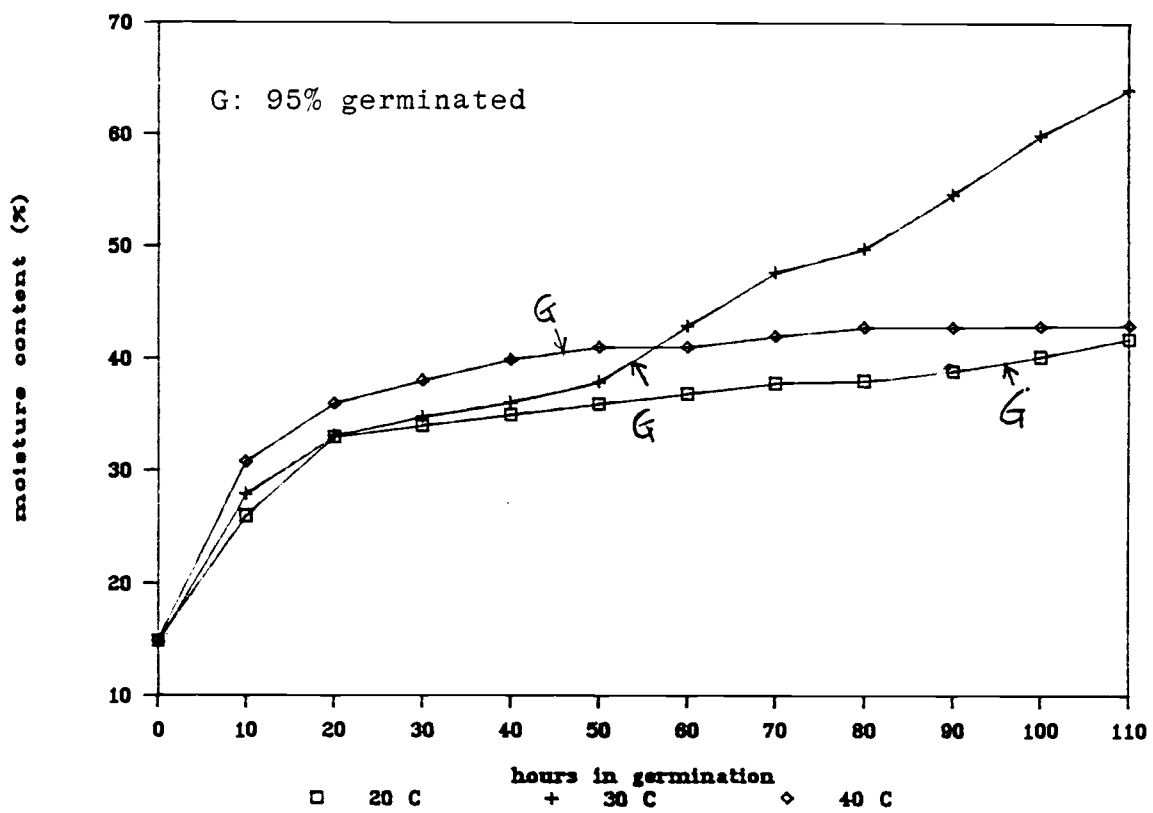composition and modulated by ripening environmental condition; and c)

Figure-1 Moisture content of rice seed germinated at 20, 30, and 40 C for 110 hours

the diffusion rate of water molecules that is mainly governed by temperature. In this study, water was applied equally to each germination box, the seed were grown and harvested at one location at the same time from a single certified cultivar. The observed variation in water uptake is the only effect resulting from the 3 temperatures. From the imbibition curve of rice seed in this study (figure-1), process of water uptake at 40 C within first two days as higher and faster than that at either 30 or 20 C. After emergence of radicles, the water uptake at 40 C was restricted because of the limitation of further seedling growth. The water uptake at 30 C reached a faster pace as seedling growth started after 52 hours of germination. Even though the water uptake was slow at 20 C, it gradually reached as high as that at 40 C at 64 hr. It appears that high temperatures affect the water uptake of rice seed during germination by increasing the diffusion rate (Vertucci and Leopold, 1983) of water molecule at the early stage. The optimal temperature promotes the coordinated developmental and metabolic events that stimulated further water uptake by the germinated embryos and the growing young seedlings (Ching, 1975).

II: CHANGES IN MORPHOLOGY AND GROWTH RATE

Temperature affects both the capacity for germination and the rate of germination (Bewley and Black, 1985). It is well known that the growth of coleoptile, mesocotyl, and radicle of rice seedling, is mainly influenced by light, moisture, temperature and nutrition (Takahashi, 1984). The temperature dependence of plant seed for germination is the result of evolutionary adaptation to the wide range of natural environments. The habitat of a specific plant variety usually is compatible to the varietal characteristics. However, in a laboratory, most favorable conditions for seed germination can be provided to gain faster and better germination and seedling growth.

The morphological changes during 7 days of germination at the three temperatures are shown in figure-2. The speed of emergence was proportional to the temperature as shown in figure-1 and figure-2 and the seedling shoot growth was highest at 30 C (figure-3). It is clearly shown in these figures that temperature affects rice seed germination process (radicle emergence) in a different manner from that of the seedling growth. The supraoptimal temperature of 40 C caused earlier radicle emergence or germination at 46 hr. The radicle emergence was observed 52 and 96 hours at 30 and 20 C, respectively. For germination to be completed, the radicle must expand and penetrate the surrounding medium to anchor the seedling. Over the years, there has been considerable debate whether this occurs solely by cell expansion, or if cell division is required too. It is now generally conceded that cell division is neither correlated with nor necessary for radicle expansion. In the absence of mitosis or increase in tissue's cell numbers, some cereal grains with chromosome breakages

20 C

30 C

40 C

1   2   3   4   5   6   7
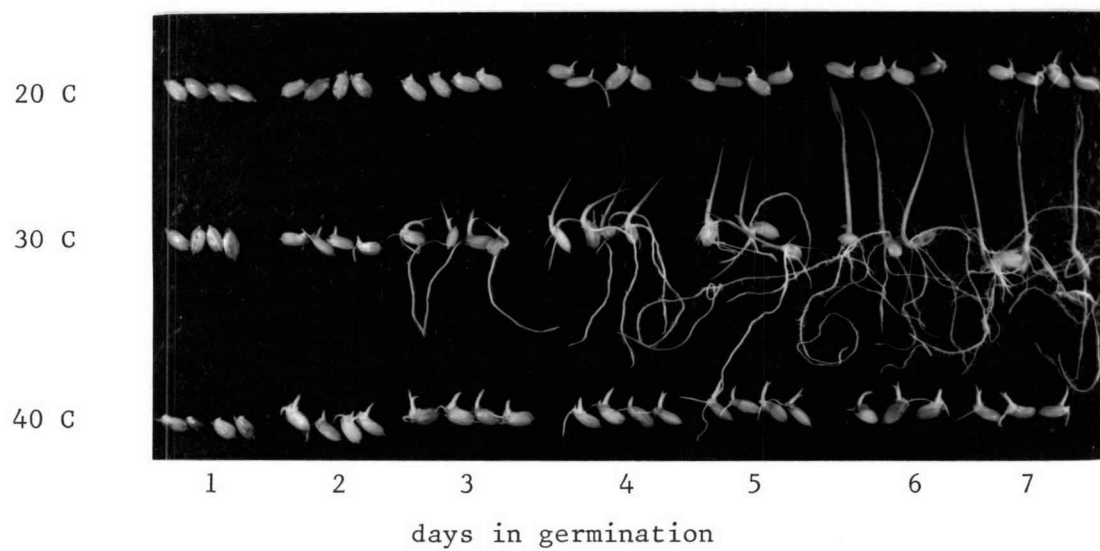
days in germination

Figure-2 Morphological changes in germinating rice seed at 20 C, 30 C, and 40 C for 7 days

are still able to germinate (Bewley and Black, 1985). Rogan and Simon (1975) concluded that extension of the radicle of Vicia faba seed occured at two phases. There is initially a slow phase up until about 48 hours by which time the root has pierced the testa in about 30% of the seeds. This is followed by a rapid phase in which there is an increase in the number of cells through mitosis in the apical region of the radicle. These two phases, the initial cell elongation and the subsequent radicle growth by increased cell number and size were shown to be different in sensitivity to the changes in environmental factors, such as water stress. This implies that the initiation of cell elongation is controlled in a different manner from later events of mitosis. The embryo or seedling growth rate at 30 C was faster and greater than those at 40 and 20 C. What specific events are essential for the radicle emergence to take place, and what internal signals are responsible for setting cell division and growth in motion? Further detailed and targeted investigations would be needed to answer this questions.

III: CHANGES IN SYNTHETIC ABILITY OF DNA, RNA AND Protein

A) DNA synthesis and seedling growth

In the germinating seeds, as soon as seed imbibition starts, the activation of preexisting systems, e.g. respiratory pathway, other enzyme and organellar activities, RNA and protein synthesis commences and the repair of macromolecular or organellar damages begins. The oxidation of sugar in the fully activated and repaired embryo provides substrates and energy for the extension of the radicle. The catabolic activities in storage tissue sustain the supply of substrates (sugar, fatty acids, amino acids, etc.), energy (ATP) and reductants (NADH or NADPH) for further biosynthetic processes in the germinated embryo. Finally the seedling emerges through a genetically directed, metabolically coordinated growth and developmental program (Ching, 1972).

The synthetic ability of DNA, RNA, and protein is of major importance in growth in terms of cell number and size. The DNA synthetic ability of embryo and seedling was traced by the ^{3}H-thymidine incorporation for two hours at different times after imbibition under different temperatures, and the results are summarized in figure-4. There was a low rate of DNA synthesis inversely proportional to temperatures at 16 hr of germination. This synthesis probably was for the repair of existing DNA at this early stage though the temperature effect was difficult to explain. After 40 hours of germination, the synthetic ability of DNA at 30 C was two-fold higher than that at 40 and 20 C. This higher synthetic ability coincides with the increased growth rate of seedlings (figure-3 and figure-4), indicating that they are synchronously occured events.
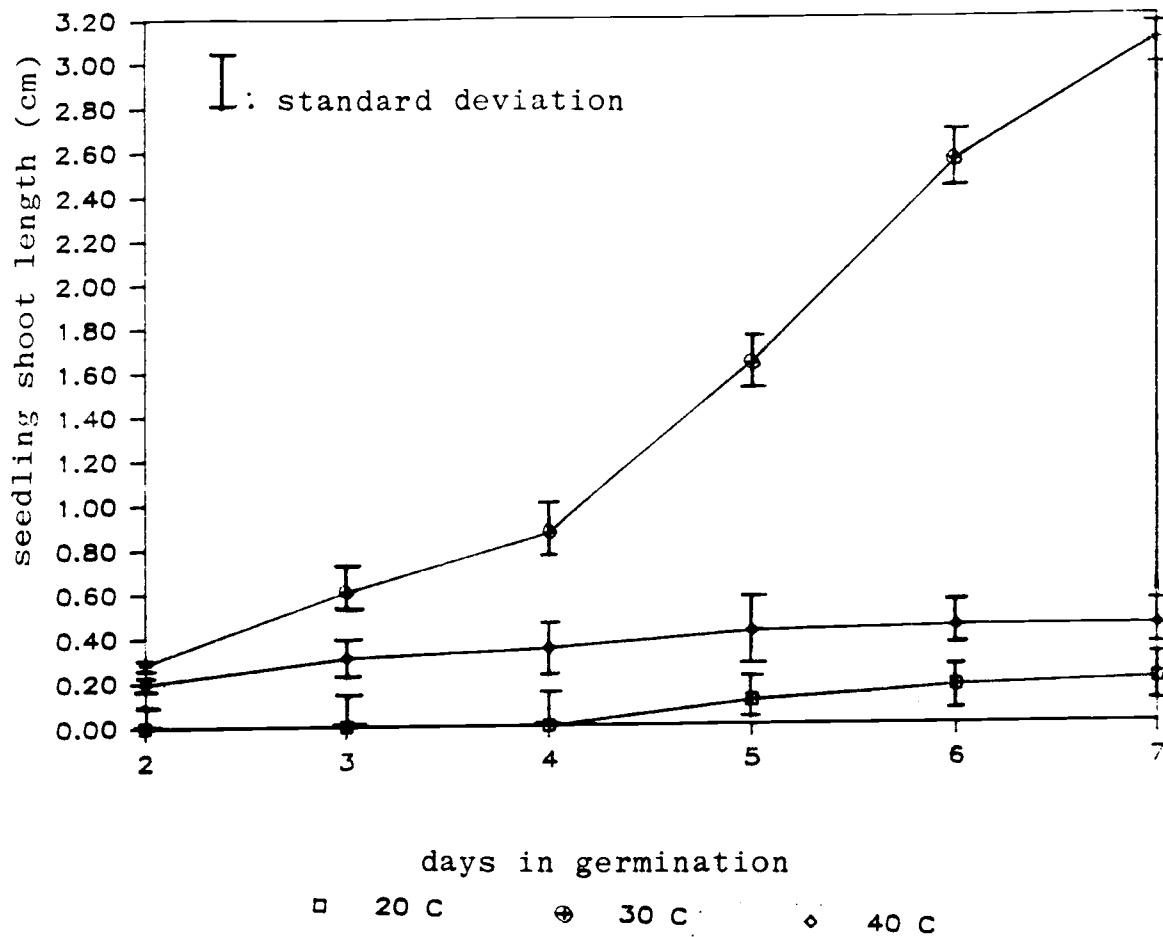
Figure-3 Growth curve of seedling shoot length (cm) in germinating rice seeds at different temperatures

0.65

0.6

0.55

0.5

0.45

0.4

0.35

0.3

0.25

$\mathrm{uMole}\,^3\mathrm{H-Thymidine\ incorporated/embryo}$

$\underline{\mathrm{I}}$: standard deviation

16   40   64

hours in germination
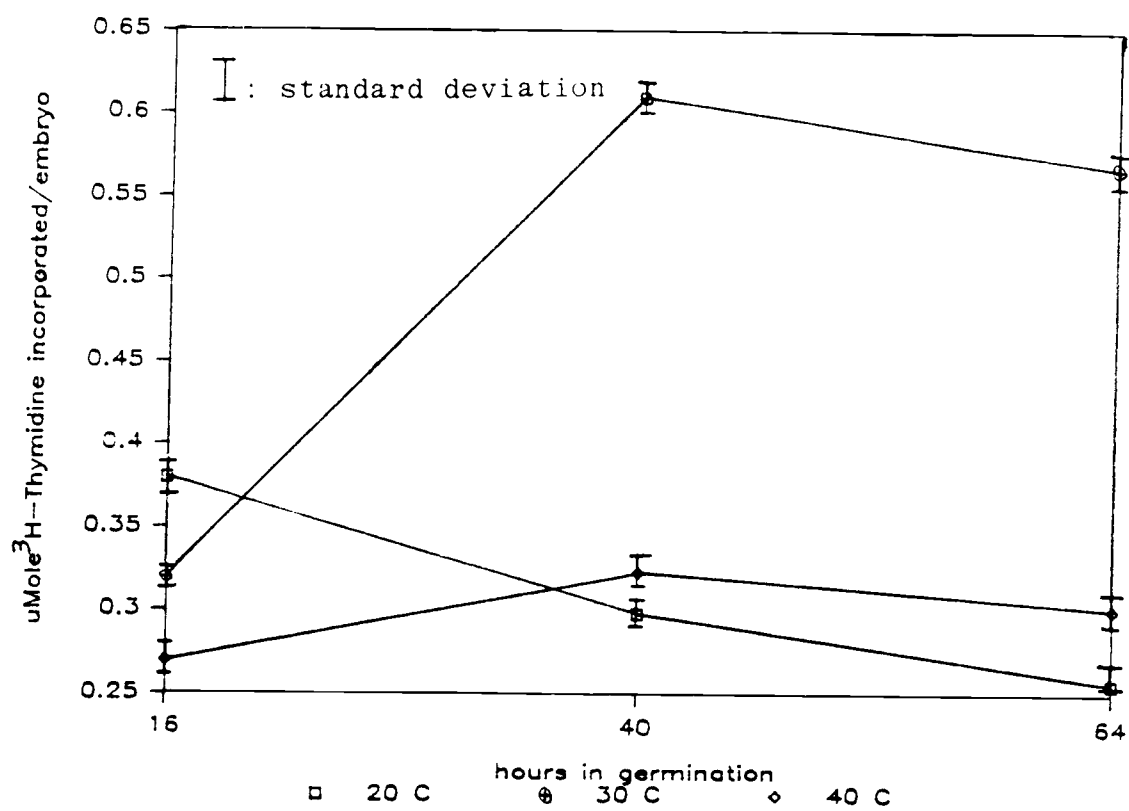
□   20 C   ⊕   30 C   ◇   40 C

Figure-4   DNA synthetic ability in the embryo or seedling of
germinating rice seed at 20, 30, and 40 C

DNA synthesis requires several enzymes, at least four common deoxyribonucleotides, nuclear RNA, energy, cofactors and the most important of all, the signal for genomic replication. Under sub- and supra-optimal temperatures, all above requirements may not be met, thus the synthetic rate was low and little growth was observed.

B) RNA and Protein Synthesis

RNA synthesis was traced by ^{3}H-uridine incorporated in embryos or young seedlings at 20, 30, and 40 C for two hours after 16, 40, and 64 hours germination. RNA synthetic ability as shown by the total incorporation of ^{3}H-uridine is summarized in figure-5. Higher synthesis of RNA at 30 C was observed at all times during the germination period. This indicates that the coordinated metabolic activities required for RNA synthesis are being met fully only at the optimum temperature.

There was no difference in RNA content at 40 C and 20 C through all the stages of germination (figure-6), probably because the sub- and supra-optimal temperature supressed or inhibited RNA synthesis due to lack of growth signal in embryos or seedlings. The total RNA content was the highest at 64 hr in the seedling grown at 30 C indicating the characteristics of a growing organ with an increasing number and size of cells.

The protein synthetic ability is summarized in figure-7. At 16 hr of germination, protein synthetic ability of imbibed embryos at 40 C and 30 C germination was higher than that at 20 C. At 40 hours
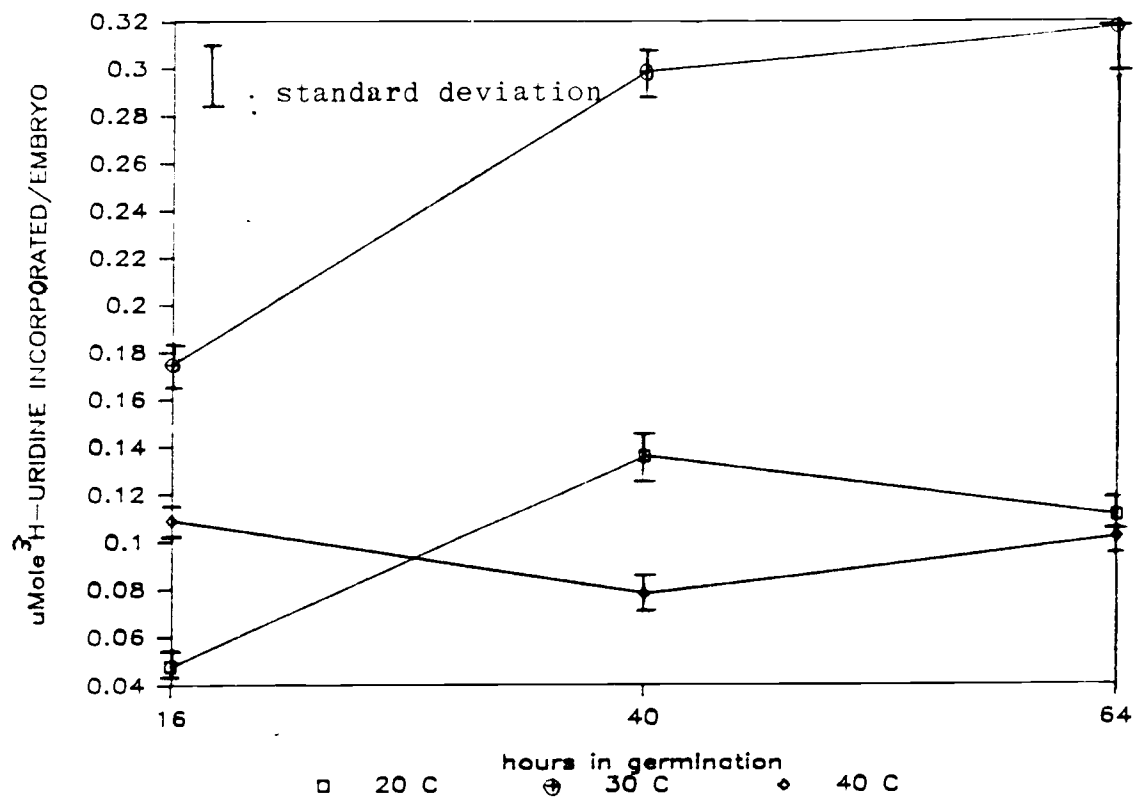
Figure-5   RNA synthetic ability in the embryo or seedling of
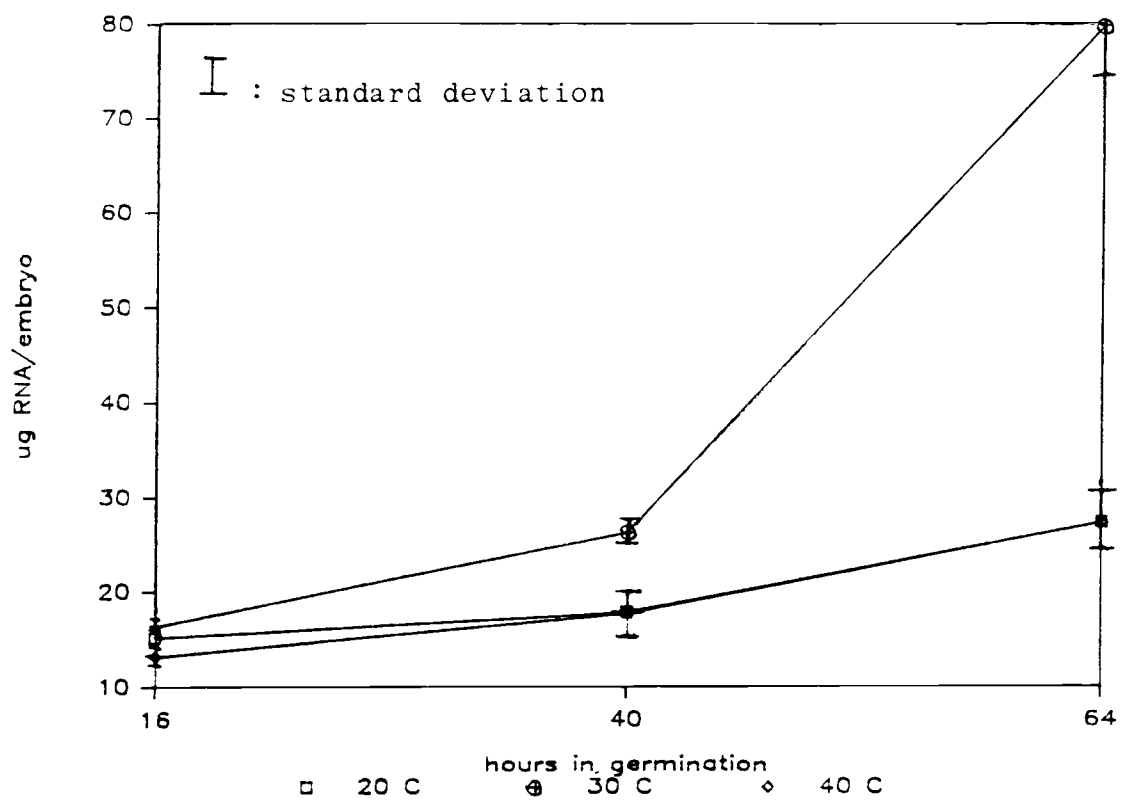germinating rice seed at 20, 30, and 40 C

Figure-6   RNA content in the embryo or seedling of germinating
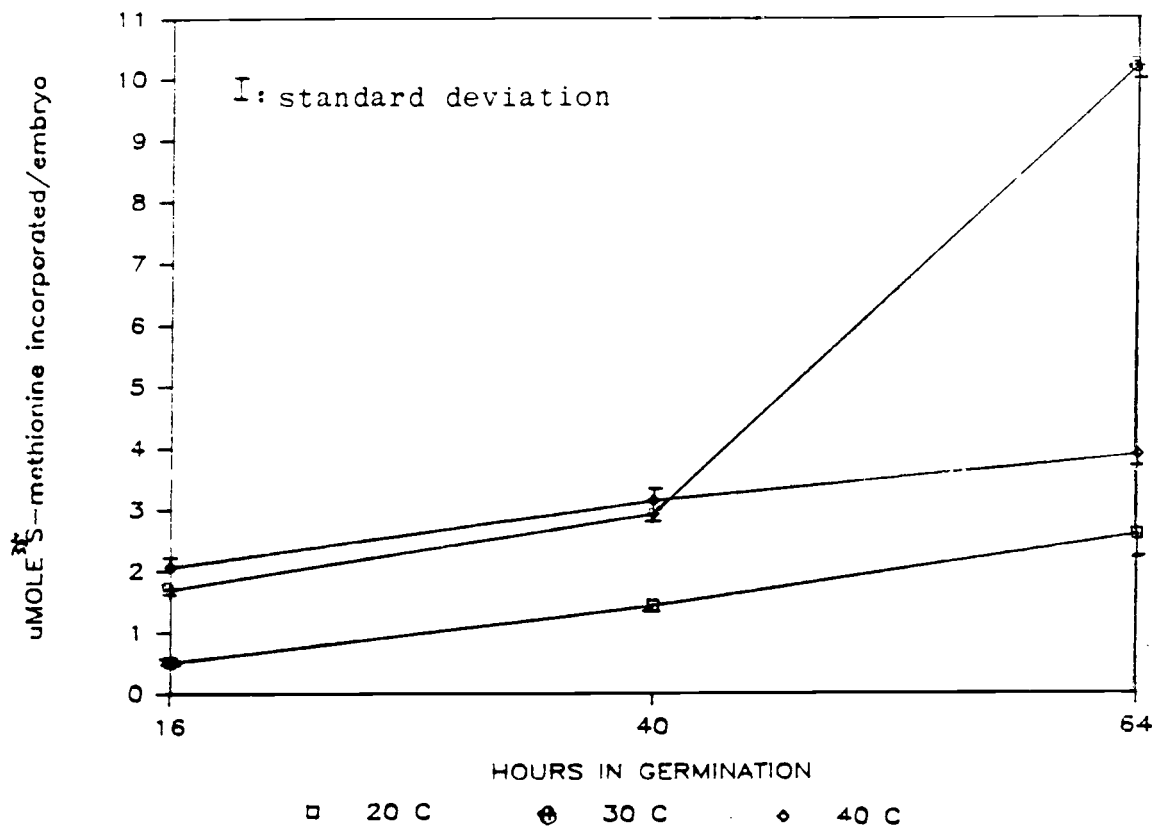rice seed at different temperatures

Figure-7 Protein synthetic ability in the embryo or seedling of germinating rice seed at different temperatures

of germination, there was no significant difference in protein

synthesis between 30 and 40 C, but they were higher than at 20 C. The

protein synthetic ability at 30 C increased to 3-fold at 64 hours

germination, whereas at 40 or 20 C the ability only elevated slightly

compared to earlier stages. It seems that rapidly stimulated protein

synthesis occured only at the optimal temperature in growing seedlings

regardless of the germination process.

The synthesis of structural, enzymatical, and regulatory

proteins is an essential event for increase in cell number and size

required for germination and for sustained seedling growth. At the

early stage, long-lived mRNA as well as newly synthesized mRNAs direct

the protein synthesis needed for early repairs and metabolic

activities. At supraoptimal temperature, not only is active mRNA

unavailable but abnormal kinds of mRNA would be synthesized resulting

in abnormal growth (Riley, 1981ab). Again the optimal temperature

elicits proper genetic expression of the species' adaptational

process. Apparently, no one single process, site, step, or enzyme

could be responsible for the composite response toward one

temperature.

IV: CHANGES IN ENZYMATIC ACTIVITIES

Temperature affects not only the synthesis of RNA and protein, but also affects many other metabolic activities, such as various enzyme pathways, organellar and membrane activities. For example, the activities of reserve degradational enzymes (alpha-amylase), respiratory enzyme (fumarase), and purine synthesis (glutamine synthetase) were all higher in crimson clover seed germinated at optimal temperature of 20 C than they were at 10 or 30 C (Ching, 1975). On the other hand, the activities of ribonuclease in 24 hours germinated crimson clover seeds was higher in imbibed seed germinating at sub- and supraoptimal temperatures than that at optimal temperature. In this experiment, the activity of acid phosphatase, glutamine synthetase and sucrose synthetase were studied.

A) Acid Phosphatase

Acid phosphatase activity is affected by temperature in various ways because the enzyme is non-specific or may be composed of many enzymes acting at various sites at different growth and development stages. Both the total and specific acid phosphatase activity changes with germination time and with different temperatures (figure-8 and figure-9). At 16 hours, the total acid phosphatase activity was not different among seeds grown at different temperatures. But, the specific activity of acid phosphatase was highest at 40 C; perhaps indicative of the participation of some degradational phosphatase at this high temperature. At 40 hours of germination, the total activity at 40 C was similar to that at 30 C
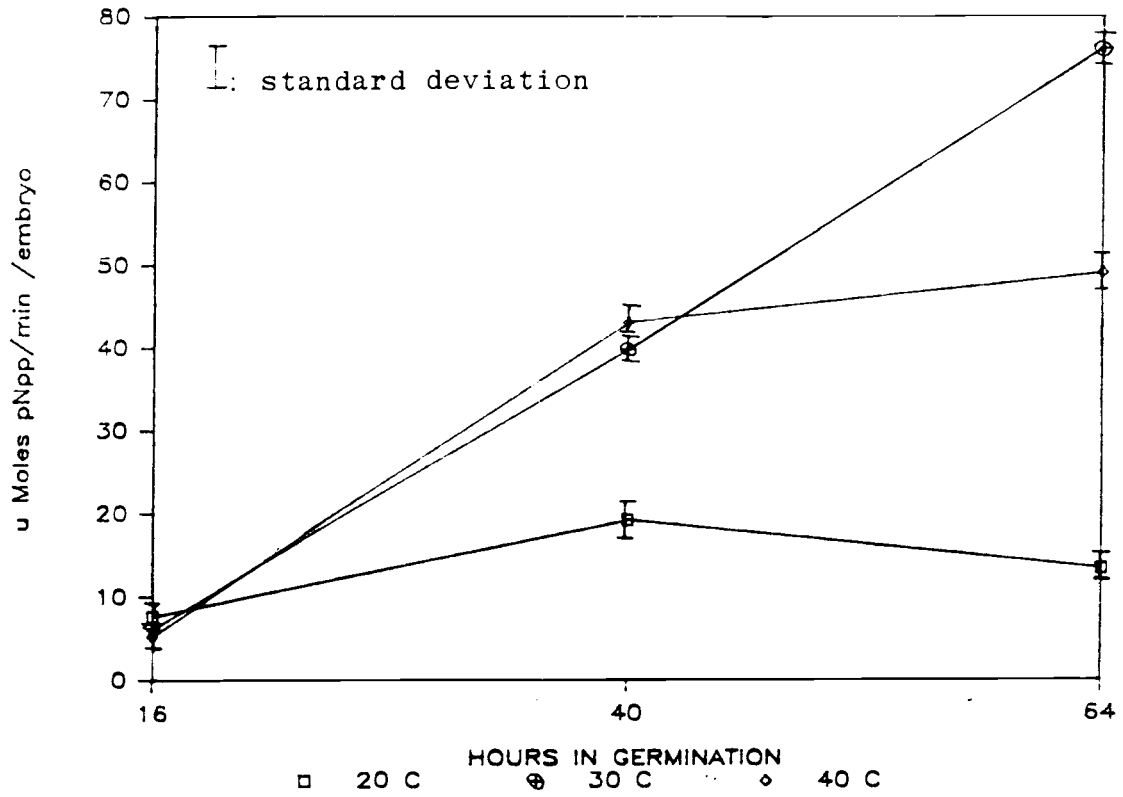
Figure-8   Total acid phosphatase activity in the embryo or seed-
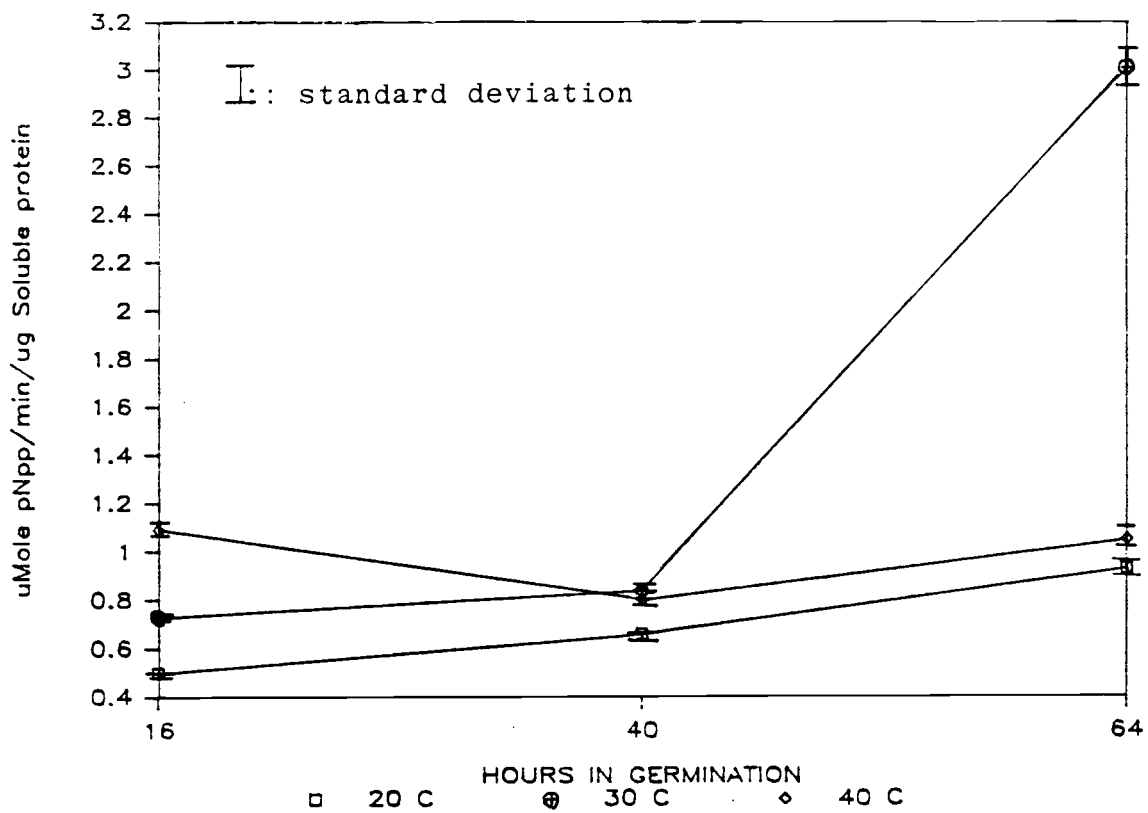ing of germinating rice seed at 20, 30, and 40 C

Figure-9 Specific activity of acid phosphatase in the embryo or
seedling of germinating rice seed at different temperature

and 2-fold higher than that at 20 C; the specific activity was similar at 3 temperatures. At 64 hr, even though the total acitivity at 40 C remained high, the activity increased linearly at 30 C and declined slightly at 20 C. Highest total and specific acid phosphatase activity was found in growing seedling at 64 hours at 30 C. Again high acid phosphatase activity was correlated with high growth rate of rice seedlings, and the highest growth rate was reached at the optimal temperature. The specific kinds of the enzyme, as well as their location and functions are unknown, and further research to obtain a better understanding on this group of enzymes would be needed to illucidate this function at various temperatures.

B) Sucrose Synthetase

The changes in sucrose synthetase activity during 64 hr of germination at different temperatures are shown in figure-10. At 16 hr of germination, the enzyme activity was low, less than 20 micromoles sucrose were cleaved or synthesized per minute per embryo. At all the sampling times, the total activity of sucrose synthetase in embryo or seedling was higher at 30 C than at 40 and 20 C. The total enzyme activity was parallely increased with germination time at all three temperatures. The specific activity of sucrose synthetase is shown in figure-11. The pattern was the reverse of that of total enzyme activities; specific activity was highest in 20 C grown embryos, medium in 40 C, and lowest at optimum, 30 C. Sucrose synthetase is a reversible catalyst. It is a major enzyme in the
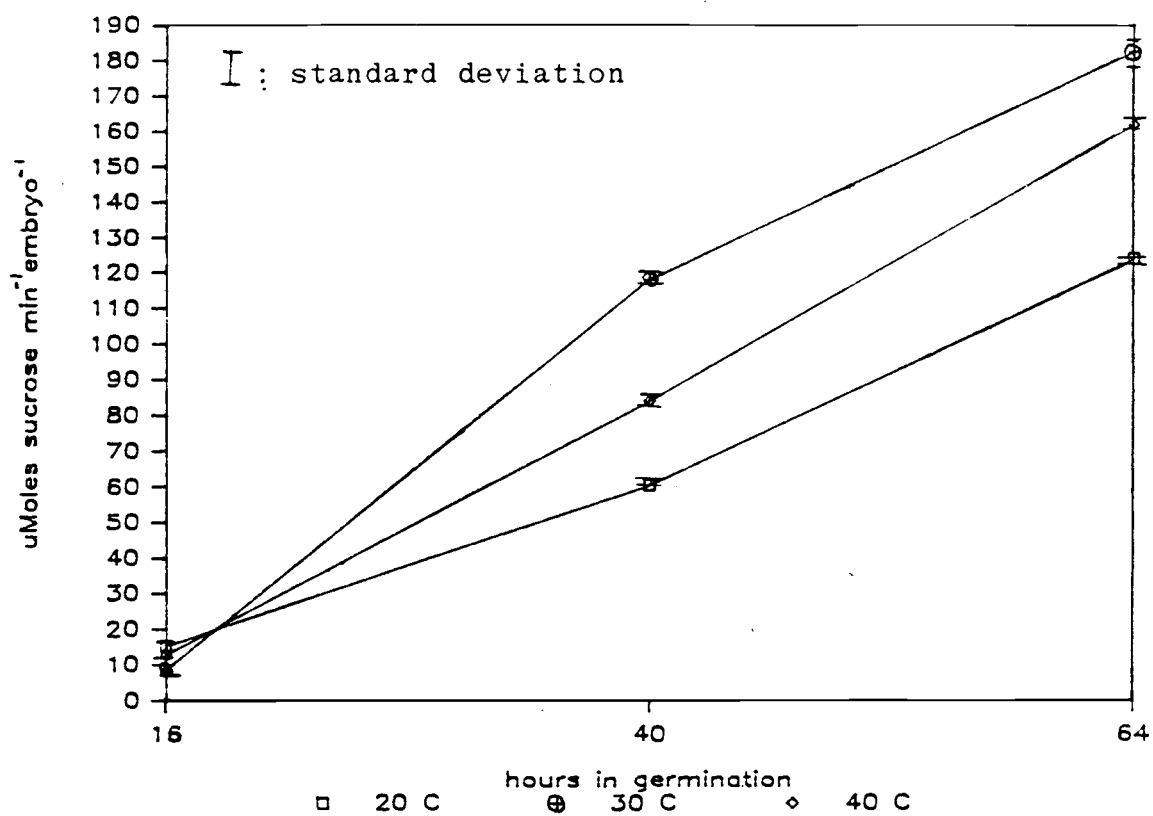
Figure-10 Total activity of sucrose synthtase in the embryo or seedling of germinating rice seed at different temperatures
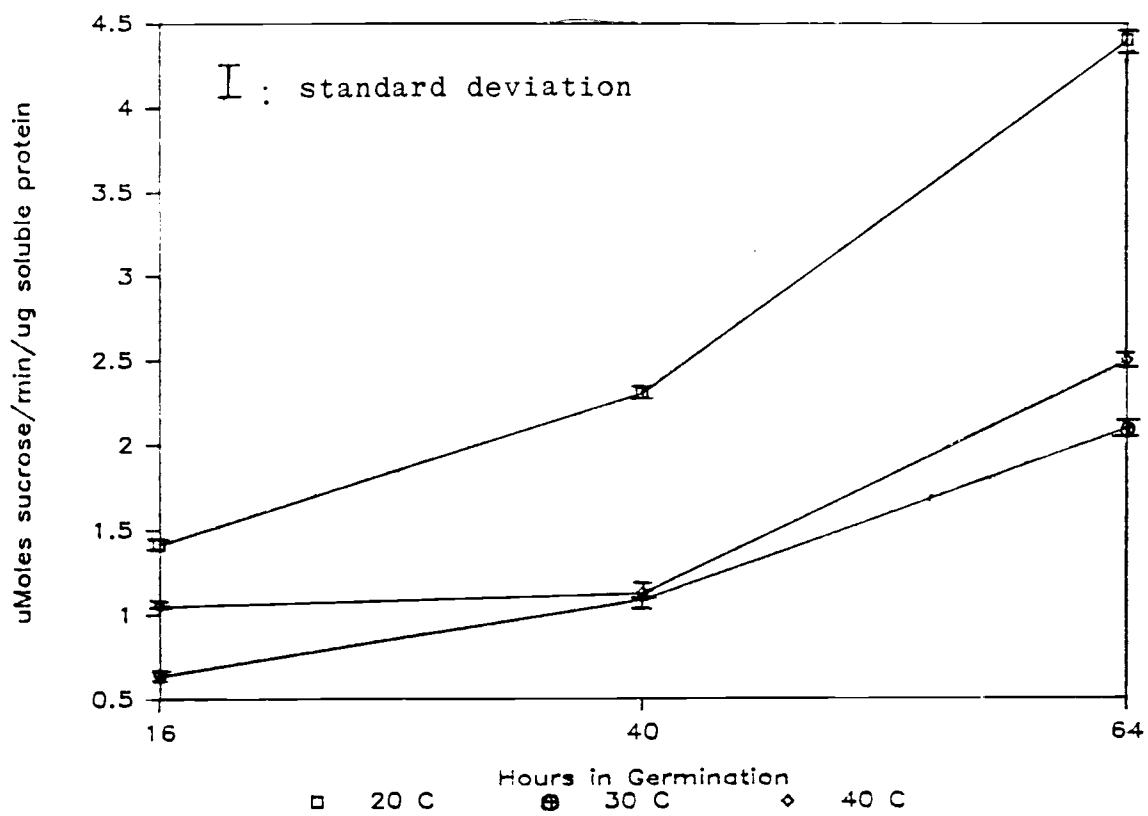
Figure-11 Specific activity of sucrose synthetase in the embryo or seedling of germinating rice seed at 20, 30, and 40 C

cereal grain, in converting the products of starch and cell wall hydrolysis, glucose and fructose respectively after one of which becomes ADP- or UDP-sugar, to sucrose. In rice seed the scutellum of the embryo is a special organ with vescular bundles for the transport the sucrose to radicle and shoot for synthetic and energy needs (Akazawa, 1976). The hydrolysis of starch and cell wall in the endosperm probably are proportional to temperature optimal curve of the growth and resulting in a proper temperature performance pattern of the total activity of SS, shown in figure-10. Therefore a highest total SS activity was observed in embryos or seedlings grown at 30 C, and lowest at 20 C.

The changes in the pattern of the specific activity changes at the three temperatures may be explained by the differences in embryos or seedling growth rate (figure-2 and -3). The embryo grown at 20 C was not germinated yet during the 64 hours of experimental period, or the cells were only hydrated without sufficient expansional growth or increased in soluble protein content. The SS appeared to be preexisting in scutellum, the influx of endosperm supply of substrates (glucose and fructose) probably stimulated the activity of SS, thus resulting in higher specific activity of SS. The same reasoning can be applied to embryos or seedlings grown at supra- and optimal temperatures.

C) Glutamine Synthetase

The changes in total activity of glutamine synthetase (GS) are shown in figure-12. At 16 and 40 hours of germination, the total enzyme activity was the highest in embryos grown at 40 C, followed by 30 C and then 20 C. At 64 hours of germination, the total enzyme activity in seedling grown at 30 C remained as high as before, but that in seedling grown at 40 C decreased rapidly to 50 % level, and embryos grown at 20 C kept a gradual increase and became higher than that of 40 C. The specific activity of the enzyme is shown in figure-13. The specific activity was the highest in the embryos grown at 40 C after 16 hours of germination. At 64 hours, in contrast, the specific activity of the enzyme at 40 C decreased to the lowest.

Glutamine synthetase is an important enzyme in N-assimilation during plant growth and seed development during utilizing N-fertilizers and biologically fixed nitrogen. During seed germination it resumes additional responsibilities in a) converting catabolically hydrolyzed reserve protein amino acids to required kinds for new protein synthesis for cell expansion and cell division in germination; b) providing substrate for purine biosynthesis. Purine is in great demand for the synthesis of vast amounts of adenine and guanidine nucleotides needed in the synthesis of RNA and DNA during cell expansion and division in germination and seedling growth. Changes in the activity pattern occured in the growth of rice embryo or seedling at these temperatures are difficult to explain. More precise and specific studies will be needed to explain its function and temperature regulation in vivo.
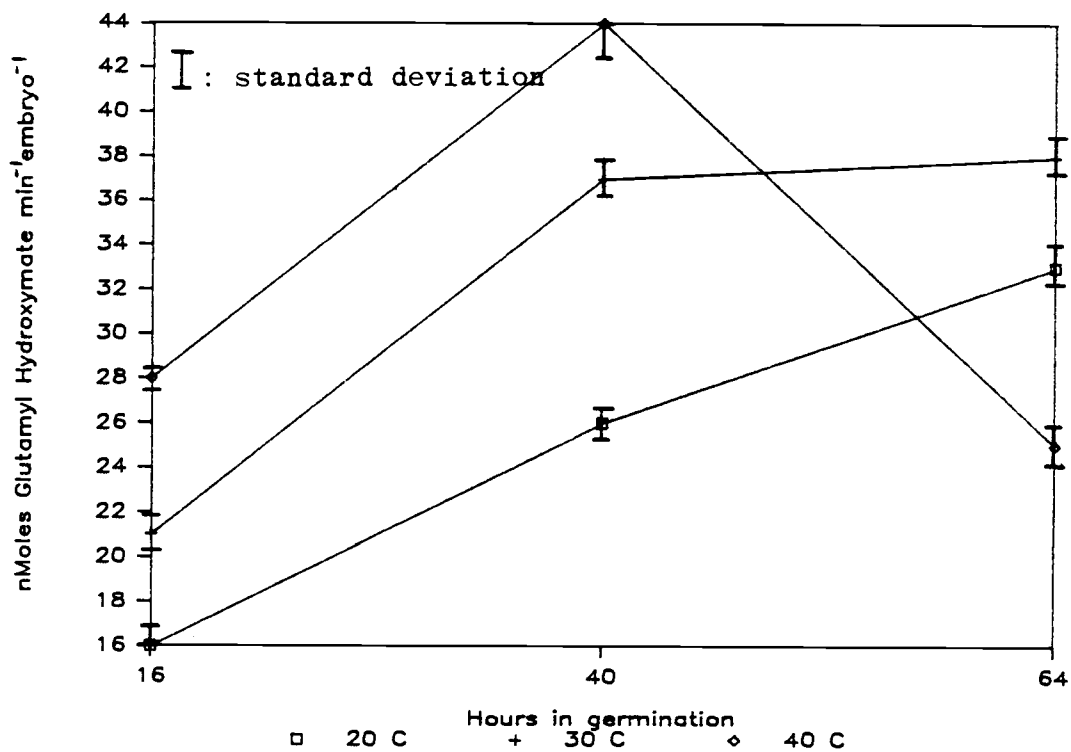
Figure-12   Total activity of glutamine synthetase in the
embryo or seedling of germinating rice seed at
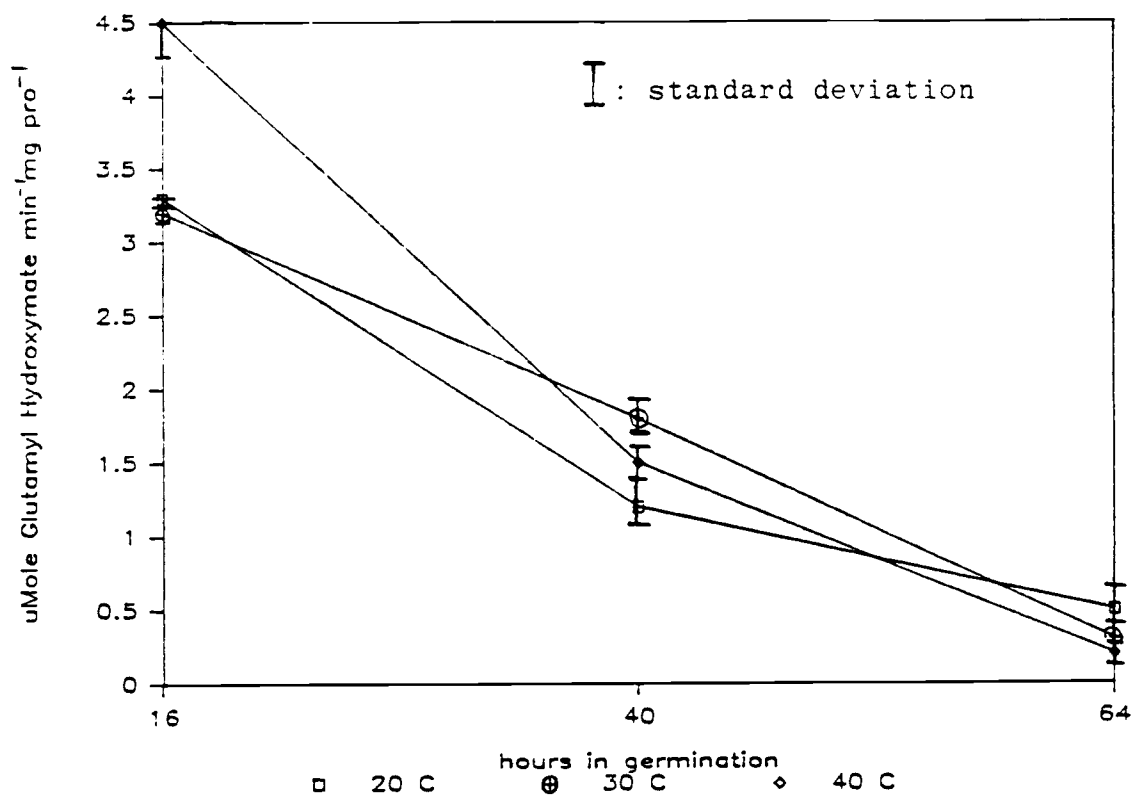20, 30, and 40 C

Figure-13 Specific activity of glutamine synthetase in the
embryo or seedling of germinating rice seed at 20,
30, and 40 C

V: CHANGES IN SOLUBLE METABOLITES

A) Soluble Sugar Content----The changes in soluble sugar
content in the embryo or seedling of germinating rice seeds at the
three temperatures is shown in figure-14. At 16 hours of germination,
the content of soluble sugars was higher at 20 C and lowest at 40 C,
with that at 30 C being intermediate. At 40 hours of germination,
embryos grown at 20 C showed a decreased sugar content and continued
to decrease to 64 hr of germination. A slight decline of sugar
content was observed in embryos grown at 30 C at 40 hours in
germination, followed with a rapid increase at 64 hours. A continuous
increase was observed in embryo or seedlings grown at 40 C, at 40 and
64 hours of germination, the soluble sugar content reached 1.3 mg per
embryo.

Soluble sugars are present in embryos of ungerminated rice
seeds as readily oxidizable and utilizable substrates for energy and
growth at the early stages of germination. Upon hydration or
imbibition the reserve starch in endosperm become hydrolyzed by
various amylases and glucosidases, the resulting sugars are
transported to the embryos and later to the seedling for its growth.
Therefore the sugar content in germinating embryos or seedlings
constitutes the balance of what is preexisted and transported in from
the endosperm and what is utilized for respiration and synthesis. The
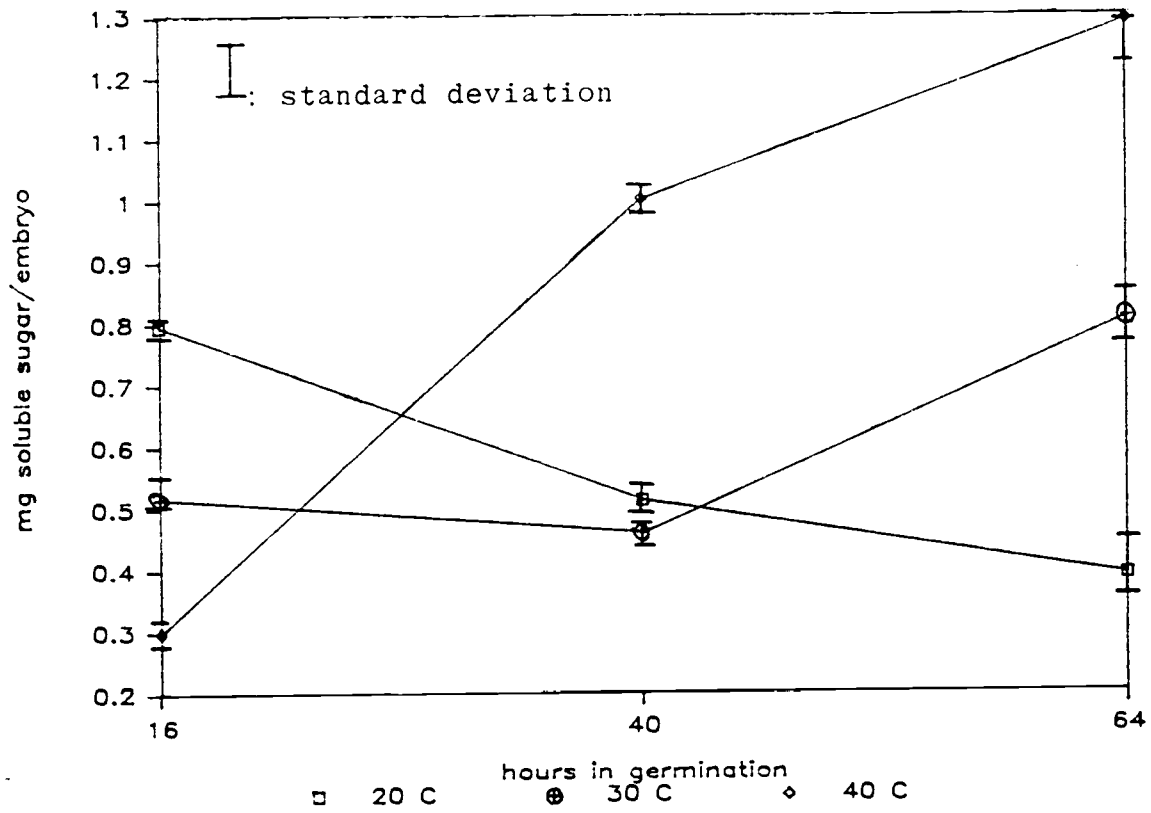data in figure 14 indicated that at 16 hours of germination, the

Figure-14   Soluble sugar content in the embryo or seedling of germinating rice seed at 20, 30, and 40 C

metabolic demand of sugar was lowest at 20 C thus the balance was highest, when the demand was intermediate at 30 C and largest at 40 C resulting in a intermediate and lowest balance, respectively. At 40 and 64 hours of germination, the supply of sugar from the endosperm and the demand of seedling growth produced the differences observed. A high balance at 40 C resulted from a high supply from the endosperm and a low demand of embryonic growth; the intermediate balance at 30 C was due to highest supply from the endosperm though the demand was high; and the lowest balance at 20 C was due to low supply as well as low demand. These speculations, however, should be verified by tracing the utilization of radioactive sucrose and by quantifying the sucrose supply from the endosperm.

B) Amino Acid Content----The changes in amino acid content during the 64 hr of germination are shown in figure-15. At 16 hours of germination amino acid content was highest at 40 C. At 40 and 64 hours of germination time, the content increased to the highest at 30 C, whereas that at 20 C it remained lowest. The content of amino acids were increased with germination time and elevated at optimal temperature of germination indicating the demand of amino acids for the seedling growth.

C) Soluble Protein----The content of soluble protein followed the increase in the protein synthetic ability (figure-16 and 7). The embryos grown at 40 C had a higher content of soluble protein at 16 hours of germination than those at 30 and 20 C. At 40 and 64 hours of
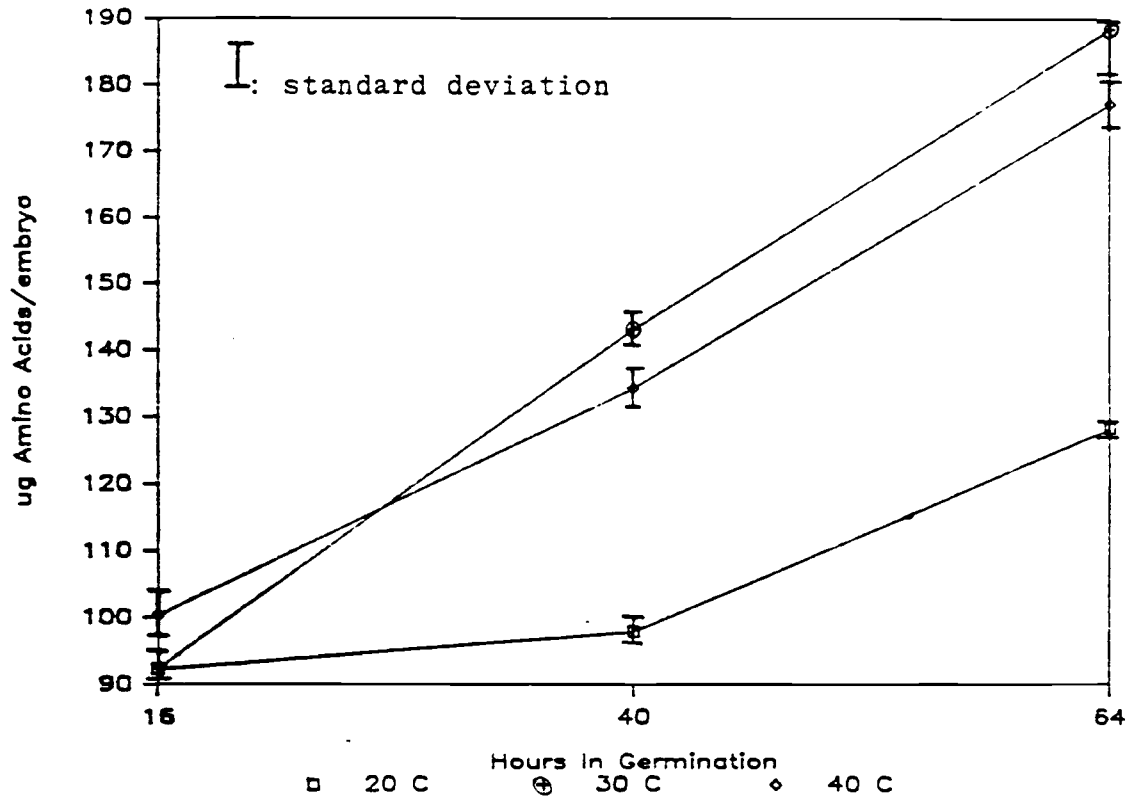
Figure-15   Amino acid content in the embryo or seedling of germinating rice seed at 20, 30, and 40 C
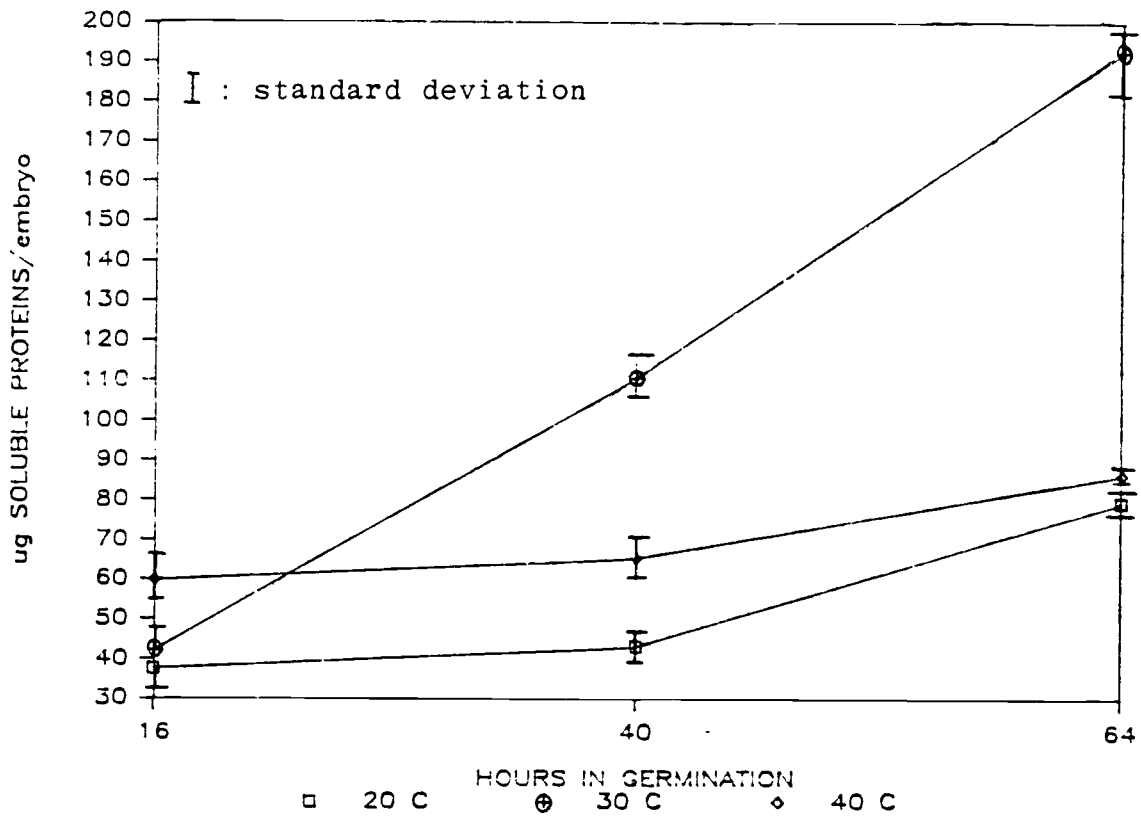
Figure-16   Soluble protein content in the embryo or seedling of germinating rice seeds at 20, 30, and 40 C

germination the quantity of soluble proteins increased continuously and became the highest in seedling grown at 30 C. Again this highest protein content represents active seedling growth activities at optimal temperature (Ching, 1975).

Although the content of soluble metabolites does not directly indicate the metabolic activities, the results of the synthetic activities and various enzymes activities provide an overview of the temperature effects on the biochemical activities of embryos or seedlings of germinating rice.

CONCLUSION

The following conclusions can be drawn from the experimental

results:

A) supraoptimal temperature stimulates the water uptake of

rice seed and results in an early germination. Continued uptake of

water depends upon the seedling growth which is highest at optimal

temperature.

B) The synthetic abilities of DNA, RNA and protein are the

major events, among those studied, being affected by temperatures

during the rice seed germination and the seedling growth. All

synthetic events are synchronously correlated to the seedling growth

rate which is highest at the optimal temperature.

C) Functions of various enzymes studied result in different

patterns exhibited by the embryo or seedling grown at the 3

temperatures. High activities always relate to high germination rate

or seedling growth. It, therefore, can be assumed that a coordinated

and concerted activity of different enzymes results in a maximal

growth processes at the optimal temperature. An optimal temperature

for a specific plant is generally the products of a long period of

evolution during which all the synthetic systems and individual

enzymes had been adjusted and complemented to the utmost harmony for

the survival of the organism. In the case of rice, which is a warm

season crop, 30 C appeard to be the optimum among the 3 constant

temperatures tested.

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