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Effect of Gibberellic Acid and Chilling on Nucleic Acids During Germination of Dormant Peach Seed

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EFFECT OF GIBBERELLIC ACID AND CHILLING ON NUCLEIC ACIDS DURING GERMINATION OF DORMANT PEACH SEED

by

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Yuh-nan Lin

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by

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Major Professor: Dr. David R. Walker
Department: Plant Nutrition and Biochemistry

A study of nucleic acid changes influenced by gibberellic acid and chilling treatments in peach seed was performed in an attempt to reach a better understanding of the mechanism involved in breaking seed dormancy.

Gibberellic acid and the chilling treatment increased the RNA content. These two treatments which break dormancy also increased RNA, suggesting a similar mechanism involving RNA. Chilled seeds contained more RNA than did the gibberellic acid treated seeds.

DNA content remained unchanged regardless of treatment.

Dry seed had a greater ribonuclease activity than with soaked seeds. Enzyme changes did not correlate well with the RNA content in gibberellic treated seeds.

Deoxyribonuclease activity was higher in dry seed than with soaked seeds. Enzyme activity change did not correlate well with the DNA content.

The phosphorus content of the seed in regard to the gibberellic acid and chilling treatments was difficult to evaluate. There were no major relationships established. Phosphorus in the methanol fraction from the chilled seed increased some as the storage period increased.

(69 pages)

INTRODUCTION

Many species of seed do not germinate when placed under conditions which are regarded as favorable for germination, namely an adequate water supply, a suitable temperature and the normal composition of the atmosphere. These seeds are viable, and can be induced to germinate after various treatments. Such seeds are said to be dormant or in a state of dormancy.

Seed dormancy is a well known phenomenon in deciduous trees. Peach seeds are dormant at the time of fruit harvest and normally require stratification at 2 C to 5 C under moist conditions for 10 to 12 weeks to bring about the resumption of growth (Carlson and Tukey, 1945). This phenomenon has also been observed in some vegetable crops and ornamental flowers.

There are several methods for inducing or promoting the germination of dormant seed. The after-ripening process as described above, and the application of growth regulators, like gibberellic acid and kinetin are two ways of breaking dormancy of both fruit buds and seed. Light and temperature also exhibit a great influence on the germination of dormant seed. The nature of the mechanism involved in the breaking of dormancy by gibberellic acid and by the chilling process is an intriguing problem.

Biochemical and physiological changes in peach seed as affected by gibberellic acid or chilling has been investigated but our knowledge of the dormancy mechanism is still incomplete. Nucleic acid metabolism may be involved in gibberellic acid-induced germination and chilling may also involve

a stimulation of nucleotide synthesis. These discoveries linking nucleic acid metabolism to seed germination have led to the present investigation.

Objective

The purpose of this study was to elucidate possible relationships between levels of nucleic acids and the germination of peach seed as affected by gibberellic acid and chilling treatments.

LITERATURE REVIEW

Concept of Seed Dormancy

Types of seed dormancy

Two basic kinds of dormancy have been recognized. One is the influence of external factors such as light, temperature, water, etc. The other inherent dormancy, is a condition brought about by or accompanying the ripening of the ovule and/or the maturation of the embryo. Environmental conditions and genotype are factors which influence seed dormancy, and these two influences may be mutually dependent and sometimes can not be separated.

The causes of seed dormancy are varied and may be quantitative, but in general fall into the following major classes: 1. rudimentary embryos, 2. physiologically immature embryos (inactive enzyme system), 3. mechanically resistant seed coats, 4. impermeable seed coats, and 5. presence of germination inhibitors (Amen, 1963).

In the case of a rudimentary embryo, an after-ripening requirement must be satisfied to allow time for the post harvest maturation of the embryo. In other instances, a low temperature may be necessary to bring about physical changes in order that the seed can germinate.

The seed coat can prevent germination either by limiting the permeability of water and gases as in the case of legumes or mechanically limiting the enlargement of the embryo. Removal of these mechanical barriers by

chemical or mechanical treatments may allow germination to proceed in some seeds. In some cases, seed coats alter the growth substance relationships of the enclosed tissues, hence they are closely interrelated with seed dormancy (Leopold, 1964).

Dormant seed subjected to an optimal dormancy breaking treatment, may lose their ability to germinate even under favorable conditions. This phenomenon is called secondary dormancy. Secondary dormancy may develop spontaneously in seed due to changes occurring in them, as in some species of Taxus and Fraxinus. Sometimes secondary dormancy is induced if the seed are given all the conditions required for germination except one. Among the factors which have been shown to induce secondary dormancy are restriction of gaseous exchange, high or low temperature, prolonged exposure of light-requiring seed to darkness and of dark-requiring seed to light.

Function of seed dormancy

Seed dormancy is considered as an aspect of growth cessation. A dormant system has only two possible immediate fates: resumption of growth or death. The occurrence of seed dormancy has a significant advantage to plants by preserving the potential for growth during unfavorable conditions.

The adaptational significance of seed dormancy might be considered an ecological mechanism in which a more favorable time for germination may result in greater survival of seedlings.

Mechanism of seed dormancy

Amen (1968) considered the control of seed dormancy from the view point of cybernetics, and proposed a hormonal regulation of four phases of dormancy. There are: 1. inductive, 2. maintenance, 3. trigger and 4. germination.

Inductive phase. Inductive phase is characterized by a marked decline in the hormone level, and is present during the development of the seed. Little is known about the development of dormancy in the seed, but certain events during the maturation of seed inevitably lead to the onset of dormancy. These events may be environmentally triggered--e.g. photoinduction, thermo-induction, or chemoinduction.

Onset of dormancy may be controlled by the critical balance of an inhibitor-promotor complex(es). During seed maturation, the balance between an inhibitor and promotor may be shifted in favor of the inhibitor component thus imposing dormancy. This shift may be accomplished by a decrease in the synthesis of the promotor, a build up of inhibitory intermediate metabolites, or by a direct antagonism. These events have been shown by Pillay (1966) in cherry, Roberts (1964) in rice, and Lipe and Crane in peach (1966), respectively.

Maintenance phase. The maintainance phase of seed dormancy constitutes an indefinite period of partial or specific metabolic arrest. However, Bradbeer and Colman (1967) showed that the cytoledonary and embryonic axis of dormant Corylns avellana L. seed exhibited an active TCA enzyme system

and possibly lipid and protein synthesis. They suggested that seed dormancy is not due to a general metabolic arrest.

The formation and maintenance of metabolic blocks, is presumably associated with the presence of endogenous inhibitors, promotors, and the relationship between them. Functional inhibitors either are directly antagonistic with endogenous promotors, or interfere with their synthesis. Thus a shift in relative balance between a promotor and an inhibitor may modify a physiological response. In all probability, different inhibitor-promotor complexes regulate specific metabolic pathways, e.g., the catabolism of starch, protein, or lipid reserves.

Kahn and Tolbert (1966) elucidated the regulatory mechanism of the inhibitor-promotor complex by inhibiting lettuce seed germination with exogenous coumarin. The subsequent addition of cycocel reversed this effect while GA_3 and IAA were unable to reverse the coumarin inhibition. They postulated that coumarin and other germination inhibitors participate in the photochemical system. In this instance, cycocel was antagonistic to an inhibitor, whereas in the work on Pharbitis seed Zeevaart (1966) found that cycocel was antagonistic to endogenous gibberellin. Based on these and other findings, a chemical may act as an inhibitor or a promotor of a regulatory complex, depending on what particular substance with which it is interacting. The relative concentration of these substances likely determines whether they are inhibitory or stimulatory to a particular process.

Trigger phase. This phase of seed dormancy represents a period of sensitivity to a specific environmental condition. A triggering agent may be

responsible for inducing germination, but need not be present continually. The triggering agents are varied depending on the different types of seed dormancy. It may be a photochemical one as in photoblastic seeds, a thermo-chemical reaction as in after-ripening (stratification) or an inhibitor-removal by scarification, leaching or seed coat removal.

The germination of lettuce seed associated with the photoblastism can be illustrated by the photochemical nature of the trigger agent. Ikuma and Thimann (1964) have postulated a scheme for the germination process in lettuce seed by showing the promotion action by red light and inhibition by far red light. Although the nature of the pigments involved are not understood, Shain and Mayer (1965) were able to elucidate some of the biochemical detail of the hydrolytic phases of the termination of dormancy in lettuce seed. They proposed that the trigger mechanism activates an existent protoeolytic enzyme which then inactives a protease inhibitor, resulting in increased protease activity. Further studies suggest that the photochemical conversion results in the production of an enzyme-releasing hormone which in turn activates an inhibitor removing enzyme.

It is reasonable to assume that removal of an inhibitor is one of the triggering mechanisms for some types of seed dormancy. Luckwill (1952) reported that the removal of an inhibitor in dormant apple seed during stratification was likely responsible for the breaking of dormancy. The completion of dormancy may occur either when the inhibitor is eluted, metabolized, or after intervention by a growth stimulating substance. Essentially, the

function of the triggering agent is in the shifting of the relative balance between an inhibitor and a promotor complex to favor the promotor.

Germination phase. The subsequent process after dormancy is seed germination. This phase is marked by an increase in hormone and enzyme activity. The early stage of germination seems to involve enzyme activation and degradative reactions, while the later stages are associated with the translocation, mobilization and assimilation of organic nutrients.

A germination agent, presumably a naturally occurring hormone (auxin, gibberellin and/or cytokinin) is believed to be required. It appears to function via an inhibitor-promotor complex. Several such complexes may be involved in the germination response of any one species, with each complex being responsible for some specific process, such as degradation of seed coat or mobilization of nutrients.

From the above observations, the over-all control of seed dormancy seems to involve a reduction in the growth-promoting hormone content during maturation, i.e., dormancy onset. Under suitable environmental conditions a trigger factor is activated which increases the hormone content. The hormones (germination agents) then perform some functions, probably activate preexistent hydrolytic enzymes, and/or stimulate the synthesis of additional enzymes via DNA depression. These degradative reactions supply appropriate monomers for the respiratory activity of the embryo, resulting in germination.

Gibberellin and Seed Dormancy

Endogenous gibberellins as functional hormones

Although gibberellins were orginally discovered as products of a fungus which parasitizes a higher plant, it is now recognized that gibberellins are a constituent of normal green plants. This finding comes from the bio-assays of extracts of seeds of various species (West and Phinney, 1956), and pea shoots (Radley, 1956), which all show similar physiological effects from gibberellin or gibberellin-like substances.

Circumstantial evidence has been accumulated showing that there is a positive correlation between endogenous gibberellin levels and certain developmental trends. Treatments of gibberellin can induce flowering of some photo-periodically sensitive and some cold-requiring plants (Lang, 1956; Lane et al., 1957). It has also been shown that the induction of flowering may bring about a natural rise in endogenous gibberellin content (Lang and Reinhard, 1961). Brian (1966) pointed out that Kato and Ito have reported that the gibberellin levels are higher in expanding leaves of apple than in those that have completed expansion, and that levels are higher in terminal buds of vigorous shoots than in those of weak ones.

The changes in gibberellin content during development of seed and fruit have been investigated by Corcoran and Phinney (1962) in Echinocystis macrocarpa, Lupeinus succulentus, and Phaseolus vulgaris. In all three cases by far the highest concentration of gibberellin was in the seed. Even

in the seed, gibberellin levels remained very low until fruit growth had nearly been completed, maximum levels always being reached after fruit growth had ceased. The marked rise in gibberellin level which occurred at that time was very strongly correlated with the period of maximum growth rate of the seed; after seed growth was complete the endogenous gibberellin levels declined rapidly. In regard to the seed dormancy and gibberellin levels, Kahn et al. (1957) reported gibberellin treatments can overcome some types of dormancy, and as seeds emerge from the dormant condition there may be a natural rise in endogenous gibberellin contents (Naylor and Simpson, 1961; Smith and Rappaport, 1961). Fraskland and Wareing (1967) showed that gibberellin content increased during the chilling process of hazel seed, and suggested that the gibberellin was possibly responsible for overcoming dormancy. The fact that gibberellins are present in dormant seed in many cases, and that gibberellin breaks seed dormancy lead one to assume that an effective or critical gibberellin level is involved in breaking dormancy.

Possible roles of gibberellic acid in the breaking of seed dormancy

Promotive effects of exogenous gibberellin in the germination of nondormant seeds have been reported by Hayashi (1940) in wheat, barley, and rice. Similar responses have also been reported in many plant species. Yet, one of the most dramatic effects of gibberellin is the breaking of seed dormancy. Kahn et al. (1956, 1957) have reported that gibberellic acid will break dormancy in light-dependent seed. They found lettuce seed germinates spontaneously when pretreated with gibberellic acid solution.

Subsequent studies show that the application of exogenous gibberellin induces the germination in many dormant seeds. For example, Curtis and Cantlon (1965) demonstrated that GA_3 substitutes for the after-ripening requirement in Melanpyrum lineare Desr. This is also the case for cold-requiring peach seeds (Donoho and Walker, 1957; Chao and Walker, 1966).

As to the role of gibberellins in promoting seed germination, many hypotheses have been proposed. Gibberellin may influence the elongation of the embryonic axis (Ikuma and Thimann, 1960); it can promote the release of readily soluble food material from food reserves (Naylor and Simpson, 1961); or it can induce quantitative and qualitative changes of protein, amino acids and auxin in the embryo (Paleg, 1961; Koller et al., 1962; Kuraishi and Muir, 1962). Gibberellins also play an important role in the regulation of nucleic acid synthesis (Naylor, 1966). In reviewing the problem of dormancy within the framework of molecular biology, Tuan and Bonner (1964) were able to demonstrate that the genetic material of the buds of dormant potato tubers is largely in a repressed state, and that the breaking of dormancy is accomplished by derepression of the genetic material. This finding strongly suggests that the mechanism of breaking dormancy is closely related to gene action.

In their study with germinating barley, Varner and Chandra (1964) were able to illustrate that GA_3 acted as a chemical signal, that activates the cells of the aleurone layer into secreting a hydrolytic enzyme (α-amylase). The activity of α-amylase in isolated barley endosperm increased markedly in

response to an application of GA_3. In addition, they noted that the synthesis of α-amylase was inhibited by actinomycin D (Varner, 1964). These observations led them to postulate that gibberellic acid controlled the synthesis of α-amylase in aleurone cells by causing the production of specific messenger RNA.

Working with embryos of Avena fatua which were excised after different periods of dry storage of the seeds, Naylor and Simpson (1961) showed the effectiveness of gibberellin in breaking dormancy in embryos from fresh seeds was greatly increased by the presence of saccharose in the medium. They also reported that in partially after-ripened embryos, germination was promoted by saccharose even in the absence of gibberellin. From these and other experiments they concluded that part of the dormancy-breaking effect of gibberellin consists of a promotion of sugar formation and sugar utilization by the embryo. They also believe that the effect of gibberellin is not direct but via reversion of the effect of an inhibitor.

Ingle and Hageman (1965) reported that endosperm carbohydrate and protein catabolism is stimulated by exogenous GA_3 in corn, concluding that exogenous gibberellin replaces a component normally supplied by the embryo.

Ribonuclease has been reported to be associated with the triggering mechanism of seed germination (Nezgovorova and Borisova, 1967). Subsequently, Chrispeels and Varner (1967) reported that GA_3 stimulates the synthesis of ribonuclease in barley endosperm. They concluded that ribonuclease is retained in the early stages of germination but is later

actively secreted. Although the precise role of the secreted ribonuclease is not clear, it presumably functions in a manner similar to the amylases and proteases and supplies specific nucleotides to the embryo. These findings strongly suggest GA_3 may perform a role by providing soluble food for the embryo.

The available evidence suggests that there are two distinct modes of action for gibberellic acid: 1. releases latent hydrolytic enzymes; 2. initiates enzyme synthesis presumably via RNA control.

Chilling and Seed Dormancy

Temperature and seed germination

Different seed have different temperature ranges within which they germinate. At a very high or low temperature seed germination is prevented.

Low temperature may be necessary or at least favorable for securing good seed germination for some plant species. This is particularly true for forest and fruit tree seed. In some cases only a brief exposure to temperature near freezing is needed to break dormancy; in others an extended period is needed; and in yet others, dormancy is not actually broken until two winters have passed (Crocker and Barton, 1957). Removal of dormancy at low temperature characteristically takes place between temperatures of 1 C and 10 C and is usually most rapid between 2 C and 5 C.

Some seed require alternating temperatures before they germinate. Morinaga (1926) first observed this and suggested that two temperatures were

required for the mechanical modification of some limiting feature of the seed or seed coat. Toole et al. (1955) worked with Lepidium seed and proposed that the alternating temperature effect was a quantitative alteration of some regulating substance. Cohen (1958), on the other hand, has observed that the elevation of temperature brings about some structural change which enhances germination. Seasonal changes of temperatures may influence germination by affecting the actual development of the embryo.

Metabolic effects of chilling as an agent breaking seed dormancy

Biochemical changes as affected by chilling have been reported by many investigators. A number of enzymes have been shown to change during stratification. Catalase and peroxidase in particular increase enormously in Sorbus aucuparia, Rhodotypos kerrioides and Crategus (Flemion, 1933; Eckerson, 1913) during chilling. Crocker and Harrington (1918) have demonstrated that low temperature is more conclusive to high catalase activity than at high temperature in peach seed embryos. These changes in enzyme activity may be the direct cause of emergence from dormancy, but it seems much more likely that they are the secondary result of other changes in the seed. For example, Barton (1934) was able to show a complete absence of correlation between an increase in catalase activity during after-ripening and the completion of the after-ripening of Tilia seed.

Low temperature after-ripening is accompanied by a low respiration rate and a low respiratory coefficient (RQ). A rise in temperature causes

an increase in the rate of respiration in the seed. Ranson (1935) reported there was a progressive increase both in rate of respiration and RQ with temperatures of 6, 12, 18, and 30 C in Polygonum scandens seed. However, the temperature effect on respiration also depends on the length of time the seeds are exposed to a given temperature (Fernandes, 1923), and the presence or absence of the testa (Spragg and Yemm, 1959).

Olney and Pollock (1960) reported that during after-ripening at 5 C, nitrogen and phosphorus are translocated to the developing tissues of the cherry seed. The translocated phosphorus moves through normal synthetic pathways into all phosphate compounds in the cells. However, in unchilled seeds, phosphorus tends to shift from compounds such as nucleic acids and accumulates as inorganic phosphate. They, therefore, suggest that the rest period may be associated with a block in the metabolism of phosphorus in the cell. Metabolism of phosphate may play a role in the dormancy breaking process as was shown by Bradbeer and Floyd (1964). They reported that an increased incorporation of labelled adenine was metabolized into adenosine-5'monophosphate at an early stage of the chilling process.

Chilling influences nucleic acid metabolism. Wood and Bradbeer (1967) reported that there was little nucleic acid synthesis in Gorylus avellana L. seed which were stored at either 4 C or 20 C for the first 5 to 10 days, however there was an increase of RNA in seed that had been chilled at 4 C for 20 days. They suggested that RNA may not be an important factor in the early stages of stratification, but likely has an important part

during the late stages of after-ripening. They also pointed out that a structural modification of RNA may be involved in the early stages of after-ripening rather than a de novo RNA synthesis.

The low temperature treatment apparently does depress a growth inhibitor in dormant seed. Lasheen and Blackhurst (1956) studied the changes in ether-soluble growth substances occurring in blackberry (Rubus sp.) seed during after-ripening. The relative concentration of the growth-inhibitory material was highest in the endosperm, lower in the teste and lowest in the embryo. The inhibitors disappeared during low temperature after-ripening of the seed, and the disappearance of the inhibitor was correlated with the breaking of dormancy. On the other hand, there was little correlation between the inhibitor content of the embryos and their state of dormancy. Similar results were obtained with peach seed by Flemion and DeSilva (1960).

From these observations we may assume that endogenous inhibitors are important in controlling seed dormancy. Yet, it can not be stated that the reduced inhibitor level is a direct or an indirect result of chilling.

Growth substances other than the inhibitors may be involved in the chilling process. Gibberellic acid applied externally will break the dormancy of many seeds having a chilling requirement (Fogle, 1958; Villiers and Wareing, 1960; Frankland, 1961). It has also been shown by Frankland and Wareing (1962) that chilling Corylus seed results in a significant increase in the gibberellin content of the embryos. Thus, it would seem that the dormancy breaking effect of chilling is due to the accumulation of a germination-promotor such as GA which enables the embryo to overcome the effect of inhibitor.

MATERIALS AND METHODS

Treatment of Seeds

Unchilled peach seeds obtained from Rudy Bonzi Enterprises, Modesto, California, with their pericarp removed were used in this investigation. One lot of dry seeds was used as the untreated control and a second lot was soaked in deionized water for ten hours at room temperature.

One group of water soaked seed (about 1,000 seeds) was soaked for an additional hour in either deionized water, or a 2,000 ppm solution of gibberellic acid (GA active ingredient 80 per cent, Merck and Company, Inc., Rahway, New Jersey). They were then soaked for ten minutes in a 5 per cent calcium hypochlorite solution to kill any organisms on the surface. The seeds were then placed in petri dishes and stored at room temperature in the dark. Every 24 hours, for a period of two weeks, a sub-lot of seed (about 20 seeds) were removed and boiled in methanol for two minutes, then stored at 2 C until they were analyzed for nucleic acids.

Another group of the water soaked seeds (about 1,000 seeds) was treated with calcium hypochlorite, placed on a 1 per cent agar media at either 7.2 C or 22.5 C. At two week intervals, for a period of ten weeks, about 20 seeds were removed, boiled in methanol for two minutes and stored until analyzed for nucleic acids.

Seeds used for enzyme activity measurement were processed immediately after removal from the storage treatments.

Extraction and Measurement of Nucleic Acids

The procedures for extraction and measurement of nucleic acid were, in general, the methods of Holdgate and Goodwin (1965) and Wheeler and Boulter (1966). The seed coats were separated from the cotyledon portion of the seed. Approximately one gram (2 seeds) of cotyledon was homogenized with 10 ml methanol at 2 C. After centrifugation at 12,000 rpm for 10 minutes, the residue was re-extracted with two more 10 ml aliquots of methanol, and the supernatent fluids combined (methanol fraction). The residue was then stirred for one minute with 10 ml of 10 per cent (W/V) trichloroacetic acid at 2 C and this slurry was centrifuged. This procedure was repeated twice and the supernatant and fluids were combined (TCA fraction).

The residue was then extracted serially with 10 ml of each of the following solvents, and separated from the supernatant by centrifugation between each treatment: 1. twice with 90 per cent ethanol saturated with sodium acetate, 2. once with ethanol, 3. twice with ethanol:chloroform (3:1), 4. twice with ethanol:ether (1:1), 5. once with ether. These procedures were done in order to remove the lipid materials. The supernatants from each extraction were combined and are referred to as the lipid fraction.

The dried residue was hydrolyzed for 18 hours with 10 ml 0.3 N KOH at 37 C, cooled to 0 C, acidified to pH 2 with perchloric acid and left for 30 minutes at 2 C. The precipitate which formed was removed by centrifugation and washed two times with 0.5 N perchloric acid solution. The supernatant solution and washings were combined and adjusted to pH 7 with

KOH and allowed to stand for two hours at 2 C. The solution was centrifuged and the potassium perchlorate precipitate was separated from the supernatent. The supernatant contained mononucleotides (RNA fraction). The RNA fraction was then subjected to purification by passing through an ion-exchange column as described by Smillie and Krotokov (1960). The RNA content was then determined by using the Beckman-DU spectrophotometer with the wave length at 260 mμ.

The initial precipitate containing the DNA, which formed when the alkaline hydrolysate was adjusted to pH 2, was hydrolyzed for 20 minutes with 3 ml of 5 per cent (V/V) perchloric acid at 70 C. The hydrolysate was cooled to room temperature and centrifuged. This hydrolysis was repeated twice. The supernatants which contained the hydrolyzed DNA were combined and made to a known volume (DNA fraction). The DNA content was then determined by the indole reaction described by Keck (1956).

Enzyme Assays

One gram fresh weight of seed without their seed coats was homogenized in ice-cold water. The homogenate was then centrifuged at 10,000 x g for 30 minutes at 0 C. Activity of ribonuclease and deoxyribonuclease were assayed in the aqueous extract. The protein content of the homogenate was determined by the method of Lowry et al. (1951).

Activity of ribonuclease was determined following the method of Johri and Maheshware (1966). The reaction mixture contained 0.3 ml of RNA

solution (5 mg/ml dissolved in 0.2 M phosphate-citrate buffer at pH 5.0), 1.0 ml of phosphate-citrate buffer (0.15 M, pH 5.0) and 0.5 ml of aqueous crude homogenate which was incubated at 25 C for 30 minutes. The reaction was stopped with 0.5 ml of 0.75 per cent uranyl acetate in 25 per cent perchloric acid. The test tubes were then stored over-night in a refrigerator and the precipitate removed by centrifugation. The increase in optical density at 260 mμ in the reaction tubes were compared with the untreated control samples and the data expressed as mg protein per 30 minutes.

The activity of deoxyribonuclease was estimated following the method of Sung and Laskowski (1962). The specific activity was expressed as an increase in optical density at 260 mμ per mg protein per 30 minutes.

Analysis of Total Phosphorus

The total phosphorus in the seed and of the dried aliquots of the various extracted fractions was determined by the method of Wheeler and Boulter (1966).

Statistical Analysis

There were three replications for the nucleic acid measurements and four replications for the enzyme assays. Data were analyzed statistically with the LSD and coefficient of variation values calculated. There were no replications for total phosphorus measurements.

RESULTS AND DISCUSSION

RNA Changes

The RNA in gibberellic acid treated seed was statistically significantly greater than in the water treated or untreated dry seed (Figure 1). The RNA increase in gibberellic acid treated seed, suggests the possibility that gibberellic acid may activate a hydrolytic enzyme system.

The increase in RNA was observed the fourth day after treatment with gibberellic acid. Subsequently, it was followed by a higher level of RNA than at day zero. Seed germination began the fourth day and increased continually during the experiment as shown in Figure 1. The RNA increase resulting from gibberellic acid treatment may account for the increase in germination when gibberellic acid is applied. It is unclear whether this is a primary response of the gibberellic acid application, or it is a subsequent biochemical change of development. However, since the measurable increase in RNA preceded the increase in germination by approximately 48 hours, a direct involvement of GA_3 in stimulating RNA synthesis seems probable.

It was rather surprising to observe that gibberellic acid treated seed did not show a linear increase in RNA. Instead, a fluctuation in RNA with the higher peaks occurring the fifth, eighth, and thirteenth days was observed. This has made the interpretation difficult since the variability among

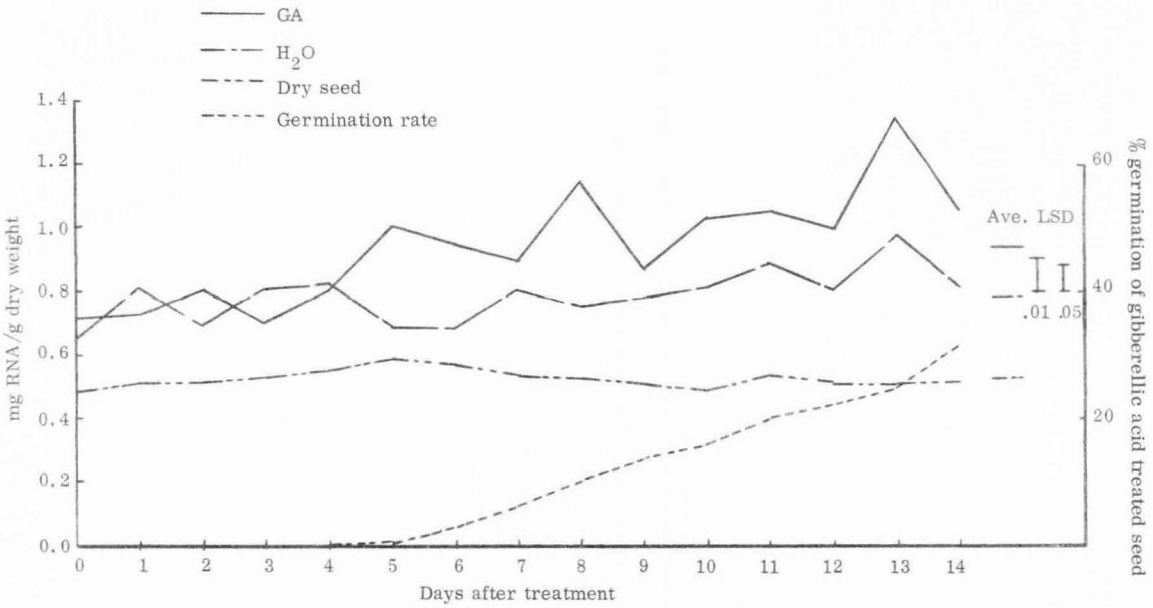

Figure 1. The effect of gibberellic acid and water on the RNA level and time of germination of peach seed.

replications for a given day was small. Possibly it is a result of different developmental stages within the seed.

Water soaked seed had a larger RNA content than did the dry seed (Figure 1). Thus, water likely activates the formation of RNA, very likely, messenger RNA. Similar results were also observed by Rast (1966).

A linear increase of RNA occurred in each of the five successive samplings of seed which were held at 7.2 C during the ten weeks (Figure 2). RNA increased two fold in chilled seeds as compared to the seeds held at 22.5 C after ten weeks of storage. Seeds soaked in water and held at 22.5 C contained much less RNA than in seed held at 7.2 C but more than in the dry seed. These results strongly suggest that the RNA metabolism was associated with the chilling treatment, and the synthesis of nucleic acid occurred prior to seed germination (Figure 2).

The gibberellic acid and chilling treatments resulted in a higher RNA content in treated seed. This indicates that a different triggering agent may initiate the same mechanism to increase RNA via activation or synthesis of m-RNA. A lower RNA content in gibberellic acid treated seed (Figures 1 and 2) may account for the slender seedlings as induced by gibberellic acid, although there is no data known to support this possibility.

DNA Changes

The DNA content of the seeds was not affected by the gibberellic acid, or water soaked treatments when compared with the dry seed (Figure 3).

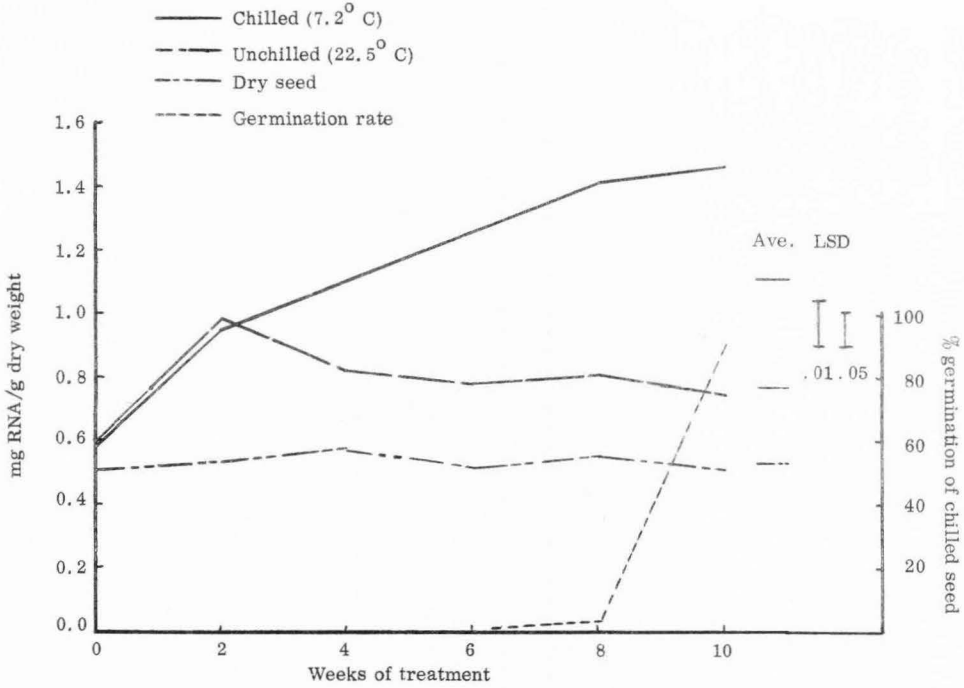

Figure 2. The effect of chilling on the RNA level and time of germination of peach seed.

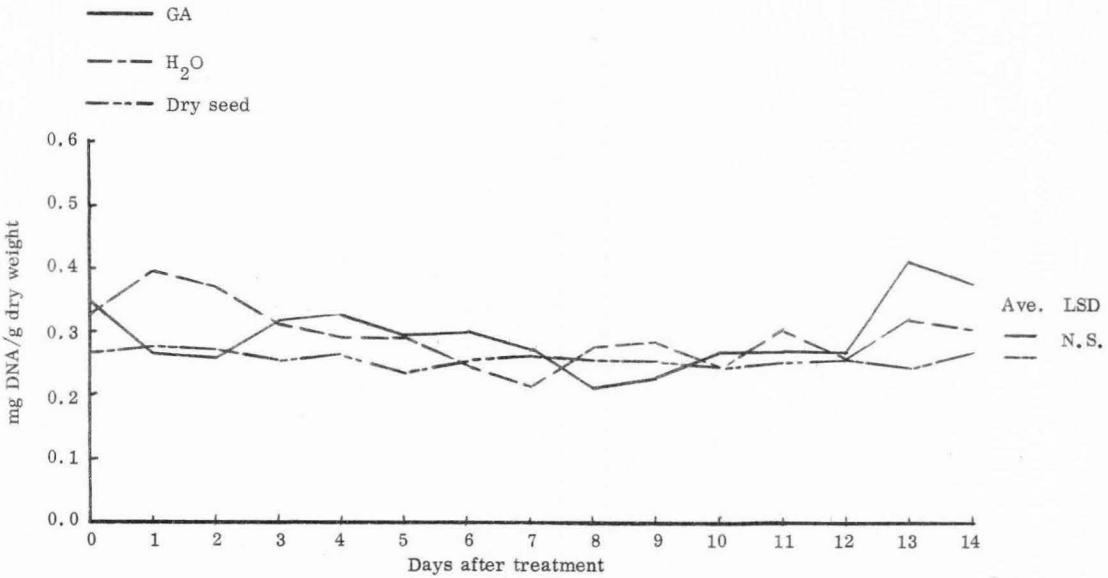

Figure 3. The effect of gibberellic acid and water on the DNA level of peach seed.

Since DNA is regarded as containing the genetic information, it would remain constant in the amount per nucleus. It might be expected, therefore, that prior to germination the DNA would remain unchanged.

Water soaked seed held at 7.2 C and 22.5 C had a similar amount of DNA as did the untreated seed during the 10 weeks of this study (Figure 4). Statistical difference at the 5 per cent level for either experiment was not present.

Ribonuclease Activity

Naggovorva and Borisove (1967) suggested that ribonuclease is related to the triggering mechanism of the germinating seed after demonstrating that imbibition of water decreases ribonuclease activity. This decrease in ribonuclease activity was observed in this study in soaked peach seed as compared to the dry seed (Figures 5 and 6). The subsequent changes did not correspond with the changes of RNA in gibberellic acid treated seed.

Ribonuclease activity in chilled seed correlated somewhat with the RNA content (Figures 2 and 6), except there is a contradiction at the fourth week's sampling. At this sampling, the highest ribonuclease activity occurred in chilled seed, even though an increase in RNA content was observed. This contradiction may be a result of synthesis and decomposition of RNA.

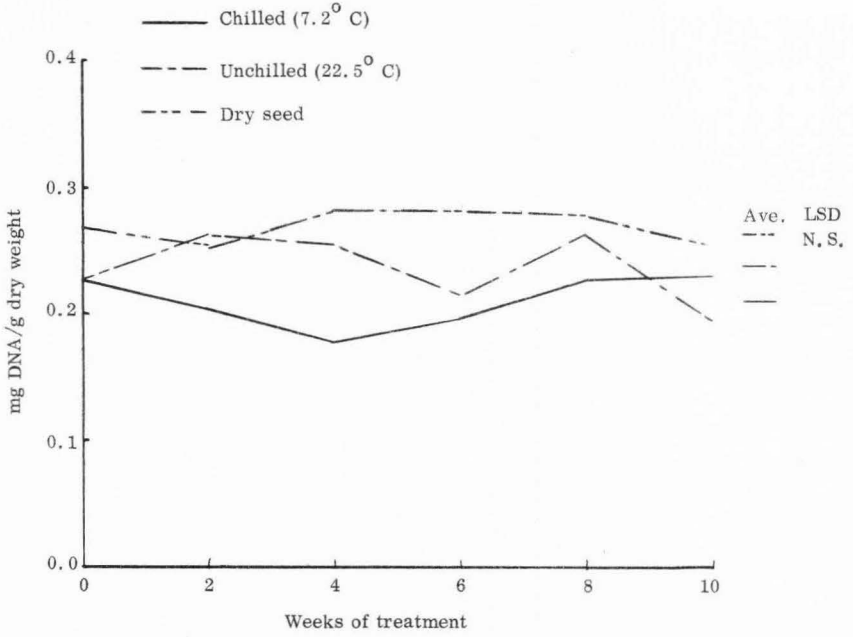

Figure 4. The effect of chilling on the DNA level of peach seed.

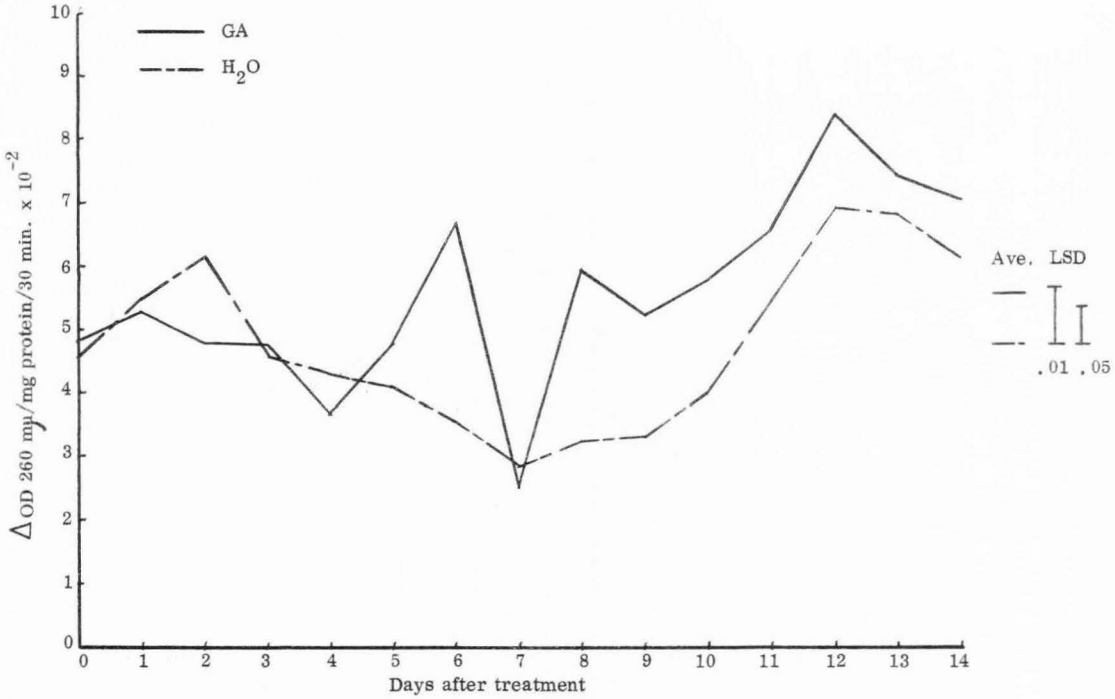

Figure 5. The effect of gibberellic acid and water on the RNase of peach seed.

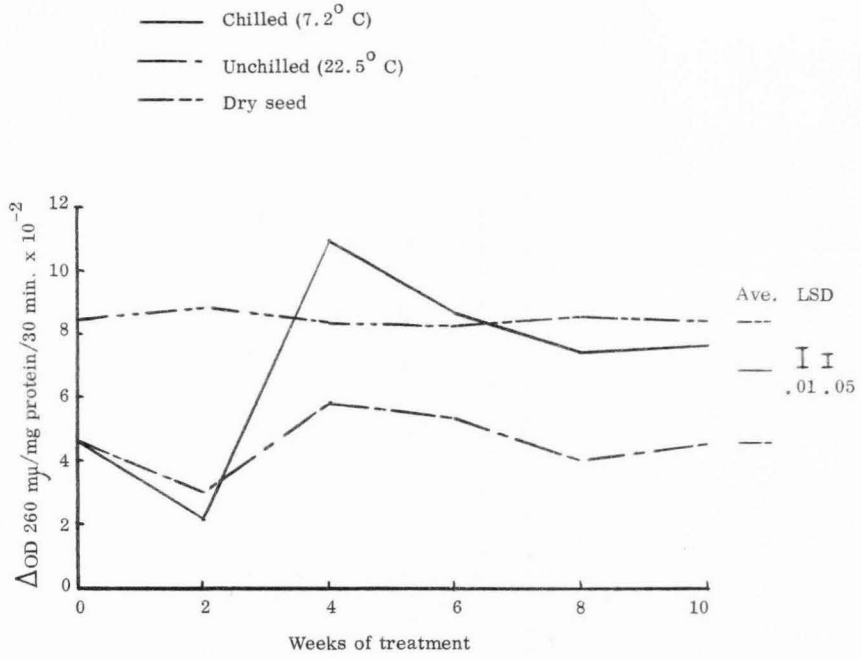

Figure 6. The effect of chilling on the RNase of peach seed.

Deoxyribonuclease Activity

Gibberellic acid and water treated seed showed less deoxyribonuclease activity than the dry seed (Figures 7 and 8). The gibberellic acid treated seeds had slightly more activity than did the water soaked seed. The relatively constant DNA level in these seeds was not correlated with the change in enzyme activity (Figures 3 and 7).

Deoxyribonucleases in the chilled and unchilled seed had the same pattern of changes during the storage period, and were significantly different at the .05 level (Figure 8). Enzyme activity changes in seed receiving these two treatments were not correlated well with the relatively constant DNA level (Figures 6 and 9).

Total Phosphorus

The phosphorus content of seed receiving the various temperature and soaking treatments are shown in Tables 1 and 2 of the Appendix. Seed phosphorus in the TCA and methanol fractions, which is contributed from the low molecular nucleotides and inorganic phosphates, was influenced by the treatments (Figures 9 and 10).

An increase in phosphorus in chilled cherry embryos was observed by Olney and Pollock (1960). In this study, with the whole seed involved, total phosphorus did not increase in chilled peach seed. The phosphorus in the methanol fraction did increase when the seeds were held at 7.2 C compared with seed held at 22.5 C (Figure 10).

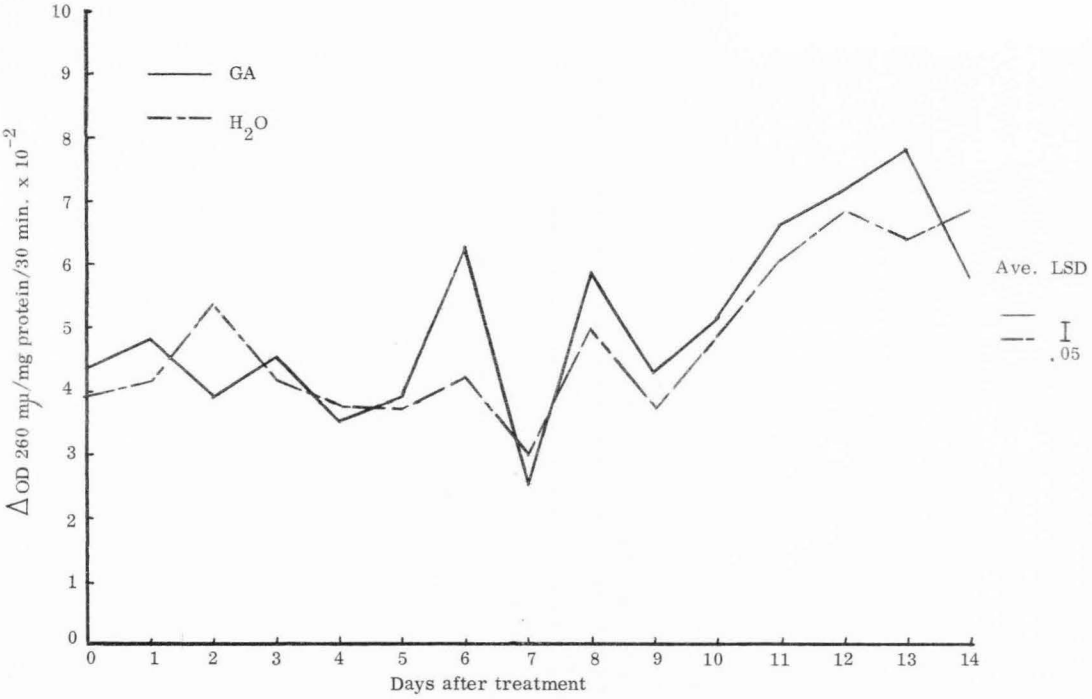

Figure 7. The effect of gibberellic acid and water on the DNase of peach seed.

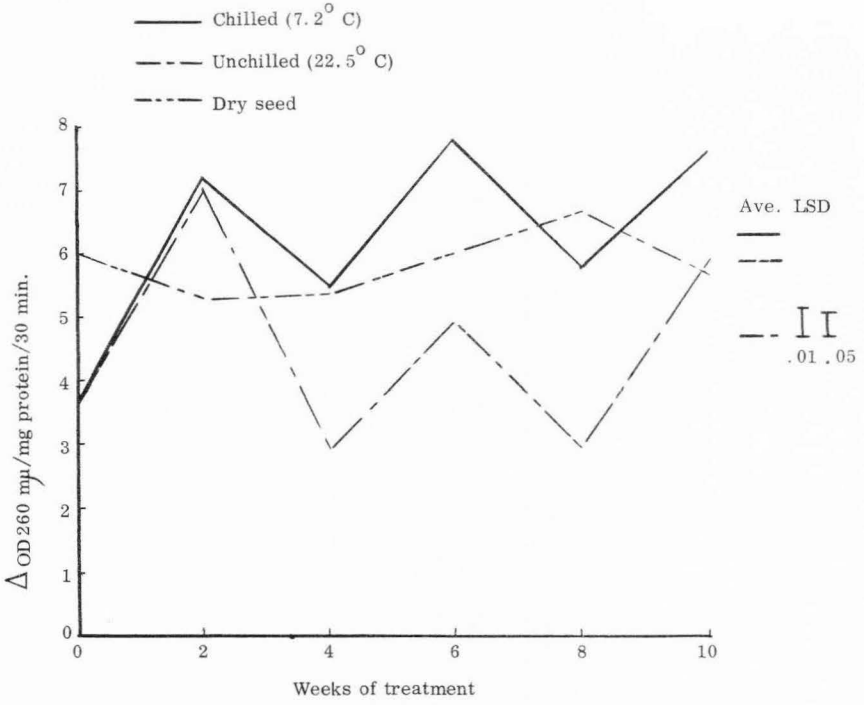

Figure 8. The effect of chilling on the DNase of peach seed.

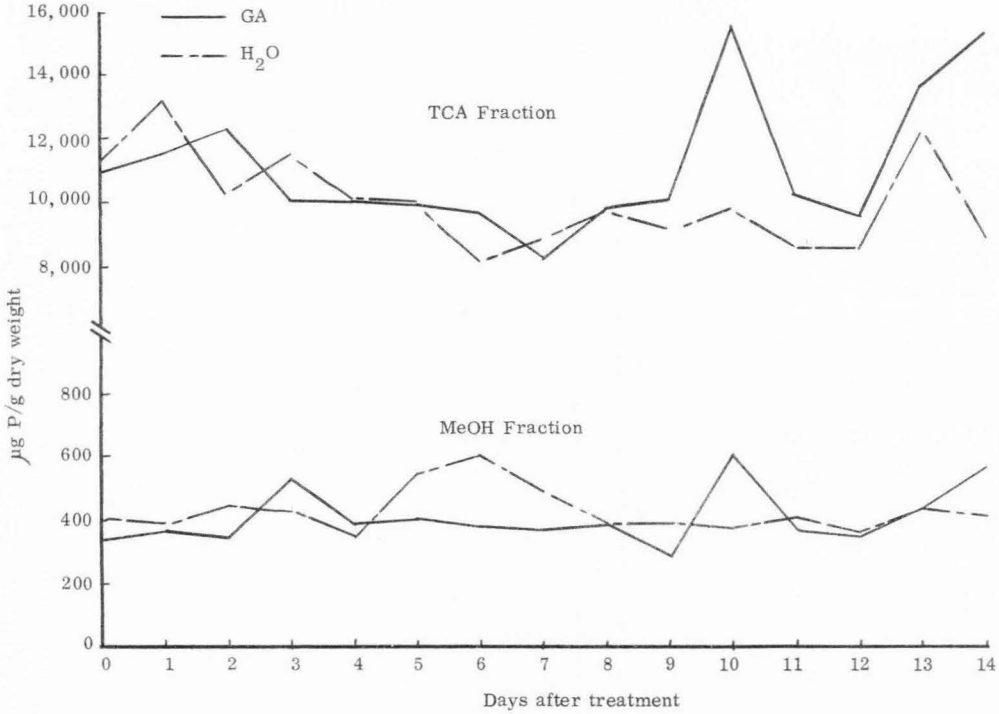

Figure 9. The effect of gibberellic acid on phosphorus content in trichloroacetic acid and methanol fractions.

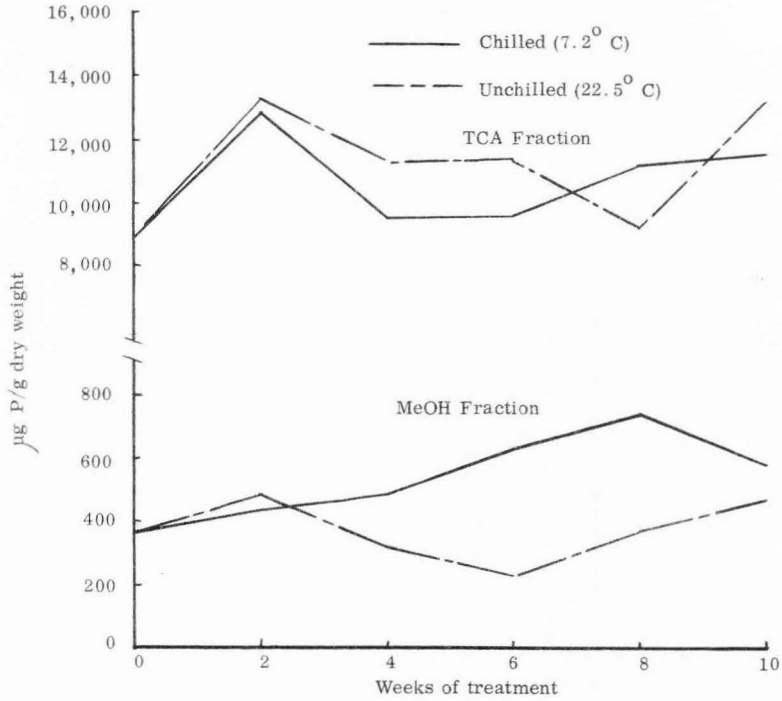

Figure 10. The effect of chilling on the phosphorus content in trichloroacetic acid and methanol fraction.

Statistical Analyses

The statistical analyses are indicated in the Tables (see Appendix). The coefficient of variation was less than 17 per cent and in some cases below 10 per cent which indicates the samples treated in a like manner were very uniform for such a biological study. This indicates that the seeds used were quite similar in the chemicals measured.

SUMMARY

1. A study of nucleic acid changes influenced by gibberellic acid and chilling treatments in peach seed was performed in an attempt to reach a better understanding of the mechanism involved in breaking seed dormancy.

2. Gibberellic acid and the chilling treatment increased the RNA content. These two treatments which break dormancy also increased RNA, suggesting a similar mechanism involving RNA. Chilled seeds contained more RNA than did the gibberellic acid treated seeds.

3. DNA content remained unchanged regardless of treatment.

4. Dry seed had a greater ribonuclease activity than with soaked seeds. Enzyme changes did not correlate well with the RNA content in gibberellic treated seeds.

5. Deoxyribonuclease activity was higher in dry seed than with soaked seeds. Enzyme activity change did not correlate well with the DNA content.

6. The phosphorus content of the seed in regard to the gibberellic acid and chilling treatments was difficult to evaluate. There were no major relationships established. Phosphorus in the methanol fraction from the chilled seed increased some as the storage period increased.

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APPENDIX

Table 1. The effect of gibberellic acid and water on various phosphorus fractions within peach seed. Data expressed as μg P/g dry weight

Days after treatment	Phosphorus fractions								
	Methanol	TCA	Lipid	RNA	DNA	Residue	Sum of fract.	Total Anal.	% Recovery
Water soaked									
0	407.75[a]	11478.84	17.98	35.45	8.18	----	11948.20	11613.75	102.8
1	387.38	13347.86	57.27	27.72	7.10	----	13827.33	13318.13	103.8
2	445.99	10276.25	41.08	33.99	8.68	----	10805.79	11395.26	94.8
3	438.53	11557.90	33.97	47.30	8.54	6.12	12092.36	11665.74	103.6
4	368.81	10582.69	18.57	29.74	7.71	9.65	11017.17	11450.48	96.2
5	555.47	10132.16	19.57	28.24	12.28	----	10747.72	10991.52	97.7
6	612.44	8151.88	20.56	30.57	9.56	5.72	8830.73	9596.34	92.0
7	496.33	8832.40	70.32	22.31	7.74	5.01	9384.11	10013.79	93.7
8	393.99	9805.14	23.20	35.18	7.61	4.27	10269.39	949.31	108.0
9	389.09	9258.27	96.15	19.02	8.88	----	9771.41	10785.57	90.6
10	382.52	9947.56	22.02	18.69	8.41	----	10379.20	11437.13	90.7
11	409.61	8551.00	26.17	28.68	7.88	7.15	9030.49	9535.55	94.7
12	378.66	8677.75	30.28	40.08	8.86	5.35	9140.98	9960.02	91.7
13	432.79	12376.89	44.11	29.08	12.53	----	12896.12	11621.75	110.9
14	415.34	8892.00	44.53	37.55	7.51	10.11	9407.04	9870.84	95.3

Table 1. Continued

Days after treatment	Phosphorus fractions								
	Methanol	TCA	Lipid	RNA	DNA	Residue	Sum of fract.	Total Anal.	% Recovery
GA soaked									
0	342.68	10969.36	15.93	28.05	10.77	----	11367.39	12112.62	93.8
1	373.81	11451.13	36.08	38.54	8.46	9.27	11917.29	11091.90	107.4
2	351.85	12314.22	18.31	19.21	8.49	----	12712.08	11527.53	110.2
3	509.60	10037.61	25.54	35.06	10.39	5.83	10644.63	11706.15	90.9
4	392.42	10520.95	15.80	28.75	12.82	10.57	10931.31	9961.48	110.0
5	405.95	9951.70	15.58	34.27	8.10	7.34	10422.99	10429.13	99.9
6	391.65	9690.81	33.14	25.85	11.07	6.11	10158.63	10286.65	98.7
7	365.20	8274.14	25.77	29.64	6.68	4.50	8705.93	9706.67	90.0
8	383.29	9854.11	16.42	19.70	7.67	----	10281.19	9374.65	109.0
9	281.04	10963.82	31.19	22.16	11.17	----	11309.38	10205.96	110.8
10	615.48	15644.62	31.61	43.08	21.69	10.27	16306.75	15027.85	108.9
11	388.58	10170.24	25.89	71.06	16.32	----	10672.09	13700.79	90.0
12	361.90	9613.23	30.49	70.26	12.45	4.57	10092.90	11256.71	90.0
13	429.52	13776.83	61.58	31.10	12.61	9.50	14321.14	14245.20	100.5
14	670.57	15471.11	55.42	41.37	12.34	----	16250.81	14711.38	110.4

[a]Each value represents 1 determination.

Table 2. The effect of chilling on various phosphorus fractions within peach seed. Data expressed as μg P/g dry weight

Weeks after treatment	Phosphorus fractions								
	Methanol	TCA	Lipid	RNA	DNA	Residue	Sum of fract.	Total Anal.	% Recovery
Unchilled									
0	360.42[a]	8931.20	55.08	19.66	8.70	----	9375.06	8138.30	115.1
2	481.71	13358.51	43.70	44.68	13.26	13.09	13954.95	12803.57	108.9
4	316.23	11278.69	52.59	30.93	9.30	9.40	11697.14	10701.37	109.3
6	230.53	11527.73	41.47	26.77	8.53	----	11835.03	10771.58	109.8
8	371.40	9019.98	87.00	25.47	8.70	4.76	9517.31	10535.74	90.3
10	463.50	13334.35	143.48	28.44	10.11	8.74	139882.62	12876.31	108.6
Chilled									
0	360.42	8931.20	55.08	19.66	8.70	----	9375.06	8138.30	115.1
2	438.75	12852.30	48.37	35.78	8.17	10.34	13393.71	12788.44	104.7
4	481.06	9447.49	35.91	37.54	9.03	4.85	10015.88	8851.01	113.0
6	619.02	9581.85	27.17	25.36	8.24	6.94	10268.58	10824.15	94.8
8	728.93	11204.88	25.29	27.57	9.19	8.29	12113.15	11258.76	107.5
10	567.91	11486.32	45.94	38.28	9.47	7.70	12155.62	10872.56	111.8

[a]Each value represents 1 determination.

Table 3. The effect of gibberellic acid, water soaking and chilling treatments on the RNA content of peach seed

Days after treatment	RNA measurements (μg/g dry weight)								
	GA treated seed			Water soaked seed			Untreated seed		
	1	2	3	1	2	3	1	2	3
0	787.06[a]	741.16	622.25	752.03	597.53	609.41	503.42	425.96	515.39
1	711.82	648.14	817.01	844.74	795.93	805.91	483.56	456.78	573.81
2	944.68	770.34	691.13	805.67	749.07	510.36	472.46	502.38	564.32
3	790.89	774.03	554.73	995.39	730.63	680.40	582.66	482.19	505.83
4	985 29	783.25	645.63	670.38	854.62	941.79	583.74	583.91	496.79
5	1162.51	832.99	1025.90	549.35	730.84	805.50	602.74	584.73	562.49
6	1063.86	810.20	953.52	730.85	671.94	647.87	512.46	608.73	601.72
7	885.66	885.83	909.35	785.00	715.75	903.15	486.72	589.64	537.85
8	1231.59	1308.83	908.27	881.13	643.05	735.79	442.65	612.00	496.84
9	974.60	761.72	869.77	721.00	713.09	907.35	512.44	474.69	528.35
10	909.80	1201.14	970.76	908.98	822.55	720.00	472.45	532.49	441.66
11	1179.09	1045.37	909.92	1009.62	835.51	798.57	495.73	535.04	563.96
12	1147.13	997.89	846.95	817.87	793.23	805.08	492.76	571.26	478.85
13	1738.04	1251.10	1066.10	925.34	879.80	1137.07	562.05	495.65	478.75
14	844.74	1134.83	1195.51	862.22	766.36	813.07	574.72	482.57	484.92

Table 3. Continued

Weeks after treatment	RNA measurements (μg/g dry weight)								
	Chilled seed			Unchilled seed			Untreated seed		
	1	2	3	1	2	3	1	2	3
0	504.63	536.78	509.24	552.19	498.76	511.77	420.77	498.22	595.16
2	1078.58	912.61	871.16	1151.83	942.81	866.71	603.42	469.43	499.81
4	1086.95	1070.55	1048.31	1014.88	756.33	701.47	582.07	495.44	645.40
6	1780.25	1351.46	1204.60	854.38	787.56	680.26	543.85	520.94	474.94
8	1284.35	1421.05	1530.83	940.93	694.22	797.35	600.74	582.32	481.38
10	1436.21	1632.20	1273.42	784.32	647.20	792.80	468.74	498.72	548.05

[a]Individual determination with 3 replications.

Table 4. The average RNA content in peach seed treated with gibberellic acid, soaked in water and chilled

A. RNA changes as affected by gibberellic acid and water treatments.

Treatment average	Ave. content (μg/g dry weight)
GA	940.0
H_2O	786.2
Dry seed	524.1
L.S.D. .05	82.1
.01	109.8

Days after treatment	Ave. content (μg/g dry weight)
0	617.1
1	681.9
2	667.8
3	677.9
4	727.3
5	761.9
6	733.5
7	744.3
8	810.0
9	718.1
10	775.5
11	820.5
12	772 3
13	948.2
14	795.4
L.S.D. .05	111.6
.01	162.4
C.V.	13.6%

Table 4. Continued

B. RNA changes as affected by chilling treatment

Treatment average	Ave. content (µg/g dry weight
Chilled	1115.7
Unchilled	776.4
Dry seed	529.4
L. S. D. .05	108.7
.01	149.3

Weeks after treatment	Ave. content (µg/g dry weight)
0	520.7
2	838.5
4	813.1
6	585.1
8	909.4
10	903.4
L. S. D. .05	90.6
.01	149.3
C. V.	10.4%

Table 5. The effect of gibberellic acid, water soaking and chilling treatments on the DNA content of peach seed

Days after treatment	DNA measurements (µg/g dry weight)								
	GA treated seed			Water soaked seed			Untreated seed		
	1	2	3	1	2	3	1	2	3
0	489.13[a]	245.77	335.11	460.96	214.87	305.88	302.13	262.75	238.75
1	262.04	222.29	318.95	478.20	333.26	384.20	218.74	315.28	200.35
2	296.17	214.41	268.75	323.49	397.82	402.88	256.25	234.10	327.90
3	313.41	340.69	302.76	287.17	326.58	318.26	198.93	266.42	204.15
4	282.11	319.39	380.20	248.06	318.42	315.28	242.68	292.54	261.29
5	263.20	261.97	356.06	218.96	308.91	347.40	182.54	265.89	265.56
6	244.98	285.66	370.04	175.28	244.01	329.60	224.73	296.16	235.49
7	277.75	216.35	330.60	254.84	222.47	163.48	301.25	261.74	225.64
8	218.95	214.25	199.29	331.79	225.92	272.92	214.75	259.79	298.71
9	263.40	203.66	208.27	272.29	249.45	337.19	283.64	168.29	301.22
10	259.89	214.73	332.64	266.72	225.17	244.59	295.41	341.80	199.77
11	232.25	344.87	234.41	272.63	345.52	297.32	286.74	238.00	243.51
12	179.89	266.66	346.86	221.06	252.72	306.89	198.86	277.60	306.54
13	320.90	387.37	523.54	225.08	297.23	439.02	286.72	380.92	262.49
14	368.93	389.50	368.52	306.01	316.70	288.40	242.35	266.90	297.74

Table 5. Continued

Weeks after treatment	DNA measurements (µg/g dry weight)								
	Chilled seed			Unchilled seed			Untreated seed		
	1	2	3	1	2	3	1	2	3
0	215.26	237.42	225.81	235.55	254.67	199.69	273.48	205.42	324.53
2	240.62	198.14	168.03	301.46	244.40	261.79	213.32	214.32	335.74
4	243.38	152.05	136.73	329 54	221.03	215.17	310.42	267.48	270.56
6	260.92	163.22	166.27	254.90	196.15	198.76	296.78	212.56	234.86
8	288.45	180.52	205.33	281.86	294.28	212.92	302.42	243.62	288.53
10	216.29	226.76	248.53	166.52	175.18	244.04	240.40	212.42	303.63

[a]Individual determination with 3 replications.

Table 6. The average DNA content in peach seed treated with gibberellic acid, soaked in water and chilled

A. DNA changes as affected by gibberellic acid and water treatments

Treatment average	Ave. content (μg/g dry weight)
GA	295.1
H_2O	297.2
Dry seed	262.6
L.S.D. .05	N.S.

Days after treatment	Ave. content (μg/g dry weight)
0	317.3
1	313.0
2	302.4
3	284.4
4	295.6
5	274.5
6	267.3
7	250.5
8	248.5
9	254.2
10	264.5
11	277.3
12	261.9
13	347.0
14	316.1
L.S D .05	N.S.
C.V.	16.1%

Table 6. Continued

B. DNA changes as affected by chilling treatment

Treatment average	Ave. content (μg/g dry weight)
Chilled	238.2
Unchilled	210.3
Dry seed	263.9
L.S.D. .05	N.S.

Weeks after treatment	Ave. content (μg/g dry weight)
0	241.3
2	241.9
4	238.5
6	220.5
8	256.6
10	226.0
L.S.D. .05	54.3
.01	79.0
C.V.	12.5%

Table 7. RNase measurements made in this study

Days after treatment	RNase measurements (ΔOD 260 mμ/mg protein/30 min)							
	GA treated seed				Water soaked seed			
	1	2	3	4	1	2	3	4
0	0.0402[a]	0.0462	0.0568	0.0494	0.0454	0.0516	0.0394	0.0448
1	0.0508	0.0394	0.0676	0.0526	0.0496	0.0614	0.0448	0.0628
2	0.0400	0.0572	0.0540	0.0378	0.0414	0.0620	0.0848	0.0566
3	0.0600	0.0650	0.0312	0.0338	0.0562	0.0550	0.0398	0.0300
4	0.0384	0.0366	0.0346	0.0368	0.0428	0.0428	0.0446	0.0446
5	0.0468	0.0512	0.0444	0.0486	0.0390	0.0424	0.0396	0.0364
6	0.0660	0.0640	0.0684	0.0664	0.0342	0.0330	0.0378	0.0366
7	0.0274	0.0274	0.0222	0.0222	0.0340	0.0326	0.0238	0.0230
8	0.0530	0.0578	0.0604	0.0660	0.0476	0.0388	0.0192	0.0234
9	0.0490	0.0564	0.0490	0.0564	0.0330	0.0246	0.0320	0.0430
10	0.0620	0.0582	0.0582	0.0548	0.0384	0.0434	0.0416	0.0368
11	0.0592	0.0570	0.0708	0.0732	0.0482	0.0660	0.0628	0.0458
12	0.0880	0.0856	0.0828	0.0800	0.0702	0.0702	0.0690	0.690
13	0.0686	0.0712	0.0790	0.0737	0.0668	0.0550	0.0856	0.0692
14	0.0720	0.0700	0.0690	0.0790	0.0654	0.0640	0.0588	0.0576

Table 7. Continued

Weeks after treatment	RNase measurements (Δ OD 260 mμ/mg protein/30 min.)											
	Chilled seed				Unchilled seed				Untreated seed			
	1	2	3	4	1	2	3	4	1	2	3	4
0	0.0426	0.0426	0.0426	0.0426	0.0426	0.0426	0.0426	0.0426	0.0903	0.0845	0.0721	0.0899
2	0.0316	0.0316	0.0170	0.0170	0.0288	0.0288	0.0336	0.0336	0.0912	0.0975	0.0892	0.0761
4	0.1264	0.0964	0.1240	0.0940	0.0600	0.0618	0.0572	0.0590	0.0832	0.0892	0.0783	0.0885
6	0.0872	0.0872	0.0872	0.0872	0.0550	0.0532	0.0552	0.0536	0.0906	0.0821	0.0876	0.0749
8	0.0732	0.0732	0.0766	0.0766	0.0386	0.0386	0.0428	0.0428	0.0921	0.0894	0.0801	0.0834
10	0.0770	0.0770	0.0770	0.0770	0.0532	0.0496	0.0424	0.0410	0.0908	0.0786	0.0732	0.0862

[a]Individual determination with 4 replications.

Table 8. The average RNase content in peach seed treated with gibberellic acid, soaked in water and chilled

A. RNase as affected by gibberellic acid and water treatments

Treatment	Ave. content ($\triangle$OD 250 mμ/mg protein/30 min.)
GA	0.056
H_2O	0.048
L.S.D. .05	0.006
.01	0.009
Days after treatment	**Ave. content ($\triangle$OD 260 mμ/mg protein/30 min.)**
0	0.047
1	0.054
2	0.054
3	0.046
4	0.040
5	0.044
6	0.051
7	0.027
8	0.046
9	0.043
10	0.049
11	0.060
12	0.077
13	0.071
14	0.066
L.S.D. .05	0.011
.01	0.016
C.V.	13.9%

Table 8. Continued

B. RNase as affected by chilling treatment

Treatment	Ave. content (△OD 260 mμ/mg protein/30 min.)
Chilled	0.069
Unchilled	0.046
Dry seed	0 085
L.S.D. .05	0.004
.01	0.006

Weeks after treatment	Ave. content (△OD 260 mμ/mg protein/30 min.)
0	0.057
2	0.048
4	0.085
6	0.075
8	0.067
10	0.069
L.S.D. .05	0.004
.01	0.006
C.V.	10.0%

Table 9. DNase measurements made in this study

Days after treatment	DNase measurement (ΔOD 260 mμ/mg protein/30 min.)							
	GA treated seed				Water soaked seed			
	1	2	3	4	1	2	3	4
0	0.0488[a]	0.0452	0.0420	0.0390	0.0383	0.0438	0.0346	0.0396
1	0.0456	0.0609	0.0500	0.0375	0.0400	0.0557	0.0400	0.0323
2	0.0329	0.0472	0.0452	0.0316	0.0460	0.0600	0.0630	0.0482
3	0.0458	0.0496	0.0453	0.0417	0.0475	0.0413	0.0431	0.0417
4	0.0372	0.0354	0.0340	0.0357	0.0344	0.0353	0.0358	0.0369
5	0.0356	0.0388	0.0396	0.0433	0.0324	0.0340	0.0394	0.0376
6	0.0648	0.0625	0.0648	0.0625	0.0436	0.0412	0.0425	0.0402
7	0.0265	0.0218	0.0222	0.0275	0.0294	0.0280	0.0334	0.0317
8	0.0755	0.0690	0.0630	0.0690	0.0551	0.0551	0.0449	0.0449
9	0.0391	0.0447	0.0405	0.0463	0.0416	0.0314	0.0300	0.0398
10	0.0555	0.0518	0.0505	0.0470	0.0476	0.0538	0.0518	0.0459
11	0.0698	0.0672	0.0658	0.0635	0.0470	0.0642	0.0765	0.0558
12	0.0789	0.0716	0.0688	0.0666	0.0700	0.0700	0.0675	0.0675
13	0.0801	0.0761	0.0801	0.0761	0.0710	0.0621	0.0675	0.0552
14	0.0606	0.0535	0.0606	0.0535	0.0691	0.0675	0.0791	0.0675

Table 9. Continued

Weeks after treatment	DNase measurement (△OD 260 mμ/mg protein/30 min.)											
	Chilled seed				Unchilled seed				Untreated seed			
	1	2	3	4	1	2	3	4	1	2	3	4
0	0.0374	0.0435	0.0393	0.0346	0.0374	0.0435	0.0393	0.0346	0.0691	0.0565	0.0556	0.0604
2	0.0698	0.0690	0.0760	0.0760	0.0719	0.0719	0.0631	0.0691	0.0580	0.0580	0.0480	0.0480
4	0.0482	0.0632	0.0615	0.0470	0.0309	0.0300	0.0296	0.0287	0.0472	0.0507	0.0593	0.0572
6	0.0768	0.0768	0.0794	0.0794	0.0539	0.0565	0.0555	0.0530	0.0662	0.0632	0.0567	0.0543
8	0.0563	0.0560	0.0600	0.0600	0.0304	0.0304	0.0296	0.0296	0.0780	0.0703	0.0631	0.0598
10	0.0765	0.0765	0.0749	0.0749	0.0580	0.0584	0.0595	0.0600	0.0518	0.0681	0.0514	0.0635

[a]Individual determination with 4 replications.

Table 10. The average DNase content in peach seed treated with gibberellic acid, soaked in water and chilled

A. DNase as affected by gibberellic acid and water treatments

Treatment		Ave. content (Δ OD 260 mμ/mg protein/30 min.)
GA		0.052
H_2O		0.048
L. S. D.	.05	0.003
	.01	N. S.

Days after treatment	Ave. Content (Δ OD 260 mμ/mg protein/30 min.)
0	0.041
1	0.045
2	0.047
3	0.044
4	0.036
5	0.038
6	0.053
7	0.028
8	0.060
9	0.039
10	0.051
11	0.064
12	0.070
13	0.071
14	0.003
L. S. D. .05	0.007
.01	0.011
C. V.	7.2%

Table 10. Continued

B. DNase as affected by chilling treatment

Treatment	Ave. content (OD 260 mu/mg protein/30 min.)
Chilled	0.063
Unchilled	0.047
Dry seed	0.059
L. S. D. .05	0.0038
.01	0.0042

Weeks of treatment	Ave. content (OD 260 mu/mg protein/30 min.)
0	0.046
2	0.065
4	0.046
6	0.064
8	0.052
10	0.065
L. S. D. .05	0.003
.01	0.004
C. V.	8.8%