5-1966

A Study of Amino Acid, Protein, Organic Acid and Carbohydrate Changes Occurring During Germination of Peach Seeds

Lee Chao
Utah State University

A STUDY OF AMINO ACID, PROTEIN, ORGANIC ACID AND CARBOHYDRATE CHANGES OCCURRING DURING GERMINATION OF PEACH SEEDS

by

Lee Chao

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Nutrition and Biochemistry

UTAH STATE UNIVERSITY
Logan, Utah

1966

ACKNOWLEDGMENT

The writer expresses sincere appreciation to those who made this study possible.

Special appreciation is expressed to Dr. David R. Walker, the writer's major professor and advisor, for his assistance, encouragement, and invaluable suggestions during this study.

The writer is deeply indebted to the members of the writer's advisory committee, Dr. Gene W. Miller, and Dr. Anthony T. Tu, for their timely advise and helpful suggestions.

The writer is greatful to his parents for their encouragement throughout the graduate study.

Lee Chao

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Conditions required for seed germination are different among species. Some seed of tropical and subtropical plants may germinate before the maturation of their fleshy fruit, e.g. papaya species, while seed from most deciduous trees have a period of after-ripening before germination. The after-ripening period, also referred to as rest or dormancy has also been observed in some vegetable crops and ornamental flowers.

Under natural conditions, light is absolutely required for the germination of lettuce seed, whereas low humidity is required for the germination of garden balsam, Impatiens balsamina. Tubers of Solanaceae require low oxygen content for germination. Among the factors required for breaking the rest period, the most important two are low temperature and moisture and have been the two most intensively studied.

In certain plants, this inability of germination is due to a hard seed coat impermeable to water (e.g. Canaceae, Corvallariceae, Malvaceae and Leguminoceae). A satisfactory percentage of germination would be reached if this seed coat barrier was removed. There are some plant species somewhat different, their inability of germination is not due wholly to a hard seed coat (e.g. Rosaceae, Juniperus virginian and garden beet). This type of seed will start

to germinate only when a certain amount of low temperature and a suitable amount of moisture are provided (e.g. stratification). The former is termed dormant seed and the later is termed resting seed.

Biochemical and physiological changes during the period of stratification have not been extensively investigated from different points of view. Most studies suggest that a large amount of lipids break down to fatty acids through B-oxidation and are incorporated into the tricarboxylic acid cycle via the glyoxylic acid cycle. It is also evident that most of the insoluble fraction of carbohydrates and nitrogenous compounds are converted to soluble sugars and amino acids. Some of the enzymes that catalyze these changes have been isolated and identified.

The processes of growth are basically due to cell division, cell differentiation and cell enlargement, hence it is evident that a certain amount of soluble nutrients must be supplied to the actively dividing growing point.

Some early studies (Luckwill, 1952) have indicated that some growth-inhibiting chemicals are present in resting plants. This has led to a conclusion that growth and rest are controlled by an auxin/inhibitor balance.

Recent studies (Trelawny and Ballantyne, 1963) show a stimulating effect of gibberellic acid on seed germination. This demonstrated a physiological role of gibberellic acid in that it replaced the cold requirement of some resting seeds and induced germination.

The objective of this study was to evaluate the biochemical changes occurring during cold and gibberellic acid treatment of peach seeds. The biochemical pattern in normal, dwarfed, and gibberellic acid induced germinating peach seed was compared in order to more fully understand the mechanism of rest.

REVIEW OF LITERATURE

Growth Substances and the Rest Period

In a study of the rest period and it's physiological and chemical interactions Denny and Stanton (1928) reported that tree buds remain in a resting condition even when roots, bark and conductive tissues are supplied large quantities of nutrients. They found also, that ethylene chlorohydrin, ethylene dichloride and ethyl iodine could to a certain extent, break this resting condition.

Luckwill (1952) extracted a growth-inhibiting substance from mature apple seeds. This substance gradually disappeared and a growth-promoting substance appeared prior to seed germination. He concluded that the formation of the growth promoting substance may be necessary to break seed dormancy.

Donoho and Walker (1957) reported that gibberellic acid apparently activated the metabolic processes or nullified the effect of an inhibitor on growth of young Elberta peach trees and seeds, and thus replaced the cold requirement for breaking the rest period.

Blommaert (1959) reported that a growth inhibiting substance in peach buds decreased when dormant trees were subjected to low temperature treatment, and concurred that the rest period was controlled by an auxin/inhibitor balance.

Hendershott and Walker (1959) identified a chemical, naringenin, as a growth inhibitor from dormant peach flower buds. In another article (1959b), they reported that naringenin was present in high concentrations in peach flower buds in August, decreased in October, increased in November and remained rather high during December, January, February. The inhibitor decreased during March and completely disappeared from the buds about two weeks before bloom. This investigation indicated a close relationship between the growth inhibitor and the rest of peach buds. Dennis and Edgerton (1961) also confirmed the presence of naringenin in peach buds and that it inhibited Avena coleoptile growth but they failed to correlate it with rest. Corgan (1965) also reported that the naringenin content in peach flower buds was high throughout the dormant season, and remained at a high level more than 30 days after the rest was terminated. He concluded that the dilution of naringenin occurred when buds expanded, and that some naringenin loss may have occurred during flowering.

Philips (1961) examined the interaction between naringenin and gibberellic acid in lettuce seed germination and reported that naringenin induced the light requirement of lettuce seeds. This effect could be reversed by gibberellic acid. He further noticed (1962) that when various concentrations of naringenin in gibberellic acid solution

were applied to dormant peach buds, a competitive effect on growth of the dormant tissues between naringenin and gibberellic acid occurred.

Flemion and de Silva (1960) reported that both growth promoting and inhibiting substances were obtained from peach seeds by paper chromatographic separation. The growth reaction was measured by the wheat coleoptile test. No direct evidence, however, has linked these growth substances with seed germination. They also reported that the concentration and translocation of amino acids, organic acids and phosphates in seeds were increased gradually under low temperature treatment (90 days at 5°C). Dwarfed seedlings appeared when treated under inadequate cold conditions (shorter than 90 days at 5 C).

Kawase (1961) reported that Betula Pubescens Ehrh. produced a growth inhibiting substance in leafy buds during short day treatment, which finally led to the dormancy of the buds. When dormant plants were subjected to long day or to gibberellic acid treatment, the growth inhibiting substance decreased and a growth promoting substance appeared, as measured by the wheat coleoptile growth test. The long day treatment also led to the breaking of dormancy, but there was no evidence that changes in growth promoting substances were the primary agents affecting dormancy in Betula.

The work of Trelawny and Ballantyne (1963) indicated that excised embryos from Moluccella laevis L. germinated

without chilling treatment, but the pericarp prevented the germination of unchilled seeds. Endosperm may have also acted as a mechanical barrier. Both the endosperm and pericarp barrier was eliminated by gibberellic acid treatment.

Physiological Effects of Growth Substances

The term growth substances includes growth promoting substances such as auxins, gibberellins, and kinins, while the term growth retarding substances include nicotinium compounds, quaternary ammonium carbamates, phosphonium compounds, choline analogues, and other naturally occurring compounds. Only the physiological effect of auxins and gibberellic acid are discussed here.

Physiological effects of auxins

Heyn (1940) reported that auxins increase the wall elasticity of many kinds of cells. The same results were confirmed by Cleland (1958) and by Tagawa and Bonner (1957) using Avena coleoptile cells.

There is evidence indicating that auxin induces pectic substance synthesis in vivo (Galston and Purves, 1960). Glasziou (1958) suggested that the growth promoting activity of auxin results from an increased binding of pectin methyl esterase, thus protecting pectin from de-esterification and maintaining cell wall plasticity.

Siegel et al. (1960) has shown that auxin inhibited peroxidase mediated lignification in a model system, and thus could conceivably serve as a mechanism of auxin induced cell elongation.

Auxin stimulates water uptake in many kinds of cells (Galston and Purves, 1960). Bonner et al. even suggested that auxin-induced water uptake against an osmotic gradient.

Masuda (1955) claimed that auxin increased the permeability of Avena coleoptile cells to water and non-electrolytes. It has also been established that auxin promotes the uptake of ions by various types of cells (Commoner and Mazia, 1942; Hanson and Bonner, 1954).

Christiansen et al. (1954) and Reinhold and Powell (1958) reported that auxin stimulated the uptake of amino acids and excretion of ammonia by cells. It was also indicated by Reinhold (1958) that auxin may affect the differential permeability of cell membranes.

Among other physical properties studies, Northen (1942) was the first to observe that auxin reduced the protoplasmic viscosity of cells of bean plant. Thimann and Sweeney (1942) also reported that auxin promoted the protoplasmic streaming of Avena coleoptile cells.

Galston and Purves (1960) summarized the effect of auxins as: indirectly increasing the respiration rate, reduction of ascorbic acid, reduction of glutathione, and related to the action of sulfhydryl containing enzymes.

In the same article they also suggested interactions between auxin and nucleic acids. At the present time, however, this has not been established.

Physiological effects of gibberellic acid

The physiological effect of gibberellic acid has been studied extensively from many different points of view. From anatomical studies of seedlings of Vigna sesquipedalis Kato (1955) reported that gibberellic acid induced growth in a longitudinal rather than in the transverse direction and that the elongation was a consequence of accelerated cell elongation rather than of cell multiplication. Similar results have been observed by Barton (1956), that gibberellic acid stimulates the extension of internodes of Malus arnoldiana from non-after ripened embryos.

Paleg et al. (1962a) reported that the embryo is responsible for the initiation of the food mobilization processes, which occur in barley endosperm during germination. Gibberellic acid hastens the response of endosperm in the presence of normal or damaged embryos and can initiate the food mobilization processes when the embryo is absent. They further postulated the possibility of an endogenous gibberellin as the endosperm mobilizing hormone (1962b).

The relation among gibberellic acid and other growth substances has become an increasingly interesting problem in recent years. Boo (1961) reported that the treatment

of resting potato tubers with an aqueous solution of gibberellic acid reduces the B inhibitor content of the tuber and thus breaks the rest.

Muir and his co-workers (1964a) studied the relationship between gibberellic acid and auxin level and reported that gibberellic acid causes an increase in diffusible auxin from the stem apex of dwarf pea, and subsequently promotes growth. They further reported (1964b, 1965) that the increase in diffusible auxin in the dwarf pea following treatment with gibberellic acid did not involve inhibition of IAA oxidase, rate of transport of basipetal auxin, decrease in growth inhibitor, formation of a complex between gibberellic acid and auxin, nor the enzymatic conversion of tryptophan to ether-soluble auxin. They demonstrated that the tryptophan conversion system from plants treated with gibberellic acid formed four times more water-soluble auxin than did the enzyme preparation from control plants.

Chemical Changes Occurring During Seed Germination

Amino acids and proteins

An increase in free amino acids, both in type and concentration is expected in germinating seeds, especially the metabolically important amino acids. It has been observed (Boulter and Barber, 1963) that glutamic and aspartic acids increase markedly in germinating *Vicia faba*

L. Both of these acids are involved in purine metabolism in other organisms, and aspartic acid is known to be a precursor of pyrimidine.

During germination of barley grain, Folkers and Yemm (1958) found that amino acids liberated by the breakdown of the endosperm proteins were translocated to the embryo and were then synthesized into embryo proteins; amino acids in excess of requirements by the embryo were transformed into aspartic acid, alanine, glycine, lysine, arginine, together with nitrogen bases and chlorophylls. These workers were specifically interested in glutamic acid, which may be involved in the synthesis of some 16 of the commoner amino acids.

Sivaramakrishnan and Sarma (1956) reported that during the germination of Phaseolus radiatus, glutamic acid was mainly degraded into aspartic acid and asparagine, and partially converted into arginine and proline. At the same time glutamic acid was synthesized from carbohydrates, especially glucose. The rapid degradation and the extensive synthesis of this acid clearly indicates its high metabolic activity in germinating seeds.

Concerning the rest problem, Barton and Bray (1962) reported that both aspartic and glutamic acids increased markedly in after-ripened tree peony seed embryos. Alanine and glutamine were also in large quantities in tissues of seedlings held at 5° C compared with those in the greenhouse. There was a rapid decrease in total protein content of

endosperm tissues in the seedlings held at lower temperature than those held at higher temperature.

More recently Paleg (1965) reported that gibberellic acid stimulates enzymatically active protein synthesis in green malt. Rai and Laloraya (1965) suggested that gibberellic acid replaced the light requirement for the germination of lettuce seeds by enhanced mobilization of reserve nitrogen from cotyledons to the growing axis of lettuce seedlings.

Organic acids

Organic acid metabolism is closely linked to sugar, amino acid, and fatty acid metabolism, and also serves as an important pool for many metabolic activities. Barton (1961) reported that malic acid accumulated in the endosperm of dormant Paeonia suffruticosa seedlings to a greater extent than in seedlings after-ripened at 5°C. Embryos held at greenhouse temperature for 4 to 8 weeks also contained more malic acid than those held at 5°C. The citric acid content present in dry endosperm tissue increased upon absorption of water by the seed, and then remained fairly constant up to 12 weeks after planting of the germinated seeds.

Sugars and starch

Since the reserve food is mainly in the form of starch or protein, resting seeds usually contain only

small amounts of soluble sugar. The release of starch and the increase of sugar supply the germinating seed both metabolic substances and an energy source.

Barton (1961) studied the chemical changes in dormant and after-ripened Paeonia suffruticosa and reported that fructose increased in the seedlings held at 5°C compared to those at higher temperatures in the greenhouse. Glucose content of tissues at the two temperatures was the same, but there was a decrease of sucrose in the endosperm with an increase of sucrose in the embryo at both temperatures.

Gibberellic acid markedly affected the starch-sugar conversion during seed germination. Clegg and Rappaport (1965) reported that gibberellin A_3 quickly stimulates respiratory activity of intact resting potato tubers, and specifically, stimulated the release of reducing sugars in excised barley endosperm. Flemion and Topping (1963) compared the starch content of normal and dwarf peach seedlings and concluded that the dwarf seedlings were apparently unable to utilize the reserve starch for development.

Nanda and Purohit (1965) reported that the gibberellic acid enhanced internodal elongation of Salmalia malabarica goes hand in hand with the disappearance of starch from corresponding untreated internodes. They also suggested gibberellic acid treatment resulted in the conversion of starch to sugar which becomes readily available for elongation of cells. The conversion is induced by

gibberellic acid enhancing the hydrolytic enzyme activities. Verner (1964) was able to demonstrate the enhanced α-amylase production by gibberellic acid in excised barley endosperm. This was also confirmed by Paleg and Hyde (1964) with barley aleurone cells.

Lipid substances

Lipid substances are reserve food for seed germination, and are also an important constituent of the cell wall and protoplasm. Its metabolism is not fully understood. Ching (1963) reported that total fats decreased rapidly with germination of Douglas fir seeds from 36 per cent to 12 per cent of the dry weight, which was a decrease from 13.1 mg. to 12.1 mg. per individual seed.

It has also been confirmed (Penny and Stowe, 1965) that exogenous lipids may induce growth and respiration in pea stem sections, since the breakdown of lipids to fatty acids may subsequently participate in carbohydrate and amino acid metabolism through the citric acid cycle.

MATERIALS AND METHODS

Treatment of Seeds

Unchilled peach seeds with pericarp removed were used in this study. The majority of them were soaked in water (25 C) for 10 hours before treatment. One lot was analyzed without water soaking as an untreated control.

After water soaking the seeds were divided into two groups for temperature and chemical treatment.

One group was subjected to different temperature treatments, namely; 32 F, 45 F and 72 F. Seeds were removed from each temperature treatment at two week intervals for a period of 10 weeks. A portion of the seeds were analyzed for amino acids, proteins, organic acids, carbohydrates and crude lipid substances while the remainder were placed in the greenhouse for germination and growth observations.

The second group of seeds were soaked for an additional hour in either distilled water, or a 2,000 ppm water solution of gibberellic acid (GA active ingredient 80 per cent, Merck & Co., Inc., Rahway, New Jersey). The seeds were analyzed for the above constituents at 30, 60, 90, 120, 170 hours after treatment. Another portion of these seeds were placed in the greenhouse for growth observations.

Preparation of Sample

Ten grams of seeds were used for each sample analyzed for the above constituents. In order to remove most of the lipid substances for amino acid and organic acid analysis, the seeds were macerated in an Omni mixer with 25 ml. of benzene, allowed to stand 30 minutes and centrifuged. The benzene was decanted and the insoluble residue was mixed with 85 per cent ethanol (pH 7) and ground thoroughly in the Omni mixer. The mixture was recentrifuged after a 10 hour extraction period. The supernatant solution was removed and the precipitate washed and centrifuged twice with additional ethanol. The ethanol solutions were combined and stored at 38 F.

The precipitate was then placed in a filter-paper thimble and extracted further with 85 per cent hot ethanol at 75 C for 48 hours in a Soxhlet apparatus.

The cold and hot extracts were combined and evaporated to dryness under reduced pressure below 30 C.

Separation of Total Amino Acids

The amino acids were separated from other constituents by use of resin columns. The resin was prepared as follows: Dowex 50W-X8 (200 to 400 mesh) cation exchange resin was soaked overnight in water, after which it was stirred with distilled water. Fine particles were removed by decanting the supernatant after 30 minutes. The process was repeated several times.

Dowex was heated for 16 hours at 100 C with 2 volumes of 1 N sodium hydroxide, and then poured into a column and drained. After the resin was washed with deionized water to remove the excess sodium hydroxide, it was treated with 6 N hydrochloric acid. The column was flushed with deionized water until the effluent was free of chloride ions (Thompson et al., 1959).

The Dowex cation exchanger in hydrogen form was introduced into a chromatographic column (10X200 mm.), and the aqueous solution (pH 7) of the total extracts was poured onto the column. Amino acids were retained by the column and were later eluted with 2 N ammonium hydroxide (Block et al., 1955). The percentage of recovery was 96 to 98.5 per cent when tested with a mixture of crystalline amino acids.

The total amino acids eluted by ammonium hydroxide was taken to dryness at room temperature and redissolved with 20 ml. of citrate buffer solution (pH 2.2). This sample was analyzed for amino acids with an amino acid analyzer in the chemistry department.

Separation of Total Organic Acids and Sugars

Rexyn CG 1 chloride form anion exchanger was suspended in distilled water and fine particles were decanted. To change it into the formate form, the Rexyn was washed several times with 6 M formic acid until it gave a negative

reaction to a chloride test. The Rexyn was then packed into a chromatogarphic column (12X150 mm.).

The effluent from the cation exchange column was poured onto this anion exchange column and organic acids were retained on this column. The total organic acid was eluted from this column by 6 M formic acid followed by three portions of deionized water washing (Jorysch et al., 1962). The percentage of recovery was 100 to 102 per cent as tested by a mixture of crystalline organic acids.

The organic acids in formic acid were evaporated in room temperature to dryness and stored in a desiccator for further separation.

The effluent from the anion exchange column was made to 100 ml. from which an aliquot was taken for sugar analysis (Colombo et al., 1960). The percentage of recovery of sugars was 94 ±2 per cent as tested by a mixture of crystalline sugars.

Determination of Individual Constituents

Separation of the individual amino acids was accomplished using the amino acid analyzer and the procedures of Moore et al. (1958) and Spackman et al. (1958). A sample containing 0.5 to 1 micromole per amino acid was first dissolved in pH 2.2 sodium citrate buffer and poured on top of the long column (Amberlite IR-120, 0.9X150 cm.) maintained at 50 C. Acidic and neutral amino acids were then subsequently eluted with pH 3.25 and pH 4.25 sodium

citrate buffers. For basic amino acid separation, the same amount of amino acid solution used in the long column was poured on top of the short column (Amberlite IR-120, 0.9X15 cm.) which was maintained also at 50 C, and eluted with pH 5.28 sodium citrate buffer. The effluent was then mixed with ninhydrin and pumped through a boiling water bath. The absorbance of the resulting solution was measured continuously at 570 and 440 mu, and peaks were recorded by a current recorder on graph paper. The quantity of individual amino acids was determined by integration of the area under the peaks.

A portion of the residues from the Soxhlet extraction was digested with 2 N sodium hydroxide at an elevated temperature. The supernatant solution obtained from the digestion was used for protein determination by the Folin-Ciocalteau method based upon the procedures of Bailey (1962) and Miller (1959).

Organic acids were separated both by paper and column chromatography. Paper chromatographic identification was based upon Buch's method (1952). A modified method of Bulen et al. (1952) was used for silica gel partition column chromatography. Boe's (1965) method was used for silica gel preparation, and the chromatographic apparatus was modified according to Marshall et al. (1952).

Dowex 1-X8 (borate form) was used for column separation of sugars as outlined by Syamananda (1962), except Parr's (1954) continuous eluting apparatus was used in place of

an automatic recording apparatus. Qualitative identification of sugars on paper chromatograms was according to Block et al. (1955).

Starch was determined spectrophotometrically after reacted with anthrone reagent, according to a modified method used by McCready (1952) and Viles and Silverman (1949).

Air dried fresh samples were analyzed for lipid content. The procedures were similar to those outlined by Wistreich et al. (1960).

Due to the complexity and precision of the amino acid analyzer, no replication was made on amino acid analysis. Three replications were made on starch and lipid analysis. The coefficient of variation was 8.55 per cent for proteins, 6.69 per cent for starch and 34.98 per cent for lipid materials. Three replications were made on sugar analysis. The coefficient of variation was 13.48 per cent for sucrose, 10.31 per cent for glucose and 4.69 per cent for fructose respectively. Two replications were made on organic acid analysis.

RESULTS AND DISCUSSION

Amino Acids

An increase in total free amino acid content was observed in all treatments with additional storage time. The total free amino acids increased three fold in seed held at 72 F as compared to the dry sample after 8 weeks of storage (Table 1). Free amino acids also increased three fold in seed held for 10 weeks at 32 F compared with those in the dry sample (Table 1 and 2), and ten fold after 10 weeks at 45 F compared with the dry sample (Table 1 and 3). The 45 F treatment was regarded as the optimum for breaking the rest. Gibberellic acid replaced the cold treatment and induced germination, it also resulted in an eight fold increase of total free amino acids above the dry sample within 170 hours after treatment (Table 1 and 4).

Glutamic acid was the most abundant amino acid present in the seed except in the case of seed held at 72 F. At this temperature, aspartic acid was the most abundant one (Table 1). Other metabolically important amino acids, such as proline, aspartic acid, alanine, serine and arginine were also present in comparatively large amounts.

In correlating amino acid content, seed germination and seedling morphology, it was interesting to note that the seed stored at 32 F grew abnormally, indicating the rest

Table 1. Amino acid changes in peach seed during storage at 72 F (Micromoles per gram fresh weight).

	Dry Seed	Water Soaked Seed	Seed held at 72 F	
			2 Weeks	8 Weeks
Glutamic Acid	0.39	1.54	3.02	1.41
Proline	nil	0.43	0.58	0.59
Aspartic Acid	0.38	1.90	3.24	2.42
Alanine	0.22	1.84	0.61	1.34
Serine	0.16	0.29	0.29	0.65
Arginine	2.23	0.10	0.22	0.12
Phenylalanine	0.08	----	0.47	0.83
Valine	0.14	0.13	0.43	0.99
Glycine	0.37	0.47	0.38	0.97
Isoleucine	0.17	0.01	0.31	0.69
Leucine	0.18	0.01	0.23	1.01
Lysine	----	0.15	0.16	0.31
Methionine	0.22	----	0.07	0.45
Tyrosine	0.06	nil	0.08	0.42
Threonine	0.14	0.22	0.27	0.70
Histidine	----	0.06	0.06	0.17
Cystine	nil	nil	0.09	nil
Total Amino Acid	4.73	7.14	10.46	14.96
Ammonia	0.06	3.80	3.05	1.98

Table 2. Amino acid changes in peach seed during storage at 32 F (Micromoles per gram fresh weight).

	Time Interval at 32 F				
	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Glutamic Acid	0.21	1.36	3.14	4.08	5.47
Proline	nil	0.09	0.38	0.32	0.75
Serine	0.08	0.31	0.56	0.19	0.78
Alanine	0.25	0.29	0.91	1.39	0.71
Aspartic Acid	0.14	1.33	2.68	0.63	0.51
Phenylalanine	0.24	0.56	0.40	0.66	0.51
Valine	0.44	1.03	0.44	0.89	0.48
Glycine	0.24	0.79	0.43	0.85	0.43
Isoleucine	0.22	0.57	0.32	0.41	0.37
Leucine	0.32	0.79	0.30	0.44	0.33
Arginine	0.19	0.19	0.20	0.22	0.31
Threonine	0.12	0.68	0.28	0.16	0.27
Tyrosine	0.13	0.29	0.12	0.25	0.12
Lysine	0.17	0.18	0.09	0.25	0.09
Methionine	0.14	0.19	0.06	----	0.05
Cystine	nil	nil	----	----	0.11
Histidine	0.67	0.07	0.08	0.19	0.03
Total Amino Acid	5.03	10.27	10.37	10.93	11.29
Ammonia	5.71	1.60	2.67	2.87	3.03

Table 3. Amino acid changes in peach seed during storage at 45 F (Micromoles per gram fresh weight).

	Time Interval at 45 F				
	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks
Glutamic Acid	5.48	7.14	20.36	13.52	21.69
Proline	0.88	3.89	6.42	3.40	6.84
Aspartic Acid	2.54	3.29	5.26	2.07	4.82
Alanine	0.71	2.44	6.98	2.90	3.38
Serine	0.84	1.67	3.73	2.36	3.12
Arginine	0.61	1.52	0.20	2.54	2.69
Phenylalanine	0.67	1.63	3.56	1.77	1.76
Valine	0.57	1.09	1.26	1.34	1.19
Glycine	0.75	1.87	1.41	1.79	1.11
Isoleucine	0.41	0.85	0.63	0.95	0.86
Leucine	0.55	1.28	0.88	1.26	0.84
Lysine	0.22	0.37	1.30	0.71	0.29
Methionine	0.05	0.44	0.94	0.50	0.45
Tyrosine	0.12	0.41	0.44	0.43	0.35
Threonine	0.42	0.75	0.72	0.85	0.66
Histidine	0.57	0.34	0.49	0.32	0.10
Cystine	----	0.30	0.15	0.72	0.05
Total Amino Acid	15.39	29.25	62.52	37.41	50.20
Ammonia	3.30	3.90	7.74	3.87	5.57

Table 4. Amino acid changes in peach seed treated with 2000 ppm gibberellic acid (Micromoles per gram fresh weight).

	Time Interval				
	30 Hours	60 Hours	90 Hours	120 Hours	170 Hours
Glutamic Acid	0.99	1.15	16.92	17.02	11.15
Proline	0.09	0.03	1.82	1.86	2.42
Aspartic Acid	1.56	0.17	4.18	0.25	5.84
Alanine	0.37	0.07	3.37	6.19	7.67
Serine	0.15	0.05	2.27	1.85	3.82
Arginine	0.06	0.03	0.53	0.11	1.22
Phenylalanine	0.17	0.23	1.14	1.51	1.37
Valine	0.09	0.03	0.49	0.54	0.60
Glycine	0.29	0.14	1.57	1.05	1.45
Isoleucine	0.07	0.03	0.25	0.17	0.29
Leucine	0.11	0.04	0.39	0.32	0.42
Lysine	0.11	0.05	0.12	0.45	0.30
Methionine	nil	nil	0.36	0.59	0.70
Tyrosine	0.05	0.08	0.12	0.20	0.22
Threonine	0.10	0.04	0.36	0.37	0.57
Histidine	0.05	0.01	0.29	0.29	0.34
Cystine	nil	nil	0.09	nil	nil
Total Amino Acid	4.30	2.16	34.61	32.75	38.38
Ammonia	2.33	1.30	6.12	2.53	3.22

Table 5. Protein content in peach seed (Milligrams per gram fresh weight).

Treatment	Interval	Protein Content
Dry sample		260.6
Soaked in water		242.4
32 F	2 weeks	218.2
	4 weeks	197.0
	6 weeks	202.4
	8 weeks	187.9
	10 weeks	169.7
45 F	2 weeks	198.8
	4 weeks	215.8
	6 weeks	242.4
	8 weeks	260.7
	10 weeks	265.4
72 F	2 weeks	236.6
	4 weeks	241.3
	6 weeks	224.9
	8 weeks	213.3
	10 weeks	215.5
Gibberellic Acid	30 hours	97.0
	60 hours	109.1
	90 hours	116.4
	130 hours	121.2
	160 hours	145.6
	190 hours	181.8
L.S.D. .05		39.1
.01		51.6

was only partially broken. This group of seed had a lower total free amino acid content but a higher glutamic acid content than did the seed held at 72 F, which did not grow at all.

Seeds held at 32 F had a lower total amino acid and higher glutamic acid content while those held at 72 F had a higher total amino acid and aspartic acid content but relatively lower in glutamic acid content. This fact indicates the importance of glutamic acid during seed germination other than transamination, which can also be achieved by aspartic acid.

Gibberellic acid treatment produced slender seedlings, and a lower proline, valine and higher alanine content compared with seed held at 45 F, which produced normal seedlings.

Proteins

Protein breakdown occurs shortly after treatment (Table 5), but a rapid resynthesis occurred after two weeks at 45 F and also after 30 hours following gibberellic acid treatment. This clearly indicates that the reserved, metabolically inactive proteins were first degraded, and then resynthesized. The abnormally short and stunted seedlings which resulted from the 32 F treatment, may possibly be due to their incapability of using reserve proteins. The protein in seed held at 72 F which gradually

decreased, may be a result of respiratory consumption by the metabolically arrested seeds.

Organic Acids

Succinic acid and malic acid were present in dry seeds in relatively large amounts (Table 6). Succinic acid remained fairly high in the seed held at 45 F, but malic acid decreased during the first four weeks and then increased to a very high level after eight weeks. Malic and succinic acid remained high, except succinic acid at 6th week was low in seed held at 32 F.

Citric, pyruvic and fumaric acids were at low level and only present after soaking. Citric acid remained very low except during the second week at 32 F, however, there was a higher citric acid in seed held at 45 F. This is probably due to the blocking of enzymes necessary for changing pyruvic to citric acid. Pyruvic acid accumulated in seed held at 32 F, but was at a considerably lower level in seed held at 45 F, except in the second and eighth week. A high fumaric acid content occurred in seeds held at 45 F, but a low content was in the 32 F treated seeds. This undoubtedly is related to the content of succinic acid, which is one step ahead of fumaric in the citric acid cycle.

A considerable amount of glyoxalic acid was present in dry seeds, but was readily metabolized when the seed were held at 45 F or treated with gibberellic acid. It remained

Table 6. Organic acid changes occurring in peach seed during different temperature treatment (Micromoles per gram fresh weight).

Treatment	Interval	Glyoxalic Acid	Citric Acid	Pyruvic Acid	Malic Acid	Fumaric Acid	Succinic Acid	Total
Dry seed		15.3	0.3	nil	28.7	nil	52.5	96.8
Soaked		12.5	10.6	2.4	47.6	1.0	127.4	201.5
72 F	8 weeks	12.3	0.1	3.9	25.8	4.0	1.5	47.6
32 F	2 weeks	15.3	9.4	2.1	41.9	nil	19.2	87.9
	4 weeks	12.4	1.1	3.5	57.0	1.7	47.9	72.3
	6 weeks	10.6	1.2	3.7	26.4	nil	9.8	51.7
	8 weeks	10.1	1.4	7.6	29.8	2.9	37.5	89.3
45 F	2 weeks	11.2	5.4	9.3	7.5	20.3	61.2	114.9
	4 weeks	8.4	2.3	0.6	12.0	4.4	52.5	80.2
	6 weeks	5.2	4.4	0.6	60.3	2.1	21.4	94.0
	8 weeks	5.7	4.6	10.4	74.6	5.6	36.1	137.0

Table 7. Organic acid changes in peach seed after a 2000 ppm gibberellic acid treatment (Micromoles per gram fresh weight).

Organic Acid	Dry Seed	Soaked	Time in Hours					
			30	60	90	130	170	190
Glyoxalic acid	15.3	12.5	12.1	10.3	6.1	5.3	3.1	2.2
Citric acid	0.3	10.6	0.3	0.1	0.9	0.9	0.8	0.7
Pyruvic acid	nil	2.4	nil	1.2	8.3	8.2	4.1	7.5
Malic acid	28.7	47.6	16.6	62.0	49.4	13.8	38.4	80.7
Fumaric acid	nil	1.0	nil	19.8	19.1	27.8	9.7	29.5
Succinic acid	52.5	127.4	24.6	15.6	30.7	40.4	34.4	122.5
Total	96.8	201.5	53.6	109.0	114.5	96.4	90.5	243.1

fairly constant in seed held at 32 F, and also in seed held at 72 F after eight weeks. The importance of the glyoxalic acid cycle during seed germination is unknown, but in this study the glyoxalic acid change indicated that the cycle may be active during the breaking of the seed rest period.

Gibberellic acid stimulated the content of all organic acid except glyoxalic acid and citric acid. This rapid increase of organic acid is probably a secondary effect of protein and starch breakdown.

Starch and Sugars

A fairly high starch content was observed in dry peach seeds (Table 8). Starch degradation was highest in seed stored at 45 F and decreased in a decending order of: gibberellic acid, 32 F and 72 F treatment. This was also exactly the sequence of an increasing percentage of abnormal seedlings and those incapable of germination.

A rapid increase in sucrose content after seed were stored 4 weeks at 45 F or 48 hours after being treated with gibberellic acid was observed (Table 9). This change correlated well with the breakdown of starch which is the most important source of glucose. The sucrose content in seed held at 32 F increased slowly and in seed held at 72 F it even decreased.

Both glucose and fructose may enter into the glycolytic sequence. It is apparent from the data (Table 10 and 11) that glucose was rapidly utilized while a small amount of

Table 8. The starch content of peach seed (Milligrams per gram fresh weight).

Treatment	Interval	Starch Content
Dry sample		47.3
Soaked in water		46.1
32 F	2 weeks	43.6
	4 weeks	46.1
	6 weeks	41.2
	8 weeks	37.6
	10 weeks	38.8
45 F	2 weeks	25.5
	4 weeks	12.1
	6 weeks	13.3
	8 weeks	9.7
	10 weeks	12.4
72 F	2 weeks	46.0
	4 weeks	42.6
	6 weeks	43.4
	8 weeks	42.4
	10 weeks	41.8
Gibberellic Acid	30 hours	42.4
	60 hours	33.9
	90 hours	33.3
	130 hours	30.3
	160 hours	26.7
	190 hours	26.6
L.S.D. .05		8.26
.01		10.96

Table 9. The sucrose content of peach seed (Micromoles per gram fresh weight).

Treatment	Interval	Sucrose Content
Dry sample		39.2
Soaked in water		45.3
32 F	2 weeks	35.9
	4 weeks	25.6
	6 weeks	48.2
	8 weeks	100.8
45 F	2 weeks	76.7
	4 weeks	587.2
	6 weeks	47.0
	8 weeks	39.4
72 F	2 weeks	18.3
	4 weeks	12.3
	6 weeks	6.7
	8 weeks	4.4
Gibberellic Acid	24 hours	3.1
	48 hours	315.5
	72 hours	812.2
	144 hours	517.7
	168 hours	20.5
L.S.D. .05		15.9
.01		21.6

Table 10. The glucose content of peach seed (Micromoles per gram fresh weight).

Treatment	Interval	Glucose Content
Dry sample		116.6
Soaked in water		66.8
32 F	2 weeks	76.6
	4 weeks	50.0
	6 weeks	58.3
	8 weeks	62.0
45 F	2 weeks	14.6
	4 weeks	83.3
	6 weeks	82.6
	8 weeks	80.0
72 F	2 weeks	85.5
	4 weeks	87.8
	6 weeks	92.5
	8 weeks	105.5
Gibberellic Acid	24 hours	2.0
	48 hours	75.5
	72 hours	52.2
	144 hours	5.1
	168 hours	91.6
L.S.D. .05		11.6
.01		15.8

Table 11. The fructose content of peach seed (Micromoles per gram fresh weight).

Treatment		Interval	Fructose Content
Dry sample			1.83
Soaked in water			2.06
32 F		2 weeks	1.78
		4 weeks	3.22
		6 weeks	2.43
		8 weeks	1.46
45 F		2 weeks	31.03
		4 weeks	1.28
		6 weeks	1.78
		8 weeks	1.17
72 F		2 weeks	1.22
		4 weeks	1.37
		6 weeks	1.16
		8 weeks	1.11
Gibberellic Acid		24 hours	0.44
		48 hours	19.32
		72 hours	1.12
		144 hours	4.50
		168 hours	1.94
L.S.D.	.05		1.35
	.01		1.83

Table 12. The crude lipid material content of peach seed (Milligrams per gram fresh weight).

Treatment	Interval	Lipid Materials
Dry sample		229
Soaked in water		178
32 F	2 weeks	153
	4 weeks	146
	6 weeks	157
	8 weeks	140
45 F	2 weeks	144
	4 weeks	134
	6 weeks	151
	8 weeks	153
72 F	2 weeks	169
	4 weeks	155
	6 weeks	131
	8 weeks	45
Gibberellic Acid	30 hours	201
	60 hours	174
	90 hours	158
	130 hours	161
	170 hours	141
	190 hours	156
L.S.D. .05		6.97
.01		9.24

fructose was accumulated after 2 weeks at 45 F and 48 hours after gibberellic acid treatment. Fructose stayed fairly stable both at 32 F and 45 F storage, glucose was utilized slowly at 32 F but even slower at 72 F storage.

The data above indicate that the results of gibberellic acid treatment followed the same pattern of 45 F storage, i.e., fast utilization of glucose and fructose apparently through glycolysis. The incorporation of glucose into the glycolytic sequence seems slowed down by 32 F and 72 F storage.

Lipid Materials

A rapid breakdown of crude lipid materials was observed in all treatments (Table 12). This breakdown soon stopped and a similar level was reached for all treatments except those held at 72 F, under which condition crude lipid materials decreased continuously.

Seedling Morphology

Seedlings from the 45 F temperature treatment were considered normal, with green leaves and long internodes. Seedlings from 32 F treatment were extremely short, with twisted, dark green leaves. Seedlings from gibberellic acid treatment were usually slender, with long internodes and narrow, yellowish green leaves. Seed held at 72 F did not germinate.

SUMMARY

A study of the changes of amino acids, proteins, organic acids, carbohydrates and crude lipid materials occurring during intervals at different temperature treatments and a 2,000 ppm gibberellic acid treatment was conducted in order to understand the chemical changes which take place during the rest period and the physiological effect gibberellic acid had on resting peach seeds.

None of the seeds held at 72 F storage germinated. Half (55 per cent) of the seeds germinated which were held at 32 F storage, but they developed into dwarf seedlings thereafter. Ninety per cent of the seeds germinated which were held at 45 F storage and developed into normal seedlings in the greenhouse. Gibberellic acid induced about 25 per cent of the treated seeds to germinate within three to five days (35 per cent total germination), but more than 50 per cent of them were abnormally slender.

Similar chemical changes occurred in seed receiving the gibberellic acid treatment as with the 45 F treatment. A rapid breakdown of proteins and lipid materials, the release of a large amount of total amino acids and sugars, and the rapid degradation of starch occurred within seed

receiving these treatments. The most drastic biochemical changes occurred after the seed were stored four to six weeks at 45 F, and 48 to 72 hours after the gibberellic acid treatment. These results indicate what chemical changes take place when gibberellic acid is applied. It partially replaces the cold treatment and induces peach seed germination within a few days. The gibberellic acid treatment differs from 45 F treatment in that gibberellic acid treated seeds were lower in total amino acids, proline, valine and citric acid content and higher in alanine and pyruvic acid content. Protein breakdown was faster in gibberellic acid treated seeds and slower in 45 F treated seeds, but the starch breakdown was just the opposite. Sucrose and glucose changes in 45 F treated seeds were less drastic compared with those of gibberellic acid treated seeds. These results may partially account for the slender seedlings induced by gibberellic acid.

The chemical changes in seed held at 32 F and 72 F indicate they were incapable of mobilizing reserve protein, starch and lipid materials rapidly. The slow consumption of reserve food did not meet the requirement of active cell division and enlargement, and led to abnormally dwarfed seedlings in the case of those held at 32 F and seeds which barely survived when held at 72 F. In addition, the results indicate that some respiratory enzyme systems are not active under these conditions. The incorporation of

glucose into the glycolytic sequence and the utilization of pyruvic acid in the citric acid cycle did not occur or occurred slowly.

LITERATURE CITED

Bailey, J. L. 1962. Techniques in protein chemistry. Elsevier Publishing Company, Amsterdam and New York. 293-294.

Barton, L. V. 1956. Growth responses of physiologic dwarfs of Malus arnoldiana Sarg. to gibberellic acid. Contributions of Boyce Thompson Institute 18(8) : 311-317.

Barton, L. V. 1961. Biochemical studies of dormancy and after-ripening of seeds. II. Changes in oligobasic organic acids and carbohydrates. Contributions of Boyce Thompson Institute 21(7) :147-161.

Barton, L. V., and J. L. Bray. 1962. Biochemical studies of dormancy and after-ripening of seeds. III. Nitrogen metabolism. Contributions of Boyce Thompson Institute 22(8) :465-472.

Block, R. J., E. L. Durrum, and G. Zweig. 1958. A manual of paper chromatography and paper electrophoresis. Academic Press, Inc., New York. 119-120. 178-189.

Blommaert, K. L. J. 1959. Winter temperature in relation to dormancy and the auxin and growth inhibitor content. South African Journal of Agricultural Science 2:507-514.

Boe, A. 1965. Personal communication.

Bonner, J., R. S. Bandurski, and A. Millerd. 1953. Linkage of respiration to auxin-induced water uptake. Physiologia Plantarium 6:511-522.

Boo, L. 1961. The effect of gibberellic acid on the inhibitor B complex in resting potato. Physiologia Plantarium 14:676-681.

Boulter, D., and J. T. Barber. 1963. Amino acid metabolism in germinating seeds of Vicia faba L. in relation to their biology. New Phytologist 62(3) :301-316.

Buch, M. L. 1952. Identification of organic acids on paper chromatograms. Analytical Chemistry 24(3) : 489-490.

Bulen, W. A., J. E. Varner, and R. C. Burrell. 1952. Separation of organic acids from plant tissues. Analytical Chemistry 24(1) :187-190.

Ching, T. M. 1963. Fat utilization in germinating Douglas fir seeds. Plant Physiology 38(6) :722-728.

Christensen, H. N., T. R. Riggs, and B. A. Coyne. 1954. Effects of pyridoxal and indole acetate on cell uptake of amino acids and potassium. Journal of Biological Chemistry 209(1) :413-427.

Clegg, M. D., and L. Rappaport. 1965. The influences of gibberellin A_3 on content of reducing sugars in excised resting potato tubers. Plant Physiology 40(Suppl.) :lxxv.

Cleland, R. 1958. A separation of auxin-induced cell wall loosening into its plastic and elastic components. Physiologia Plantarium 11:599-609.

Colombo, P., D. Corbetta, G. Euffini, and A. Sartori. 1960. A solvent for qualitative and quantitative determination of sugars using paper chromatography. Journal of Chromatography 3(4) :343-350.

Commoner, B., and D. Mazia. 1942. The mechanism of auxin action. Plant Physiology 17(4) :682-685.

Corgan, J. N. 1965. Seasonal changes in naringenin concentration in peach flower buds. Proceedings of American Society of Horticultural Science 86:129-132.

Dennis, F. G., and L. J. Edgerton. 1961. The relation between an inhibitor and rest in peach flower buds. Proceedings of American Society of Horticultural Science 77:121-134.

Denny, F. E., and E. N. Stanton. 1928. Localization of response of woody tissues to chemical treatment that break the rest period. American Journal of Botany 15(1) :337-344.

Flemion, Florence, and Carol Topping. 1963. Cytochemical studies of the shoot apices of normal and physiologically dwarfed peach seedlings. II. Starch distribution. Contributions of Boyce Thompson Institute 20(6) :365-380.

Flemion, Florence, and D. S. de Silva. 1960. Bioassay and biochemical studies of extract of peach seeds in various stages of dormancy. Contributions of Boyce Thompson Institute 20(6) :365-380.

Folkes, B. F., and E. W. Yemm. 1958. The respiration of barley plants. X. Respiration and the metabolism of amino acids and proteins in germinating grain. New Phytologist 57(1) :106-131.

Fruton, J. S. 1958. General biochemistry. John Wiley and Sons, Inc., p. 456-544. 750-769.

Galston, A. W., and W. K. Purves. 1960. The mechanism of action of auxins. Annual Review of Plant Physiology 11:239-276.

Glasziou, K. T. 1958. Effect of 2,4-dichlorophenoxyacetic acid on pectin methylesterase in tobacco pith sections. Nature 181(4606) :428-429.

Halevy, A. H. 1963. Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. Plant Physiology 38(6) :731-737.

Hanson, J. B., and J. Booner. 1954. The relationship between salt and water uptake in Jerusalem artichoke tuber tissue. American Journal of Botany 41(9) : 702-710.

Hendershott, C. H., and D. R. Walker. 1959. Seasonal fluctuation in quantity of growth substance in resting peach flower buds. Proceedings of American Society of Horticultural Science 74:121-129.

Heyn, A. N. J. 1940. The physiology of cell elongation. Botanical Review 6:515-574.

Jorysch, D., P. Sarris, and S. Marcus. 1962. Detection of organic acids in fruit juices by paper chromatography. Food Technology 16(3) :

Kato, Y. 1955. Responses of plant cells to gibberellin. Botanical Gazette 117(1) :16-24.

Kawase, M. 1961. Growth substances related to dormancy in betula. Proceedings of American Society of Horticultural Science 78:532-544.

Koller, D., A. M. Mayer, A. Poljakoff-Mayber, and S. Klein. 1962. Seed germination. Annual Review of Plant Physiology 13:437-457.

Kuraishi, S., and R. M. Muir. 1964a. The relationship of gibberellin and auxin in plant growth. Plant Cell Physiology 5(1) :61-69.

Kuraishi, S., and R. M. Muir. 1964b. The mechanism of gibberellin action in the dwarf pea. Plant Cell Physiology 5(3) :259-271.

Luckwill, L. C. 1952. Growth-inhibiting and growth-promoting substances in relation to dormancy and after-ripening of apple seeds. Journal of Horticultural Science 27:53-55.

Marshall, L. M., K. O. Donaldson, and F. Friedberg. 1952. Chromatographic resolution of organic acids with progressively changing solvents. Analytical Chemistry 24(5) :773-775.

Masuda, Y. 1955. Der einfluss des heteroauxins auf die plasmapermeabilitat fur harnstoff und alkylharnstoff. Physiologia Plantarium 8:527-537.

McCready, R. M., J. Guggolz, V. Silviera, and S. Owens. 1950. Determination of starch and amylose in vegetables. Analytical Chemistry 22(9) :1156-1158.

Meyer, R. M., D. B. Anderson, and R. H. Bohning. 1960. Introduction to plant physiology. Princeton Press. N. J. p. 510-528.

Miller, G. L. 1959. Protein determination for large numbers of samples. Analytical Chemistry 31(5) : 964.

Moore, S., D. H. Spackman, and W. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene resins. Analytical Chemistry 30(7) :1185-1190.

Nanda, K. K., and A. N. Puroht. 1965. Effect of gibberellin on mobilization of reserve food and its co-relation with extension growth. Planta 66:121-125.

Northen, H. T. 1942. Relationship of dissociation of cellular proteins by auxins to growth. Botanical Gazette 103(4) :688-693.

Paleg, L. G., D. H. B. Sparrow, and A. Jennings. 1962. Physiological effects of gibberellic acid. IV. On barley grain with normal, X-ray irridiated, and excised embryos. Plant Physiology 37(5) :579-583.

Paleg, L. G., B. C. Boombe, and M. S. Buttrose. 1962. Physiological effects of gibberellic acid. V. Endosperm responses of barley, wheat and oats. Plant Physiology 37(6) :798-803.

Paleg, L. G., and B. Hyde. 1964. Physiological effects of gibberellic acid. VII. Electron microscopy of barley aleurone cells. Plant Physiology 39(4) : 673-680.

Paleg, L. G. 1965. Physiological effects of gibberellins. Annual Review of Plant Physiology 16:291-322.

Parr, C. W. 1954. The separation of sugars and sugar phosphates by gradient elution from ion exchange column. Biochemistry Journal 56:xxvii-xxviii.

Penny, D., and B. B. Stowe. 1965. Relationship between growth and respiration induced by lipids in pea stem sections. Plant Physiology 40(6) :1140-1145.

Phillips, I. D. J. 1961. Induction of light requirement for the germination of lettuce seed by naringenin, and its removal by gibberellic acid. Nature 129(4799) :240-241.

Phillips, I. D. J. 1962. Some interactions of gibberellic acid with naringenin in the control of dormancy and growth in plant. Journal of Experimental Botany 13:213-216.

Rai, V. K., and M. M. Laloraya. 1965. Correlative studies on plant growth and metabolism. I. Changes in protein and soluble nitrogen accompanying gibberellin induced growth in lettuce seedlings. Plant Physiology 40(3) :437-441.

Reinhold, L. 1958. Release of ammonia by plant tissues treated with indole-3-acetic acid. Nature 182(4641) : 1022-1023.

Reinhold, L., and R. G. Powell. 1958. The stimulatory effect of IAA on the uptake of amino acids by tissues of Helianthus annuus. Journal of Experimental Botany 9(25) :82-96.

Sastry, K. S. K., and R. M. Muir. 1965. Effects of gibberellic acid on utilization of auxin precursors by apical segments of the Avena coleoptile. Plant Physiology 40(2) :294-298.

Siegel, S. M., P. Frost, and F. Porto. 1960. Effects of IAA and other oxidation regulators on in vitro peroxidation and experimental conversion of eugenol to lignin. Plant Physiology 34(2) :163-167.

Sivaramakrishnan, V. M., and D. S. Sarma. 1956. The metabolism of glutamic acid in germinating green grain seeds (Phaseolus radiatus). Biochemistry Journal 62(1) :132-135.

Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Analytical Chemistry 30(7) : 1190-1206.

Sweeney, B. M., and K. V. Thimann. 1942. The effect of auxins on protoplasmic streaming. Journal of Physiology 25(6) :841-854.

Syamananda, R., R. C. Staples, and R. J. Block. 1962. Automatic analysis of sugars separated by column chromatography. Contributions of Boyce Thompson Institute 21(4) :363-369.

Tagawa, T., and J. Bonner. 1957. Mechanical properties of the Avena coleoptile as related to auxin and to ionic interactions. Plant Physiology 32(3) :207-212.

Thompson, J. F., C. J. Morris, and R. K. Gering. 1959. Purification of plant amino acids for paper chromatography. Analytical Chemistry 31(6) :1028-1031.

Trelawny, J. G. S., and D. J. Ballantyne. 1963. The effect of gibberellin and temperature on the germination of seeds of Bells of Ireland. Canadian Journal of Plant Science 43(4) :522-527.

Verner, J. E. 1964. Gibberellic acid controlled synthesis of a-amylase in barley endosperm. Plant Physiology 39(3) :413-415.

Viles, F. J., and Leslie Silverman. 1949. Detection of starch and cellulose with anthrone. Analytical Chemistry 21(8) :950-952.

Walker, D. R., and C. W. Donoho. 1959. Further studies of the effect of gibberellic acid on breaking the rest period of young peach and apple trees. Proceedings of American Society of Horticultural Science 74:87-92.

Wareing, P. E., and T. A. Villiers. 1961 Growth substance and inhibitor changes in buds and seeds in response to chilling. Plant Growth Regulation. The Iowa State University Press. p. 95-107.

Wistreich, H. E., J. E. Thompson, and E. Karmas. 1960. Rapid method for moisture and fat determination in biological materials. Analytical Chemistry 32(8) : 1054.

APPENDIX

Protein Determination (Bailey, 1962)

Reagents

1. Chromogenic solution: Mix 1 ml. of 2 per cent sodium carbonate in 0.1 N sodium hydroxide with 50 ml. of 0.5 per cent cupric sulfate in 1 per cent sodium citrate solution.
2. Folin-Ciocalteu reagent: Dilute the commercially available reagent (Fisher Scientific Company, New Jersey) with one part of distilled water.

Procedure

1. Add 10 ml. of distilled water to a test tube containing 0.5 gram powdery dry sample and make a homogenous plate.
2. Add 2 ml. of 2 N NaOH to the mixture and heat the sample at 100 C for 30 minutes.
3. Filter after cooling and dilute the filtrate to 100 ml.
4. Mix 1 ml. of the sample and 1 ml. of the chromogenic solution in a clean test tube and wait for 10 minutes.
5. Add 0.5 ml. of the Folin-Ciocalteu reagent and 3 ml. distilled water.
6. After 30 minutes at room temperature, the sample is read at 570 mu with Beckman B spectrophotometer and compared with a blank.

7. Prepare a standard solution of blood albumin in the same manner and the protein content in the sample can be determined.

Silica Gel Preparation (Boe, 1965)

1. Dissolve 1 pound of pure sodium metasilicate in distilled water on a warm-water bath such that the final volume is 620 ml.
2. Cool the solution to 35 C on an ice-water bath and add enough methyl orange to give the solution a yellow color.
3. Add 10 N hydrochloric acid from a separatory funnel with vigorous stirring. Keep the temperature within the range of 36 to 38 C by controlling the rate of addition of the acid.
4. Stir the mixture while continuously adding the acid until the indicator remains pink after thorough mixing. Approximately 250 ml. of the acid is required.
5. Let the mixture stand at room temperature with an occasional stirring. Add a small amount of acid if it loses it's pink tinge.
6. Filter the silica gel using a Buchner funnel and wash it with 500 ml. portions of distilled water.
7. Resuspend the silica gel in one liter of 0.2 N hydrochloric acid and allow it to stand for 18 to 20 hours.
8. Wash the silica gel on a Buchner funnel with distilled water until the washings are about pH 4.5, then wash it with 500 mil of 95 per cent ethanol.
9. Air dry the silica gel on filter paper for 5 hours and then oven-dry it at 160 C for 4 days.
10. Store the silica gel in a desiccator before use.

Column Chromatography of Organic Acid (Bulen et al., 1952; Boe, 1965; Marshall et al., 1952)

I. Preparation of Eluting Solvents:

1. Wash Chloroform twice with distilled water and then equilibrate it against 0.5 N sulfuric acid for 4 hours in a separatory funnel. Filter the organic phase with Whatman No. 1 filter paper to remove water droplets.
2. Equilibrate 1-Butanol against 0.5 N sulfuric acid for 4 hours in a separatory funnel and then filter it with Whatman No. 1 filter paper to remove water droplets.

II. Preparation of Indicator:

Dissolve 100 mg. of the pure phenol red indicator in 5.7 ml. of 0.05 N sodium hydroxide and dilute to 100 ml.

III. Preparation of Column:

1. Place a glass wool plug in the bottom of a 12 mm. x 25 mm. chromatographic tube.
2. Mix 8 grams of silica gel with 5.5 ml. of 0.5 N sulfuric acid in a mortar.
3. Add 60 to 70 ml. of chloroform to the mixture and pour this free-flowing silica gel to the chromatographic tube in successive portions.

4. Apply pressure slightly to obtain a uniform column of 14.5 cm. long.
5. Maintain the solvent level always above the column surface.

IV. Apparatus:

1. Connect a reservoir (8 cm. I.D.) containing 450 ml. of chloroform to the top of the chromatographic tube by a capillary tube.
2. Connect another small reservoir (5.5 cm. I.D.) containing 150 ml. of 1-butanol to the above mentioned reservoir by a U shaped tube.
3. Apply a small pressure to the small reservoir to drive off the air in the U shaped tube by 1-butanol. The two solvents will thus mix together such that the ratio of chloroform to 1-butanol decreases as the elution progresses.

V. Column Chromatography:

1. Dissolve the dry sample with 5 ml. of 0.5 N sufuric acid and mix it with 1 gram silica gel.
2. Transfer quantitatively the free-flowing mixture on top of the column.
3. Cover the sample surface with a glass wool plug to avoid disturbing the silica gel column when pouring solvent.

4. Connect the reservoirs and force the solvents to transfer to the chromatographic tube by applying a pressure to the small reservoir.
5. Collect the effluent in 1 ml. aliquots with a fraction collector.

VI. Titration of Samples:

1. Add five milliliters of carbonate-free distilled water with two drops of phenol red indicator to each tube by an automatic pipet machine.
2. Titrate each tube with standarized 0.009012 N solium hydroxide.
3. Calculate each acid on a milliequivalent basis to a micromole basis.

Paper Chromatography of Organic Acids

(Buch, 1952)

Solvent system

Use the organic phase of the equal volume mixture of 1-pentanol and 5 M aqueous formic acid as solvent.

Color reagent

1. Bromophenol blue reagent 0.04 per cent bromophenol blue in 95 per cent ethanol adjusted to pH 6.7 with dilute sodium hydroxide is used for R_f determination.
2. Ammoniacal silver nitrate Mix equal parts of 0.1 N silver nitrate and 0.1 N ammonium hydroxide prior to use.
3. Acetic anhydride-pyridine reagent 10 per cent of acetic anhydride in pyridine (by volume).

Procedure

1. Organic acid samples containing 100 to 150 micrograms of acid are spotted on Whatman No. 1 filter paper strips (4X50 cm.).
2. For uniform development, the paper strips are fixed on glass racks with plastic clamps and saturated in the chamber for 3 hours before development.
3. Develop the descending chromatograms for 18 to 20 hours, and then mark the solvent front.

4. Air-dry the chromatograms at room temperature and spray the different color reagents.
5. Compare unknown acids with standard acids based upon both R_f values and specific color reactions.

Starch Determination (McCready, 1952)

Reagents

1. Perchloric acid: Add 270 ml. of 72 per cent perchloric acid to 100 ml. of distilled water to give a final concentration of 52 per cent.
2. Anthrone reagent: 2 grams of anthrone (Eastman Organic Chemicals, Rochester, New York) is dissolved in one liter of cold sulfuric acid and stored under refrigeration before use. This reagent is unstable and gives high blanks and variable results when old.

Procedure

1. Add 10 ml. of distilled water to a test tube containing 0.5 gram of the powdery dry sample and make a homogenous paste.
2. Add 10 ml. of 52 per cent perchloric acid and allow to stand for 5 hours.
3. Filter the extract with Whatman No. 1 filter paper.
4. Extract the residue again with 10 ml. of distilled water and 10 ml. of 52 per cent perchloric acid.
5. Combine the filtrate and dilute to 100 ml.
6. Mix 1 ml. of the extract with 10 ml. of the anthrone reagent and heat the mixture to 100 C for 7.5 minutes.
7. Cool the sample in a water-bath immediately to 25 C.

8. Read at 630 mu with a Beckman Model B spectrophotometer against a blank.

9. Starch content can be determined according to a standard curve of glucose solution prepared in the same manner.

Column Chromatography of Sugars

I. Preparation of Anthrone Reagent (Trevelyan and Harrison, 1952)

Dissolve 0.2 gm. of anthrone reagent in 140 ml. of 25 N sulfuric acid. The reagent can be used within two to three days if kept under refrigeration.

II. Preparation of Resin (Syamananda et al., 1962)

1. Suspend 100 grams of Dowex 1-X8 anion exchange resin (200 to 400 mesh) into 1 liter of deionized water, decant the colloidal materials and fine particles.
2. Pack the resin into a 4x50 cm. column and convert the resin from the chloride form to the borate form by passing 0.25 M potassium tetraborate through the column until the washings are negative to the chloride test.
3. Rinse the resin with 1 liter of deionized water and resuspend the resin into 0.001 M potassium tetraborate solution.
4. Pack the prepared resin into a 10x100 mm. chromatographic tube for column chromatography.

III. Eluting Apparatus (Parr, 1954)

Two reservoirs are required to maintain a changing chloride concentration during elution. Reservoir A contains 1 liter of 0.1 M boric acid adjusted to pH 7

with 0.2 M sodium hydrocloride. Reservoir B contains 1 liter of 0.1 M boric acid with 0.25 M sodium chloride at pH 7.

Connect reservoir A to the chromatographic column, and the reservoir B to reservoir A, which is stirred by a magnetic stirer.

IV. Procedure (Syamananda et al., 1962)

1. Dissolve the sugar sample in 10 ml. of 0.001 M potassium tetraborate solution.
2. Introduce the solution into the packed column with deionized water.
3. Connect the reservoirs and use an air pressure to start the eluting solvent. Maintain a speed of 5 ml. per minute.
4. Collect the effulent at 10 ml. aliquots with a fraction collector.
5. Take 1 ml. aliquot from each tube and mix with 5 ml. of the prepared anthrone reagent.
6. Heat the mixture at 100 C for 5 minutes, and then cool it in running water for 3 minutes.
7. Read at 620 mu against a blank with Beckman model B spectrophotometer.
8. Run a standard curve with different sugars for quantitative determination.

Paper Chromatography of Sugars (Block et al., 1958)

Solvent systems

1. Water saturated phenol containing 1 per cent ammonium hydroxide.
2. Mixture of 4 parts n-butanol, 1 part acetic acid and 5 parts water (by volume).

Color reagents

1. Ammoniacal silver nitrate Mixture of equal volumes of 0.1 N silver nitrate and 5 N ammonium hydroxide.
2. Anthrone reagent Dissolve 300 mg. of anthrone in 10 ml. of glacial acetic acid by warming, and 30 ml. of 95 per cent ethanol, 3 ml. of phosphoric acid and 1 ml. of water are then added to the acidic anthrone solution. This reagent may be stored under refrigeration for few days.

Procedure

1. Sugar samples in aqueous solution continaing 30 to 40 ug. of sugar are spotted on Whatman No. 1 filter paper strips (4x50 cm.).
2. After 2 hours saturation in a chamber, the chromatograms are developed (descending) for 18 hours.
3. Mark the solvent front after developed, and dry the chromatograms using a hood.
4. Spray the chromatograms with either of the two color reagents, and then heat in an oven at 105 C for 5 to 10 minutes.

5. Measure the R_f values and compare with standard sugar samples.

Crude Lipid Material Determination

1. Grind 5 grams of fresh sample with 100 ml. of ethyl ether in a homogenizer for 3 minutes.
2. Transfer the sample quantitatively into an alundum extraction thimble and extract in a Soxhlet extraction apparatus with 200 ml. of ethyl ether for 24 hours.
3. Shake the extract with 100 ml. of dionized water in a saparatory funnel to remove pigments and other water-soluble materials.
4. Discard the lower aqueous layer and filter the purified extract with Whatman No. 2 filter paper.
5. Evaporate the solvent at room temperature and then heat the residue in an oven at 80 F for 5 hours.
6. The ether-soluble water-free residue is crude lipid material.