FACTORS AFFECTING FLOWERING RESPONSE IN 'TRIUMPH 64' WINTER WHEAT

By

JAUNITA HWA-LING TU "Chin Choy

Bachelor of Science

National Taiwan University

Taipei, Taiwan, China

1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May 1970

FACTORS AFFECTING FLOWERING RESPONSE

IN 'TRIUMPH 64' WINTER WHEAT

Thesis Approved:

Edward L. Smith
Thesis Adviser

Lewis H. Edwards

Lavoy J. Croy

D. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author is grateful to the Agronomy Department for the facilities and financial assistance provided for this study.

Special appreciation is expressed to Dr. E. L. Smith, her major adviser, for his advice, guidance and counsel during the course of the study. Special thanks is also expressed to the other members of my committee, Dr. L. I. Croy, for his advice and help during this study, and to both him and Dr. L. H. Edwards for their valuable suggestions on the preparation of this manuscript.

To the author's parents, Mr. and Mrs. Chi-Lian Tu, deep gratitude is expressed for their encouragement, help and advice during the period of this study.

Special thanks is expressed to Mrs. Robert Morrison for the typing of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

One of the prime problems in the breeding of winter wheat as compared to spring wheat is that only one generation per year can be obtained under field conditions. If a procedure could be developed for obtaining two or more generations per year at an early stage in the breeding program, the efficiency of breeding programs could be increased. Controlled environmental conditions which are available today could assist in such a breeding program.

Under Oklahoma field conditions, winter wheat does not develop rapidly because of the need of vernalization, a process for the induction or acceleration of flowering by chilling or low temperature treatment. The chilling of germinated seeds prior to planting would tend to shorten the time required for producing one generation of plants of winter wheat.

Certain chemical substances, especially gibberellic acid, may offer possibilities in reducing the cold requirement needed for vernalization. Therefore, a better method of fulfilling the vernalization requirement with the subsequent handling of the plants to obtain rapid growth and development of winter cereals is of particular interest to plant breeders. Such a system would permit plant breeders to grow two or more generations of plants per year under controlled conditions.

The main objectives of this study were (1) to determine the vernalization requirement of 'Triumph 64' winter wheat and (2) to

determine the effects of selected chemicals combined with different periods of vernalization on time of floral initiation. The use of immature seeds was also investigated as a possible means of reducing intergeneration time.

LITERATURE REVIEW

Winter wheat plants normally require exposure to low temperature in order to initiate flowering. This process is called vernalization. Vernalization has been defined by Chouard (6) as the process of acquiring, or of accelerating, the ability to flower of certain winter-type plants by chilling.

Response to vernalization is under genetic control, but the exact number of genes involved and their effects are still undetermined. Various investigators, according to Halloran and Boydell (10) have shown that chromosomes 5A and 5D are directly involved in the vernalization process. Halloran and Boydell (10), using chromosome substitution lines, evaluated the effects on vernalization of each of the 21 chromosomes of the variety 'Hope' by substituting one pair at a time into the variety of 'Chinese Spring' which has a moderate vernalization response. They reported that chromosomes 2A, 5A, 3B, 6B, 7B, 1D, and 7D individually reduced the vernalization requirement, while chromosomes 4D, 2D, and 5D increased the vernalization requirement.

The effect of vernalization on the pattern of low molecular weight substances in the wheat plant has been investigated by Trione et al. (26) in an attempt to establish a cause and effect relationship. Working with both spring and winter wheat varieties, they found that glutamic acid and lysine were more abundant when plants were grown at higher temperatures than at lower temperatures. Alanine and proline were more abundant at lower temperatures. A general increase in the

neutral and acidic amino acids occurred in both types of varieties during the first two weeks when grown at 2 C. This was followed by a rapid decline during the subsequent period. Under these growing conditions, the pattern of the basic amino acid was irregular.

Effects of Light and Temperature on Vernalization

Klippart, according to McKinney and Sando (16), was one of the earliest workers to study the effects of chilling on germinating winter wheat seeds. The process which he used was to allow winter wheat to germinate in the fall or winter then restrict growth by subjecting the germinating seeds to near freezing temperatures until these seedlings were sown in the spring. Using this treatment, Klippart was able to make winter wheat react like spring wheat.

In another early study, Peltier and Kiesselbach (20) vernalized germinating seeds of 'Turkey' winter wheat by treatment at approximately 2 C for 30 to 45 days. These vernalized seedlings were then planted in the field at the same time as 'Ceres' spring wheat. Vernalized 'Turkey' headed at about the same time as 'Ceres' when both were planted in the spring. Non-vernalized 'Turkey' did not head from spring seeding. The 'Early Blackhull' variety of winter wheat when vernalized and seeded in the spring with 'Ceres' headed about a week earlier than the spring variety. It was also found that the freezing of seedlings after they had been vernalized did not result in earlier heading as compared to seedlings that had not been frozen after vernalization.

Adams (1) was the first to point out the importance of temperature in relation to the daily photoperiod in regulating the time of flowering

in winter wheat. He stated that these two factors were interchangeable. Grant (9) reported that improper control of temperature and light results in the failure or delay of floral initiation and floral development in winter wheat. McKinney and Sando (15, 16, 17) and Purvis (21) found that floral initiation of the winter wheat variety 'Harvest Queen' and the winter rye variety 'Petkus' was induced by a short photoperiod and low temperatures. However, a longer photoperiod was required for floral development.

Ahrens and Loomis (2) reported that 'Minter' winter wheat could be vernalized by soaking the kernels in water and then chilling the germinating seeds at 1 to 3 C. No vernalization effect was observed when the seeds were chilled at -2 C. They also found that chilling periods of less than 3 to 4 weeks did not result in earlier heading, whereas chilling periods of 6 to 7 weeks produced maximum effects on earliness. Chilling periods of 6 to 7 weeks at 1 C followed by growing the plants in a warm greenhouse at 24 C with a 24-hour photoperiod resulted in the earliest heading with this variety. The total elapsed time from the start of the vernalization treatment to heading was 99 days. Further increases in the chilling period of the germinating seeds merely delayed the total time necessary for heading.

Grant (9) reported that vernalization treatment of winter wheat for periods of 5 to 8 weeks at 10 C under an 8-hour photoperiod, followed by growing the plants in a greenhouse at 21 C with continuous light, resulted in an earlier initiation of flowering. The variety 'Comanche' required only 77 days to reach anthesis after the initiation of germination, including 5 weeks of vernalization. The range of the minimum number of days from seeding to anthesis for 8 varieties was

from 77 to 98 days. The varieties which were used in this study represented a wide range in level of winter hardiness and included the varieties 'Cheyenne' and 'Lutescens.' No relationship between winter hardiness and vernalization requirement was noted.

McKinney and Sando (15, 16, 17) found that the germinating seed of 'Harvest Queen,' 'Kharkov 22 M. C.,' and 'Lutescens' winter wheats when chilled for 65 days in the dark at 3 to 5 C produced plants that headed earlier than those from seedlings chilled at lower or higher temperatures.

Pauli et al. (19) reported that the stage of growth prior to the cold treatment was an important factor in the vernalization process. Using the 'Bison' variety of winter wheat, they found that delaying the cold treatment until the 1-leaf stage caused a delay of about 8 days in the time required for the plant to reach anthesis. Ahrens and Loomis (2), working with 'Minter' winter wheat, found that plants 1, 2, 4, and 11 weeks of age at the start of vernalization treatment of 7 C for 7 weeks headed in 34, 33, 30 and 27 days after vernalization, respectively. All of these differences were significant at the 5% level, indicating that the older plants headed faster following the vernalization treatment. However, the total time from planting to heading was 90, 96, 107, and 153 days, respectively, for these treatments.

McKinney and Sando (17) reported that a short photoperiod during the cold treatment decreased the time required for heading of winter wheat. Their plants were exposed for 54 days to a temperature of 11 C, which was later increased to 15 C. Their results showed that the 'Harvest Queen' variety headed in 88 days after planting when given a short photoperiod of 8 hours. Plants exposed to a photoperiod of 12 to

14 1/2 hours headed after 95 days, and under a longer photoperiod of 16 1/4 to 17 3/4 hours, plants headed after 114 days. With 'Pawnee' and 'Minter' varieties of winter wheat, Ahrens and Loomis (2) found that the photoperiod during the cold treatment had no influence on either the length of the vernalization period or the response to photoperiod following vernalization. However, the prevernalization photoperiod showed significant effects. The seedlings treated with a short photoperiod at high temperatures before vernalization resulted in earlier heading as compared to seedlings treated with a longer photoperiod.

Gott et al. (8) exposed some vernalized 'Petkus' winter rye plants to continuous light while others were exposed to short days of 10-hour light periods. Floral initiation occurred in 3 weeks under continuous light but took 6 to 7 weeks under short day conditions. Purvis (21) obtained similar results in a photoperiod experiment with several winter cereals.

McKinney and Sando (17) grew vernalized seedlings of 'Harvest Queen' winter wheat at 12 to 18 C and subjected them to two different photoperiod treatments. With 16 to 18 hours of light, plants headed in 66 days after planting, while those receiving 11 to 15 hours of light headed after 118 days. Plants which were grown from nonvernalized seeds headed 128 days after planting.

Ahrens and Loomis (2) reported that conventionally vernalized plants of 'Pawnee' and 'Minter' winter wheats headed earlier under an 18-hour photoperiod than those exposed to an 11-hour photoperiod after chilling. Pauli et al. (19) found that vernalized plants of 'Bison'

winter wheat grown in the greenhouse under a 24-hour photoperiod averaged 3 weeks earlier in heading than those under an 18-hour photoperiod.

Apparently, the response to photoperiod is conditioned by the genotype of the plant. The floral initiation of certain varieties of winter wheat is enhanced by long days, while other varieties respond to short day treatments.

Balla (3) recently reported that European varieties of winter wheat could be classified into two groups according to photoperiod response. One group comprised those types that required more than a 12-hour photoperiod, and the other group comprised those types requiring less than a 12-hour photoperiod. There were some types that tended to be intermediate to these two groups with respect to photoperiod response. Balla (3) also noted that plants with a prostrate type of growth habit tended to have a slow rate of development, while the semi-erect types had a faster rate of development.

Chemical Effects on Vernalization

In recent years, a number of chemicals have been used in attempts to promote early flowering in plants. In 1953, Leopold and Guernsey (13) found that treatment with naphthaleneacetic acid solutions followed by a brief exposure to a low temperature of 3C promoted early flowering in winter barley, 'Victory'oats, and other crops. This promotive effect was evidenced by advanced stages of flower development at time of stem dissection. When the auxin treatment was followed by an exposure to room temperature of 18 C, flowering was inhibited in 'Wintex' barley. When the plants were shaded, the stimulative effect

was inhibited. In a later study, these workers (14) found that naphthaleneacetic acid, B-naphthoxyacetic acid, thiamine and 2, 3, 5-tri-iodobenzoic acid were effective in promoting earlier flowering in the 'Alaska' pea variety.

Gibberellic acid is known to increase shoot growth. This compound can also induce other physiological responses. Barbat and Ochesanu (4) sprayed a 20 mg/liter solution of gibberellin A_3 twice weekly for 6 weeks on potted plants of the winter wheat varieties 'Antonomia,' 'Funone,' 'Bezostia' and 'A15.' An examination of the growing point revealed that gibberellin A_3 had a stimulating effect on the cells. This effect was more pronounced under short-day than long-day conditions and promoted floral initiation since plants receiving no gibberellin treatment failed to develop spike primordia. Also, the effect of gibberellin was influenced by day length as well as the quality and intensity of the light.

Pauli et al. (19) soaked germination seeds of 'Bison' winter wheat in 200 ppm gibberellic acid overnight at room temperature prior to vernalization at 7 C for varying periods of time. After vernalization, the seedlings were transferred to the greenhouse and placed under continuous light. These authors observed that the initial growth of the seedlings was stimulated but concluded that the effects of the gibberellic acid were temporary since the treatment did not affect the time of anthesis.

Weibel (28) studied the effect of gibberellic acid applied to germinating seeds during the vernalization process. He sprayed 0.05 to 0.1 mg of gibberellic acid on the germinating seedlings during the cold treatment period. Seedlings were moved to the greenhouse after varying

periods of cold treatment and grown at room temperatures. Weibel found that the check seedlings headed normally with a cold treatment of 45 days but failed to head with a shorter period of cold treatment. On the other hand, seedlings treated with giberellic acid headed with a cold treatment of 28 days or more, but failed to head with less than 28 days of cold treatment. He indicated that gibberellic acid when applied to seedlings might be used as a means of reducing the normal vernalization period of winter wheat by 4 weeks.

The application of gibberellic acid following vernalization has also been studied. Krekule (12) worked with plants of the winter wheat variety 'Hodoninska' which had been vernalized under short day conditions in the field. These plants were later transferred at various intervals to long day conditions. During the short day treatment, some of the plants were sprayed with aqueous solutions of gibberellic acid (25 mg/liter) for 4 to 5 days. He found that plants treated with gibberellic acid headed later than the nontreated plants.

Rapport and Bonner (22), working with the 'Batavian' variety of endive, treated plants biweekly with potassium gibberellate at the rate of 50 or 100 ug. The plants were treated at the 2-leaf stage following a cold treatment of 4 C for 20 days before being transferred to the greenhouse under either an 8- or 16-hour photoperiod. The plants treated with gibberellate headed earlier than the untreated control plants. Earliness was enhanced when plants were grown under a long photoperiod in the greenhouse.

Pauli et al. (19) using four hard red winter wheat varieties noted that gibberellic acid, when applied to plants at time of anthesis, failed to affect the cold requirement of the next generation progeny

grown from treated plants. However, these workers reported that gibberellic acid treatment partially compensated for the cold requirement in winter wheat. Plants headed earlier when short periods of cold treatment were combined with high levels of gibberellic acid treatment.

Studies with Immature Kernels

The use of immature kernels might aid in shortening the intergeneration time in a wheat breeding program. Germination of immature kernels of winter cereals have been studied by several workers. Wellington (29) found that immature wheat seeds would not germinate if harvested when the pericarp was still green, but seed harvested when the color had changed germinated satisfactorily. Robertson and Curtis (23) collected winter wheat kernels at 3-day intervals starting 15 days after anthesis and continuing until the kernels were ripe - about 39 days after anthesis. Kernels air-dried for 3 days after collection germinated better than the fresh kernels harvested at the same stage of development. Good germination was obtained with air-dried kernels harvested as early as 18 days after anthesis.

Weibel (27) studied the germination of immature embryos of 'Comanche' winter wheat by harvesting spikes with attached culms from 8 to 12 days after anthesis. These were vernalized by standing the culms in water and storing them in the refrigerator at 0 to 5 C for 40 to 50 days. Plants grown from these seeds headed only when grown under long day conditions.

MATERIALS AND METHODS

Three separate experiments were conducted during 1968 and 1969. Foundation seed of 'Triumph 64' hard red winter wheat was used in all experiments. 'Triumph 64' is an early maturing variety with wide adaptation, moderate to good standing ability, and good winter hardiness. 'Triumph 64' and its sister varieties, 'Triumph' and 'Improved Triumph,' are the predominant varieties grown in Oklahoma. They comprised over 50% of the wheat acreage in the state in 1969. Detailed descriptions of 'Triumph 64' have been published (5, 25).

In all experiments, vernalization treatment consisted of exposing germinating seeds to a temperature of 2 C for varying periods of time. Kernels were placed in petri dishes which had been lined with moistened 'Kimpak' germination tissue. These were left at room temperature for 24 hours to allow the germination process to start. The germinating seeds were then placed in a refrigerator compartment maintained at 2 C. The temperature was checked periodically and the petri dishes received water or solution as needed to maintain seedling growth.

After the prescribed period of cold treatment, the seedlings were transplanted to plastic pots, 9 cm in diameter. The soil medium in these pots was a mixture of greenhouse soil and perlite, at a ratio of 5:1. The pots were then placed in a growth chamber at a temperature of 20 ± 1.5 C. The light intensity in the chamber was maintained at approximately 2000 ft-candles. Three seedlings were transplanted to each pot. Plants were thinned after 2 weeks to one per pot.

Although this study was of an exploratory nature, the three experiments were designed so that the data could be analyzed statistically. However, plants in certain cold treatments failed to survive and in other treatments plants were still in the vegetative stage when the experiments were terminated. Therefore, statistical analyses were not conducted since it appeared that they would be of limited value.

Experiment I. Mature Seed Study with Gibberellic Acid Treatment

In June of 1968, mature, sound seeds of 'Triumph 64' in petri dishes containing water were placed in the refrigerator for the start of the vernalization treatments. There were nine cold treatments. These consisted of exposure at 2 C for the following number of weeks: 0, 1, 2, 3, 4, 5, 6, 7 and 8.

During the first eight weeks after the start of germination, the following gibberellic acid (GA) treatments were superimposed on each of these cold treatments:(1) check (no treatment), (2) weekly applications (total of 8), (3) biweekly applications (total of 4), (4) triweekly applications (total of 2). The following 3 rates of GA were used: (1) 0.025 mg, (2) 0.050 mg, and (3) 0.075 mg. The GA treatments were applied to the seedlings as aerosol spray. The applicator was calibrated to apply GA at the rate of 0.025 mg per second. Thus, a 1-second application would be 0.025 mg, a 2-second application would be 0.050 mg and a 3-second application would be 0.075 mg. The seedlings receiving 0 weeks cold treatment were transplanted immediately to pots and received all their GA treatment in the growth chamber. The seedlings receiving 8 weeks of cold treatment received all of their GA treatments while in the refrigerator at 2 C. Seedlings of the other

cold treatments received a part of their GA treatment while in the refrigerator and the other part in the growth chamber after transplanting.

There were a total of 90 different treatments including the checks and 2 replications. The growth chamber in which the plants were placed after being transplanted into pots was maintained with a 24-hour photoperiod.

Data were recorded on the number of days to anthesis. This was expressed as the number of days from the start of germination to anthesis and the number of days from the end of vernalization to anthesis.

Experiment II. Mature Seed Study with GA, Phosphate and Glucose

In February 1969, mature, sound seeds of 'Triumph 64' wheat were treated with 'Arasan 75' for the purpose of surface sterilization. The seeds were then soaked in germinating media at room temperature in petri dishes for 24 hours to allow germination to start. On the basis of the results obtained in Experiment I, the cold treatments were changed to 2, 3, 4, 5, 6, and 7 weeks of exposure at 2 C.

The following chemical treatments were superimposed on each of the cold treatments: (1) check (water only), (2) glucose alone, (3) GA alone, (4) phosphate (monobasic KH_2PO_4) alone, (5) phosphate + glucose, (6) GA + glucose, (7) GA + phosphate, and (8) GA + phosphate + glucose. The GA concentration was 40 ppm, phosphate was 0.02 M and glucose was 0.2 M. Chemical treatments were applied as the germinating media. A pH of 5.5 was maintained in the germinating media of those treatments containing phosphate by the use of a PO_4^{-3} buffer solution. There were 48 treatments, including a check, replicated 4 times.

During the first 3 weeks after the seedlings had been transplanted to the pots, they were supplied with a 50% Hoagland nutrient solution as necessary to maintain proper growth. The growth chamber in which the plants were placed after being transplanted into pots was maintained with a 16-hour photoperiod.

Data were recorded on the number of days to anthesis. This was expressed as the number of days from the start of germination to anthesis and the number of days from the end of vernalization to anthesis. Also, measurements were made on the length of shoots and roots of five random seedlings in each of the chemical treatments after 7 weeks of cold treatment.

Experiment III. Immature Seed Study with GA, Phosphate and Glucose

During the spring of 1969, spikes of 'Triumph 64' plants growing in the greenhouse soil bed were collected at weekly intervals of 2, 3, 4 and 5 weeks after anthesis. The kernels were mature on the last date of collection. A number of plants were tagged and labeled on the date of anthesis so that samples could be taken at a prescribed time after anthesis. After harvest, the spikes were allowed to air-dry for 1 week. The seeds were then removed from the spike and placed in petri dishes with various chemical treatments as the germinating media.

Based on Experiment II, the cold treatments were reduced to 3 1/2 weeks, 5 weeks and 6 1/2 weeks. Superimposed on each cold treatment were the following chemical treatments: (1) check (water only), (2) GA, (3) glucose, and (4) glucose + phosphate. These treatments were selected as the most promising based on the results of Experiment II. The concentrations of these solutions were 40 ppm GA, 0.02 M phosphate,

and 0.2 M glucose. A pH of 5.5 was maintained in the germinating media of those treatments containing phosphate by the use of a PO_4^{-3} buffer solution.

For each of the 4 harvest periods there were 3 cold treatment periods and for each of these there were 4 chemical treatments including the check. This resulted in 48 treatments with 4 replications.

The growth chamber in which the plants were placed after being transplanted into pots was maintained with a 16-hour photoperiod. The plants were watered as necessary to maintain adequate growth. After 2 weeks in the growth chamber, commercial fertilizer (16-20-0) was applied to the plants at a rate of half-teaspoonful per pot. This rate of fertilizer was applied at weekly intervals for a period of 1 month.

Percentage germination of the seeds after different periods of cold treatment was recorded. Plans were made to record date of anthesis; however, the plants were lost before heading occurred.

RESULTS

Experiment I. Mature Seed Study with GA Treatment

In this experiment, the response of 'Triumph 64' to varying durations of cold treatment combined with various GA treatments is recorded as days from the start of germination to anthesis. This information is shown in Table I. No data on flowering were obtained from the 0, 1 and 2 weeks of cold treatment since plants in these treatments failed to reach the heading stage.

Considering only the cold treatments, plants headed earliest from the 5 and 6 weeks exposure to low temperatures. As shown in Table I, the average time to anthesis for 3 to 8 weeks of cold treatment in the absence of GA were: 111, 90, 83, 82, 86 and 91 days, respectively. It took a shorter time from germination to anthesis with the 5 and 6 weeks of cold treatment than for any other periods of cold treatment. This same trend was also evident when GA treatments were superimposed on the cold treatments. Consequently, 5 or 6 weeks of vernalization could be regarded as critical chilling periods as further increase or decrease of the cold treatment period increased the total time necessary for anthesis.

With a few notable exceptions there was little difference between the various GA treatments and the checks. In general, no differences were observed with regard to times of application or rates applied.

However, there were two combinations of chemical treatments and cold treatments that resulted in plants which headed several days

TABLE I

EXPERIMENT I. NUMBER OF DAYS FROM START OF GERMINATION TO ANTHESIS OF 'TRIUMPH 64' SUBJECTED TO DIFFERENT PERIODS OF COLD TREATMENT COMBINED WITH VARIOUS GA TREATMENTS

Weeks of Cold Treatment	Check	GA Treatment									Average	Range
		0.025 mg/application			0.050 mg/application			0.075 mg/application				
		Weekly	Biweekly	Triweekly	Weekly	Biweekly	Triweekly	Weekly	Biweekly	Triweekly		
3	111[a]	101	106	128[a]	130[a]	107	119	117[a]	116[a]	130[a]	117	101-130
4	90	75	88	89	88	85	95	91	83	93	88	75-95
5	83	81	83	82	78	82	82	82	81	83	82	78-83
6	82	88	84	84	86	84	83	82	82	81	84	81-88
7	86	87	86	86	87	86	85	85	86	91	87	85-91
8	91	91	88	89	93	93	89	91	91	92	91	88-93
Average	91	87	89	93	94	90	92	91	90	95	91	
Range	82-111	75-101	83-106	82-128	78-130	82-107	82-119	82-117	81-116	81-130		75-130

[a]Data from one replication only; the other did not head.

earlier than the earliest check. Plants treated weekly with 0.025 mg of GA headed earliest (75 days from seeding to anthesis with a cold treatment of 4 weeks). Plants treated weekly with 0.050 mg of GA headed in 78 days from seeding to anthesis with 5 weeks of cold treatment. Thus there appeared to be a tendency for short exposure duration to low temperature combined with a frequent application of GA to induce early heading. Figure 1 compares the check treatment with weekly applications of 0.025 mg GA over the range of vernalization periods. This GA treatment induced the lowest average days from germinating seed to anthesis of all GA treatments observed. The best GA treatment, i.e., the treatment which received weekly applications of 0.025 mg GA combined with 4 weeks cold treatment, headed 1 week earlier than the earliest check treatment. Of note in this figure is that after 5 weeks of cold treatment the seeds treated with GA took a longer time to reach anthesis than the check treatments.

The number of days from planting to anthesis is shown in Table II. An increase in the cold treatment time resulted in earlier flowering after vernalization, i.e., a reduction of time from planting to anthesis. For example, plants reached anthesis in 90, 62, 48, 40, 37 and 35 days after the completion of the vernalized periods of 3, 4, 5, 6, 7 and 8 weeks, respectively, for the check treatments. For each of the GA treatments the same trend was demonstrated. An exception to this generalization is that plants treated weekly with 0.050 mg GA plus 5 weeks of cold treatment were 1 day earlier from planting to anthesis than plants from 0.050 mg GA plus 6 weeks of cold treatment. Also, plants with triweekly applications of 0.075 mg GA plus 6 weeks of cold

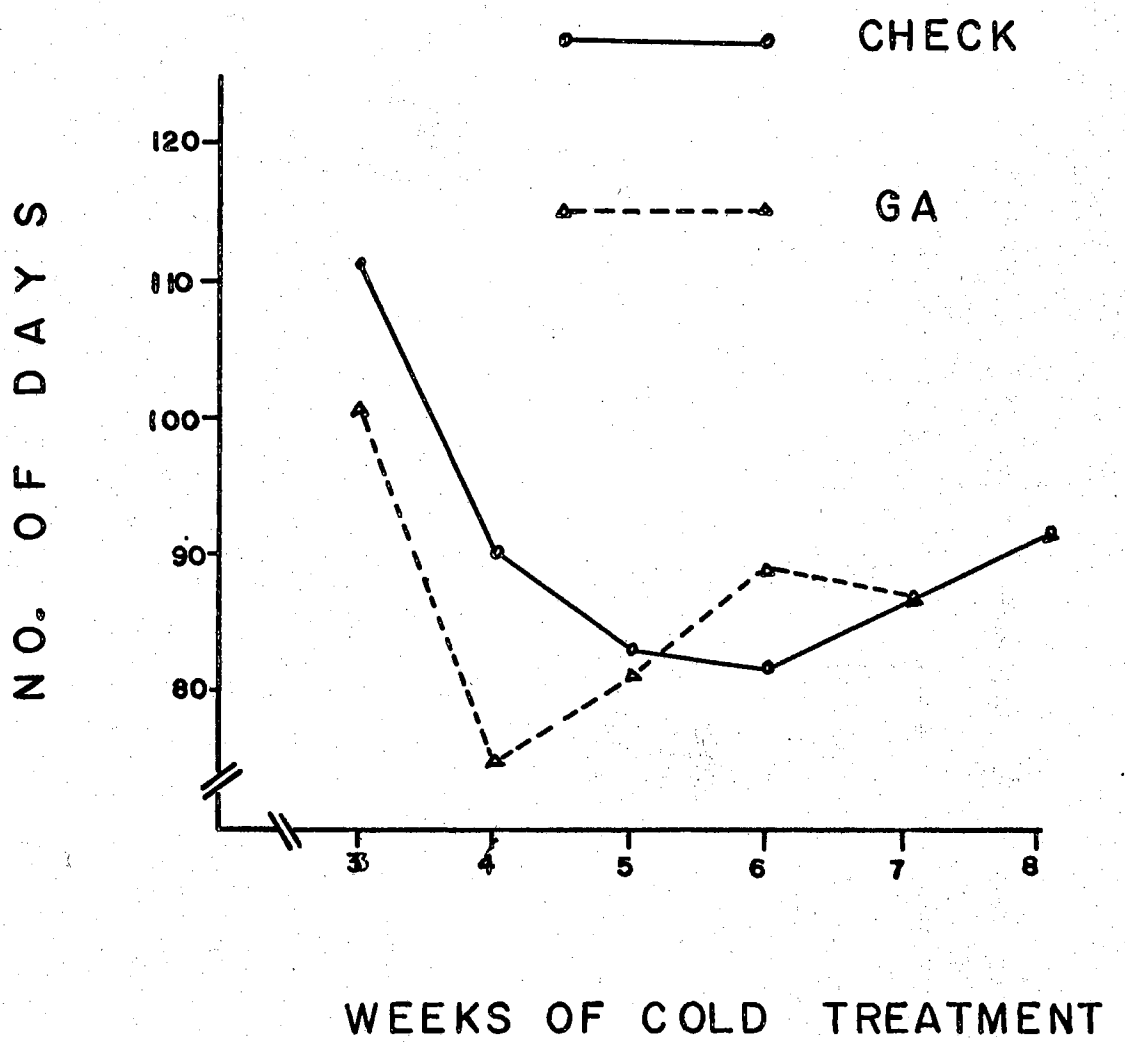

Figure 1. Experiment I. Number of days from start of germination to anthesis of 'Triumph 64' treated weekly with 0.025 mg GA compared with check at various periods of vernalization.

TABLE II

EXPERIMENT I. NUMBER OF DAYS FROM END OF VERNALIZATION PERIOD TO ANTHESIS OF 'TRIUMPH 64' SUBJECTED TO DIFFERENT PERIODS OF COLD TREATMENT COMBINED WITH VARIOUS GA TREATMENTS

Weeks of Cold Treatment	Check	GA Treatment									Average	Range
		0.025 mg/application			0.050 mg/application			0.075 mg/application				
		Weekly	Biweekly	Triweekly	Weekly	Biweekly	Triweekly	Weekly	Biweekly	Triweekly		
3	90[a]	80	85	107[a]	109	86	98	96[a]	95[a]	109[a]	95	80-109
4	62	47	60	61	60	57	67	63	55	65	60	47-67
5	48	46	51	47	43	47	47	47	46	48	47	43-51
6	40	46	42	42	44	42	41	40	40	39	42	39-46
7	37	38	37	37	38	37	36	36	37	42	38	36-42
8	35	35	32	33	37	37	33	35	35	36	35	32-37
Average	52	49	51	55	55	51	54	53	51	57	53	
Range	35-90	35-80	32-85	33-107	37-109	37-86	33-98	35-96	35-95	36-109		32-109

[a]Data from one replication only; the other did not head.

treatment were 3 days earlier than those treated with 0.075 mg GA plus 7 weeks cold treatment.

Experiment II. Mature Seed Study with GA, Phosphate and Glucose

Since seeds utilize hexose sugar (usually glucose) to start metabolism to release energy during germination, glucose treatments were applied to determine what effects they might have on floral initiation. Since phosphate is known to promote germination of certain seeds, when used with GA as an inducer (11) phosphate was included. In this experiment, these chemical treatments were used as the germinating media. After vernalization for a period of 7 weeks, the germination percentages reached the maximum and it was observed that the rate of growth for shoots and roots was differentially affected by the chemical treatments. The lengths of shoots and roots were measured, and these data are shown in Table III. For the check (water as germinating solution), the shoot length to root length ratio was 1.0. GA treatment restricted root growth but did not affect shoot growth materially. Phosphate treatment enhanced root growth but did not affect shoot growth. The glucose treatment restricted both shoot and root growth. Chemical combinations involving GA resulted in a shoot length to root length ratio of greater than 1.0.

The results on the number of days from the start of germination to anthesis of this experiment are shown in Table IV. Variation in the total number of days required from the start of germination to anthesis was large for the vernalization treatments. Considering cold treatment alone, the average number of days from seeding to anthesis were 188, 157, 123, 106, 107 and 99 for 2, 3, 4, 5, 6 and 7 weeks exposure to low

TABLE III

EXPERIMENT II. AVERAGE LENGTH OF SHOOTS AND ROOTS OF 'TRIUMPH 64' SEEDLINGS IN VARIOUS GERMINATION MEDIA WITH 7 WEEKS OF VERNALIZATION. (VALUES BASED ON 5 RANDOMLY SELECTED SEEDLINGS.)

Germination Median	Shoot Length (cm)	Root Length (cm)	Shoot/root Ratio
Check (Water)	2.41	2.41	1.0
GA	2.57	0.66	3.8
Phosphate	2.59	4.06	0.6
Glucose	1.09	1.19	0.9
GA + Phosphate	1.24	0.74	1.7
GA + Glucose	1.17	0.74	1.6
Phosphate + Glucose	0.97	0.86	0.9
GA + Phosphate + Glucose	0.74	0.33	2.2

TABLE IV

EXPERIMENT II. EFFECTS OF GA, GLUCOSE AND PHOSPHATE IN CONJUNCTION WITH VARIOUS PERIODS OF COLD TREATMENT ON NUMBER OF DAYS FROM START OF GERMINATION TO ANTHESIS OF 'TRIUMPH 64'

Weeks of Cold Treatment	Treatment Combinations									
	No GA				GA					
	No Phosphate		Phosphate		No Phosphate		Phosphate			
	No Glu.	Glu.	No Glu.	Glu.	No Glu.	Glu.	No Glu.	Glu.	Avg.	Range
2	188[a]	147[b]	155[b]	177[b]	172[b]	147[a]	170[b]	170[b]	166	147-188
3	157[a]	146[a]	160[a]	159[b]	132[b]	146[b]	193[a]	170[b]	158	132-193
4	123	126	144	151	107	154	132	130	107	123-154
5	106	108	106	98	93	--[c]	138	122	110	93-138
6	107	105	102	101	97	102	110	113	105	97-113
7	99	101	103	98	101	105	101	122	104	98-122
Average	130	122	128	131	117	130	141	158	125	
Range	99-188	101-147	102-160	98-177	93-172	105-154	101-193	113-170		93-193

[a]Data from 2 or 3 replications; the rest in booting stage.

[b]Data from 2 or 3 replications; the rest in vegetative stage.

[c]No plants survived to anthesis.

temperature, respectively. Thus generally, by increasing the length of vernalization, earlier flowering resulted. The 7 week cold treatment resulted in the earliest heading when compared to other cold treatments.

Glucose and phosphate treatment had very little effect on altering time of anthesis. Also very little effect was noted for the combinations: glucose + phosphate, GA + glucose, and GA + phosphate. The combination of GA + phosphate + glucose tended to delay time of anthesis. The GA treatment resulted in earlier time of anthesis than the check in the 3, 4, 5, and 6 week cold period treatments. The treatment resulting in earliest anthesis was the GA treatment with 5 weeks exposure to low temperature. Anthesis was reached after 93 days with this treatment compared to 106 days for the untreated check with the 5-week cold period. This GA treatment is compared with the untreated check under various cold treatment periods in Figure II.

An increase of the vernalization period decreased the time necessary for anthesis under the imposed 16-hour photoperiod for all treatments. The average times required after vernalization to anthesis were 174, 136, 95, 71, 65 and 50 days for the vernalization periods of 2, 3, 4, 5, 6 and 7 weeks, respectively, for the check treatments. For each of the chemical treatments the same trend was demonstrated. These data are shown in Table V.

Although no data are presented, it was observed that GA treated plants in the 5 to 7 weeks cold treatment periods had fewer tillers, were shorter and had fewer seeds per spike than the check plants. Nevertheless, 5 to 7 weeks of vernalization did have the highest percentage of seed set, regardless of the chemical treatment applied. The

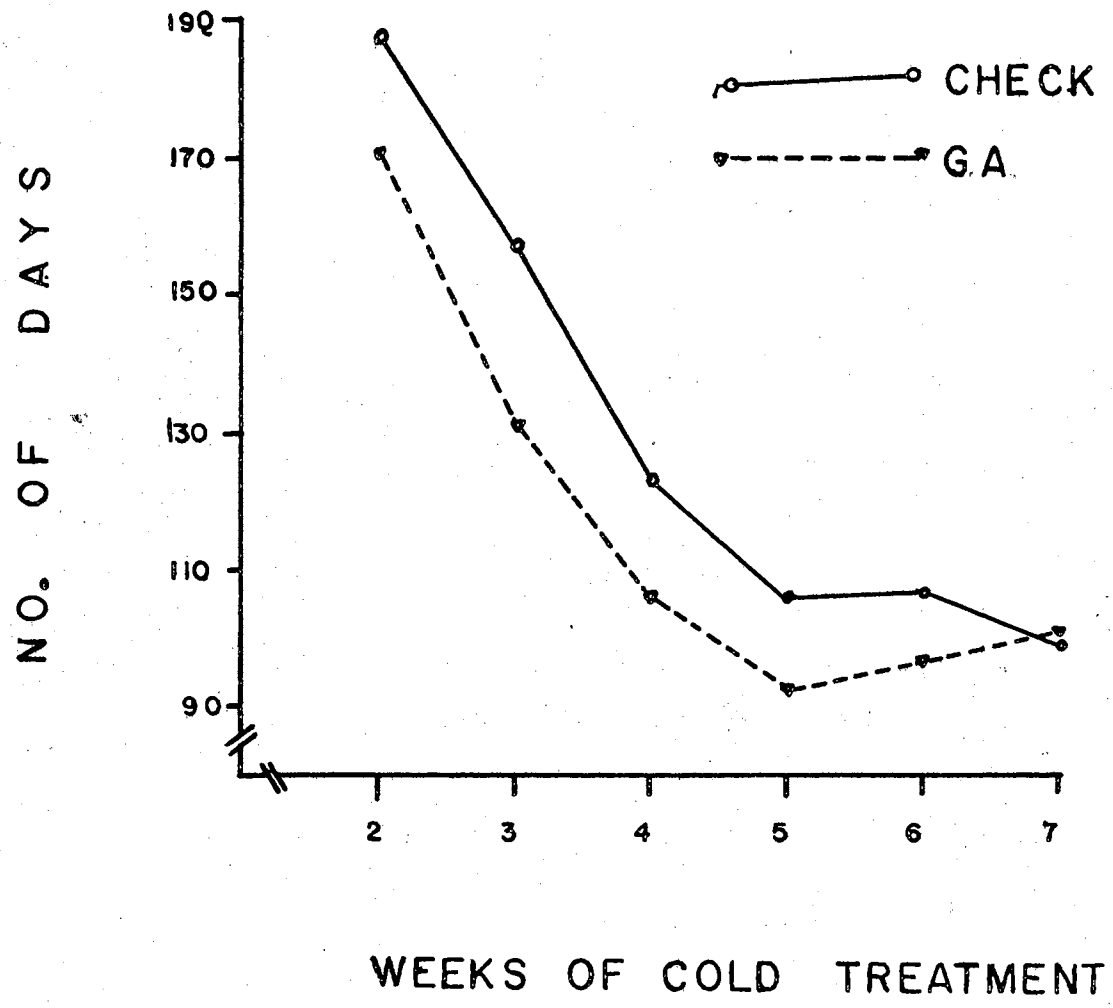

Figure 2. Experiment II. Number of days from start of germination to anthesis of 'Triumph 64' treated with 40 ppm GA as germination medium compared with check at various periods of vernalization

TABLE V

EXPERIMENT II. EFFECTS OF GA, GLUCOSE AND PHOSPHATE IN CONJUNCTION WITH VARIOUS PERIODS OF COLD TREATMENT ON NUMBER OF DAYS FROM END OF VERNALIZATION PERIOD TO ANTHESIS OF 'TRIUMPH 64'

Weeks of Cold Treatment	Treatment Combinations									
	No GA				GA					
	No Phosphate		Phosphate		No Phosphate		Phosphate			
	No Glu.	Glu.	No Glu.	Glu.	No Glu.	Glu.	No Glu.	Glu.	Avg.	Range
2	174[a]	133[b]	141[b]	163[b]	158[b]	133[a]	159[b]	156[b]	152	133-174
3	136[a]	122[a]	139[a]	138[b]	111[b]	125[b]	172[a]	149[b]	137	111-172
4	95	98	116	123	79	126	104	102	105	79-126
5	71	73	71	63	58	--[c]	103	87	75	58-103
6	65	63	60	59	55	60	68	71	63	55-71
7	50	52	54	49	52	56	52	73	55	49-73
Average	99	90	97	99	86	100	110	106	98	
Range	50-174	52-133	54-141	49-163	52-158	56-133	52-172	71-156		49-174

[a]Data from 2 or 3 replications; the rest in booting stage.

[b]Data from 2 or 3 replications; the rest in vegetative stage.

[c]No plants survived to anthesis.

plants with 2 to 4 weeks of vernalization had the lowest percentage of seed set.

Experiment III. Immature Seed Study with GA, Phosphate and Glucose

Germination of air-dried immature seeds in various chemical media combined with different cold period treatments was observed in this experiment. The results are shown in Table VI. The GA treatment resulted in 90% germination at the first harvest date with each period of vernalization. Germination was poor (0 to 5%) in the remainder of the chemical treatments of this collection time. With the second, third, and fourth collection times, 100% germination was obtained for all chemical and cold period treatments.

After vernalization, the germinating seeds were transferred into pots and placed in the growth chamber, but several weeks later the plants became severely wilted. It was presumed that wilting and eventual loss of the plants were due to either the application of an excess of fertilizer or high temperatures in the growth chamber which occurred when the electric power was off for nearly two days. Nevertheless, it was evident that the GA treated plants from the third and fourth collection times with 6 1/2 weeks of cold treatment had started the booting stage. This was observed about 137 days after anthesis of the parent plant. The remainder of the plants did not advance beyond this point in the vegetative stage. Since some of the plants were lost, the data were of questionable value and were not included.

TABLE VI

EXPERIMENT III. AVERAGE PERCENT GERMINATION OF IMMATURE KERNELS OF 'TRIUMPH 64' AT 3 PERIODS OF VERNALIZATION AND 4 DIFFERENT GERMINATION MEDIA TREATMENTS

Weeks of Cold Treatment	Time of Harvest															
	1st Collection Time (2 wk. after anthesis)				2nd Collection Time (3 wk. after anthesis)				3rd Collection Time (4 wk. after anthesis)				4th Collection Time (5 wk. after anthesis)			
	Ck.	GA	Glu.	Glu. + Phos.	Ck.	GA	Glu.	Glu. + Phos.	Ck.	GA	Glu.	Glu. + Phos.	Ck.	GA	Glu.	Glu + Phos.
	% Germination Based on 20 seeds/treatment															
3½	5	90	5	0	100	100	100	100	100	100	100	100	100	100	100	100
5	5	90	5	0	100	100	100	100	100	100	100	100	100	100	100	100
6½	5	100	0	5	100	100	100	100	100	100[a]	100	100	100	100[a]	100	100
Avg.	5	93	3	2	100	100	100	100	100	100	100	100	100	100	100	100

[a]plants in 4 replications were in booting stage after 137 days from anthesis of the parent plant. In the remainder of the treatments, the plants were in the vegetative stage.

DISCUSSION

Environmental factors affecting floral response in winter wheat can be classified into 3 groups: temperature, light, and chemical treatment. Temperature is a factor in the cold treatment necessary for vernalization as well as during the period after vernalization. Light consists of two factors, intensity and photoperiod, of which the photoperiod component is the more important. Of the chemical treatments, GA seems to hold the most promise in enhancing floral initiation.

In the present experiments, only one temperature (2 C) was used for vernalization although different durations of cold treatment were employed. The best results were produced by the 5 to 7 weeks vernalization periods. Ahrens and Loomis (2) found that vernalization periods of 6 to 7 weeks at 1 C produced the maximum effects on earliness. Pauli et al. (19) recognized that complete vernalization was achieved when as much as 6 1/2 weeks of cold treatment were employed. Grant (9) obtained the best results with a vernalization period between 5 and 8 weeks. Nevertheless, it should be recognized that the most efficient periods of vernalization are dependent on the variety being studied, and that winter hardiness and maturity are not always associated with the vernalization requirements (7, 9, 24). Pauli et al (19) found that rapid growth and floral development were induced after vernalization when the temperature was increased to 70 C with a continuous light regime.

Ahrens and Loomis (2) found that the photoperiod during the cold treatment had no influence on either the required length of the vernalization period or the response to photoperiod following vernalization. However, the prevernalization photoperiod showed significant effects.

The data obtained from the present experiments showed that plants reached anthesis in 90, 83, 82 and 86 days from start of germination to anthesis with vernalization periods of 4, 5, 6, and 7 weeks, respectively, when a 24-hour photoperiod was used after vernalization. Comparable figures with a 16-hour photoperiod were 123, 106, 107 and 99 days. From this it is evident that plants which were grown under a continuous light regime reached anthesis stage 2 to 3 weeks earlier than those grown under a 16-hour light regime. These results are in agreement with those reported by Pauli et al. (19) who found that wheat plants grown under a 24-hour photoperiod regime headed about 3 weeks earlier than those grown under an 18-hour photoperiod regime.

When GA was applied to seedlings and young plants as a spray in Experiment I of the present study, plants treated weekly with 0.025 mg of GA reached anthesis in 75 days after the start of germination with 4 weeks of cold treatment. This particular GA and cold period treatment induced heading 1 week earlier than the best check treatment (cold treatment alone) which was exposed to 6 weeks of cold treatment.

When GA was used in the germinating media, in Experiment II, the earliest anthesis occurred with 5 weeks of vernalization. Anthesis was reached after 93 days with GA treatment compared to 106 days for the untreated check at the 5-week cold period treatment. Chemical treatments of glucose, phosphate, glucose + phosphate, and GA + glucose had very little effect on altering time of anthesis. The combinations of

GA + phosphate and GA + phosphate + glucose tended to delay the time of anthesis.

When applied as the germination medium, GA restricted root growth but did not affect shoot growth materially. Phosphate treatment enhanced root growth but did not affect shoot growth. The glucose treatment restricted both shoot and root growth. Combinations of these chemicals also had a tendency to restrict both shoot and root growth.

GA is believed to function in the transcription of mRNA from DNA and in the resultant protein synthesis (18). Induction to flowering and stem elongation probably is accomplished only when sufficient GA is produced to permit the production of the appropriate message. The length of time necessary for flowering in response to the most efficient cold period is shortened by GA, indicating that the GA effect is probably quantitative. The use of glucose and phosphate gave no advantage in reducing the response time for flowering; therefore, these two components of the energy production cycle would appear to be adequate in the plants under test.

GA, used as a germinating medium, substantially increased the germination percentage of air-dried immature seeds, while glucose and glucose + phosphate combination did not. The use of immature seeds as a method for reducing the generation time seemed to be effective as shown in Experiment III of the present study. The GA treated plants from the third and fourth collection times, with 6 1/2 weeks of cold treatment, had started the booting stage in 137 days after anthesis of the parent plant. Owing to the wilting and loss of the plants, anthesis was not reached.

Although there is research being done today to explain the function of vernalization and floral initiation in the physiology of plants, not enough information is available at the present time to specify the mechanism involved. Information is being accumulated on factors that affect vernalization requirements and the compensating effect of some of these factors for cold requirement in connection with the time of floral initiation.

SUMMARY AND CONCLUSION

The flowering response of 'Triumph 64' winter wheat was found to be influenced by duration of cold exposure of germinating seeds, length of photoperiod following cold treatment, and application of GA to the germinating seeds and seedlings. Applications of glucose and phosphate in germinating media had very little effect on altering time of anthesis and in some cases even delayed anthesis.

With mature seeds, when GA was applied as a spray, 5 to 6 weeks of cold treatment was the critical chilling period and further increase or decrease of the cold treatment period increased the total time necessary for anthesis. An exception to this generalization occurred when weekly applications of 0.025 mg GA were imposed on the 4-week cold period treatment. This treatment induced the earliest heading of all the treatments employed. Plants reached the anthesis stage 75 days from the start of the germination period. It can be concluded from this that GA reduced the vernalization requirement.

GA treatment combined with 5 weeks of cold treatment resulted in the earliest heading of all the treatments involving the three chemicals used as germinating media. Plants in this treatment reached anthesis 93 days after the start of germination.

Shoot growth was unaffected but root growth was restricted when GA was applied as a germination medium to mature seeds. Phosphate enhanced root growth but did not affect shoot growth, while glucose restricted both root and shoot growth.

Plants which were grown under a continuous light regime reached anthesis 3 weeks earlier than those grown under a 16-hour light regime. From this it is evident that floral initiation and development of 'Triumph 64' winter wheat is enhanced by a long photoperiod regime.

Harvesting of plants before seed maturity is reached could be used to decrease the generation time for breeding purposes. Immature kernels harvested 2 weeks after anthesis and air-dried for 1 week germinated at a high percentage when GA was used as the germinating medium. Glucose and glucose + phosphate combination did not enhance germination of immature seeds.

From the results of this study it is evident that two generations of winter wheat could be grown in one year by combining the use of immature seeds, frequent GA applications and vernalization during the germination process.

LITERATURE CITED

1. Adams, J. 1924. Does light determine the date of heading out in winter wheat and winter rye? Amer. J. Bot. 11:535-539.

2. Ahrens, J. F. and W. E. Loomis. 1963. Floral induction and development in winter wheat. Crop Sci. 3:463-466.

3. Balla, L. 1968. Developmental physiological problems of the production of high yielding, winter-hardy wheat varieties having a short growing season. Acta Agron. Acad. Sci. Hungaricae 17:131-138.

4. Barbat, L. and C. Ochesanu. 1963. Effect of gibberellin on the variations of the growth point in winter wheat. Naturwissenschaften 50:159.

5. Briggle, L. W. and L. P. Reitz. 1963. Classification of Triticum species and of wheat varieties grown in the United States. USDA Tech. Bull. 1278.

6. Chouard, P. 1960. Vernalization and its relations to dormancy. Ann. Rev. Plant Physiol. 11:191-238.

7. Croy, L. I. 1959. The response of selected exotic and indigenous wheat varieties to changes in day length and temperatures. M. S. Thesis, Oklahoma State University, Stillwater, Okla.

8. Gott, M. B., F. G. Gregory and O. N. Purvis. 1955. Studies in vernalization of cereals. XIII. Photoperiodic control of stages in flowering between initiation and ear formation in vernalized and unvernalized Petkus winter rye. Ann. Bot. (N.S.) 19:87-126.

9. Grant, M. N. 1964. Vernalization and days to anthesis of winter wheat under controlled temperature and light. Can. J. Plant Sci. 44:446-450.

10. Halloran, G. M. and C. W. Boydell. 1967. Wheat chromosomes with genes for vernalization response. Can. J. Genet. Cytol. 9:632-639.

11. Hashimoto, T. and Y. Yamaki. 1962. Interaction of gibberellin A_3 and inorganic phosphate in tobacco seed germination. Plant and Cell Physiol. 3:175-187.

12. Krekule, J. 1962. Inhibiting effect of treatment with gibberellic acid during vernalization of winter wheat under short day conditions. Naturwissenschaften 49:164-165.

13. Leopold, A. C. and F. S. Guernsey. 1953. Modification of floral initiation with auxins and temperature. Am. J. Bot. 40:603-607.

14. _____ and _____. 1954. Flower initiation in Alaska pea. II. Chemical vernalization. Am. J. Bot. 41:181-185.

15. McKinney, H. H. and W. J. Sando. 1930. The behavior of winter wheat in artificial environment. Science. 71:668-670.

16. _____ and _____. 1933. Earliness and seasonal growth habit in winter wheat. J. Her. 24:168-179.

17. _____ and _____. 1935. Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. J. Agr. Res. 51:621-629.

18. Overbeek, J. V. 1966. Plant Hormones and Regulators. Sci. 152: 721-731.

19. Pauli, A. W., J. A. Wilson and F. C. Stickler. 1962. Flowering response in winter wheat as influenced by temperature, light and gibberellic acid. Crop Sci. 2:271-274.

20. Peltier, G. L. and T. A. Kiesselbach. 1934. Inducing early fruiting in winter wheat by seed activation. J. Am. Soc. Agron. 26:503-506.

21. Purvis, O. N. 1934. An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. 48:919-955.

22. Rapport, L. and J. Bonner. 1960. Interactions of gibberellin, vernalization, photoperiod and temperature in the flowering of endive. Plant Physiol. 35:98-102.

23. Robertson, L. D. and B. C. Curtis. 1967. Germination of immature kernels of winter wheat. Crop Sci. 7:269-270.

24. Schlehuber, A. M. 1955. Unpublished data, Oklahoma Agricultural Experiment Station (used with permission).

25. _____ and J. W. Johnson. 1965. Registration of Triumph 64 wheat. Crop Sci. 5:605.

26. Trione, E. J., J. L. Young and M. Yammamoto. 1960. Free amino acid changes associated with vernalization of wheat. Phytochemistry. 6:85-91.

27. Weibel, D. E. 1958. Vernalization of immature winter wheat embryo. Agron. J. 50:267-270.

28. Weibel, R. O. 1960. Effects of gibberellin on the vernalization period of winter wheat. Agron. J. 52:122-123.

29. Wellington, P. S. 1956. Studies on the germination of cereals. I. The germination of winter grains in the ear during development, ripening and after ripening. Ann. Bot. (London) (N. S.) 20:105-120.

VITA

Jaunita Hwa-ling Tu Chin Choy

Candidate for the Degree of

Master of Science

Thesis: FACTORS AFFECTING FLOWERING RESPONSE IN 'TRIUMPH 64' WINTER WHEAT

Major Field: Agronomy

Biographical:

Personal Data: Born in Hunan Province, Republic of China, October 15, 1944, the daughter of Chi-Lian and Lee-ing Tu.

Education: Attended elementary school and graduated from Taipei Girls' First Provincial High School, Taipei, Taiwan, China, in 1963; received the Bachelor of Science degree from the National Taiwan University in June, 1967; completed requirements for the Master of Science degree in May, 1970.

Professional Experience: High school tutor, Taipei, February, 1964, to May, 1967. Graduate Assistant, Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma, September, 1967, to May, 1970.