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PHOTOINDUCTION OF FLORAL DEVELOPMENT IN HARD RED WINTER WHEAT

BY

JOSE ERNESTO ROBLES WIJANGCO

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science
Major in Plant Science

South Dakota State University
1982

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PHOTOINDUCTION OF

FLORAL DEVELOPMENT IN HARD RED WINTER WHEAT

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the department.

D. G. Kenefick
Major Advisor

Date

M. L. Horton
Department Head

Date

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--jerw

INTRODUCTION

Yield is primarily related to assimilation of photochemically harvested CO_2 and nutrient uptake during the life cycle of crop plants. Initial directions to improve CO_2 assimilation focused on the assumption that yield was limited by net photosynthesis (in this context defined simply as the photochemical reduction of CO_2).

Recent reports, showing measurements of CO_2 uptake among various crop plants, indicate that high yielding cultivars are not always associated with high CO_2 uptake. In wheat, the CO_2 assimilation rate of high yielding modern cultivars is lower than their wild progenitors (13). Duncan and Hesketh concluded that improvements in maize were not associated with an increase in photosynthesis (7). Dantuma reported that variation in CO_2 uptake among winter wheats was not related to grain yield (6). Evans suggested that the CO_2 fixation potential of modern cultivars is more than adequate to support the plant's productive capacity (12). In wheat, high CO_2 assimilation has been measured at ear emergence (17). Evans and others have suggested that the presence of an active sink (i.e. inflorescence) is one important controlling factor in mobilizing the photosynthetic capacity of these modern crop plants (1, 12, 17). Hence, a balance between maximum CO_2 fixation and high demand for photosynthate would be an ideal situation for higher crop yield. Under field conditions in early spring,

CO_2 fixation in C_3 plants is improved by low temperatures (12). Early development of the inflorescence during this time would be advantageous since available photosynthate would not be limiting. Dantuma and others have suggested that high grain yield in winter wheat is related to early inflorescence development (6, 14, 15, 21).

The time of floral initiation also affects the yield potential of the plant by its influence on the spikelet number. Lucas (19), Thorne (27), and Williams et al. (29) have shown that photoperiod conditions prior to the initiation of the inflorescence affects spikelet number. Williams et al. (29) and Mohapatra et al. (20) showed that the longer the daylength conditions at floral initiation, the smaller the spikelet number. Rawson concluded that daylight beyond the duration required for inflorescence development reduced the spikelet number, grain number, and yield per ear (23). Cultivars with late floral initiation develop at longer daylengths in the spring compared to those with early development. Spikelet production has also been shown to be sensitive to temperature (19). Consequently, spikelet number may be reduced thereby limiting yield. Despite the obvious advantages of understanding factors influencing earliness, Evans indicated that knowledge of the precise photoperiod conditions for early floral initiation in winter wheat is limited (13).

South Dakota is at the northern limit of winter wheat

cultivation in the United States. However, it has been estimated that only 15% of the total winter wheat acreage has been planted to hardy winter wheat cultivars in this state. Records at this station indicate that grain yields of winter hardy cultivars can be 20 to 40% lower than less hardy cultivars in the absence of severe freeze stress. Low grain yields of hardy types is one reason for low preference of these phenotypes by growers.

Hardy cultivars usually head late compared to less-hardy ones. With late initiation of the inflorescence, it is important that these cultivars exhibit rapid grain development and maturity to avoid the high temperatures and low moisture conditions of mid-summer (12, 15). Thus, the grain filling period is shortened. Late initiation has an influence on spikelet number, as previously discussed, and could be another detriment to high yield among hardy types.

George has pointed out that precocious floral initiation, before the onset of winter, occurs among the high yielding soft white winter wheat cultivars grown in the Pacific Northwest (14). He suggests that such cultivars have a greater susceptibility to freeze loss. The ontogeny of the shoot apex of winter wheat during seasonal change, particularly those cultivars with early-heading traits, has not been well documented in South Dakota. It is important that heading date data be augmented by studies of floral apex development (14), to accurately reflect the growth habit of

this crop as it relates to freeze susceptibility during early spring. Hence, a challenge for the agronomist is an understanding of earliness in reproductive development and its influence on grain yield.

Precise environmental conditions which control morphogenetic events in flowering would be valuable information for future improvements of wheat. It would facilitate investigations establishing the relationship between morphogenesis and concurrent biochemical changes that take place. A previous investigation by Evans, on the biochemistry of flowering in a long-day plant, *Lolium* sp., showed protein synthesis as a primary process in the plant's transition from vegetative to reproductive growth (9). However, the methods used in Evans' study showed only the requirement for protein synthesis during floral evocation. The final phase in this research was a study of the changes that occur in the protein complement of the shoot apex of winter wheat concurrent with morphological change.

An urgent need exists to understand factors involved in floral initiation of winter wheat and the influence on grain yield (13). The phenology of the hard red winter wheat class was of interest in this study, as it relates to the range of phenotypes grown in the Northern Great Plains and the climatic adversity which exists. The objectives of this research were: 1) to compare the periods of floral initiation of field-grown hardy and less-hardy phenotypes in

South Dakota, 2) to determine the minimum photoperiod requirements for floral initiation of these cultivars, 3) to evaluate the effect of growth temperature on floral primordia, and 4) to examine the changes in protein composition of the developing apex by isoelectric focusing, as a biochemical indicator of the phasic development in winter wheat.

MATERIALS AND METHODS

Shoot Apex Survey in Field Grown Plants. Each spring, during the years 1977 through 1980, winter wheat plants were collected from variety trials at various locations in the state for apex analysis. The sites included Onida, Sturgis, Bear Butte, Quinn, Martin and Brookings. Usually, six plants per cultivar were examined at weekly intervals starting on about April 15 for a three-week interval. At least three cultivars from the less-hardy group (Scout 66, Centurk, Lancer) were selected together with three from the hardy group (Winoka, Roughrider, YTO-117) for comparison. The primary culm was used as the apex source, excised and examined according to the procedure described for environment chamber studies below. Approximately 1000 plants were evaluated during the survey.

Plant Growth. Two cultivars of *Triticum aestivum*, cv. "Winoka" and "Centurk" were chosen for the environment chamber study as representing late and early maturing types of hard red winter wheat, respectively. Seed were germinated for 16 hours at 25 C in a moist paper towel. After germination, the young plants were transferred to 2 C for six weeks of vernalization. Attention was given to the upright position of the paper towel to insure a germinated embryo with the root and shoot organs aligned in a straight axis. This procedure facilitated a more uniform emergence

of shoots from the soil after the seedlings were transplanted. Upon completion of the vernalization period, 2 plants per cultivar were transplanted into six-inch clay pots containing loam soil. The young plants were immediately watered and transferred into a controlled environment chamber. The plants were grown 4 weeks at 25/15 C (day/night) temperatures with an average of 28,000 lux intensity during the 11.5- or 8-hour light period (short day). This period of growth, prior to photoinduction, is referred to as plant entrainment. Fluorescent tubes represented 95% (26,000 lux) of the total illumination and the remaining 5% (1,500 lux) was from incandescent bulbs. Measurement of illuminance was taken at a leaf height of 27 cm. The soil was kept moist by application of deionized water once a day. Supplemental nutrition was provided by a small quantity of slow-release fertilizer two weeks after transplanting.

Photoinduction and Apex Analysis. Preceeding each major investigation for plants grown in controlled environments, pilot tests were conducted to explore entrainment, photoinduction and post-treatment conditions for subsequent experiments. For example in Test 1, Table 1, pilot tests were conducted prior to each run within Test 1.

Growth conditions during the light induction period were identical with those used to propagate the plants. Light treatments were based upon the requirements for

photoinduction. Daylight longer that 12 hours (long day) is necessary for induction of flowering in winter wheat. The red-light segment of the visible spectrum (660 nm) is responsible for this photomorphogenic response (3). Radiation of plants with red light converts the red-absorbing form of phytochrome (P_r) to the far-red form (P_{fr}). The P_{fr} form is necessary for floral induction (4, 10, 11, 25).

In this research, red light was used to supplement the short-day period (less than 12 hours) as a condition for photoinduction. Red light was supplied from a metal lantern by unidirectional transmission of light from a 75 watt flood bulb through a 10x10 cm red filter (Carolina Supply Filter, 650 nm). The lanterns were fabricated with baffles to avoid light leakage and yet to allow air flow for dissipation of heat generated by the light source. After treatment the samples were returned to the short-day chamber.

The morphological state of the shoot apex from the main culm was determined by microscopic examination. The whole plant was removed from the pot, trimmed to a 12 cm segment of the primary culm and placed in ice to prevent the dessication of the apex until dissected. Appearance of the "double-ridge" stage was used to indicate reproductive development (2). The double-ridge stage occurs when the spikelet primordium and its subtending leaf primordium have developed at opposite flanks of the elongated shoot apex.

Isoelectric Focusing of Apex Proteins. Procedures for casting the gel slabs and isoelectric focusing (IEF) of extracts from macerated shoot apices were according to a technical bulletin by the suppliers of Seakem Agarose (TM) (FMC Corp., Marine Colloids Division, Rockland, ME, 04841). The subsequent fixation and staining procedure for identifying the protein bands was based on a personal communication (Dr. M. Ferguson).

The 1% agarose solution was heated in boiling water until the mixture appeared clear (approximately 10 minutes). The solution was cooled to 60 C prior to the addition of 1.2 ml of carrier ampholytes, pH 3.5-9.5 Isogel ampholytes (TM), (FMC Corp., Marine Colloids Division, Rockland, ME 04841). The agarose-ampholyte solution was pipetted into a preheated mold. The warm solution was allowed to solidify for 30 minutes, forming a 10x8 cm slab-gel of 0.8 mm thickness. The slab was removed from the mold and stored in the coldroom (1 C) for 16 hours prior to use.

Shoot apex samples were kept in the frozen state until analyzed. Prior to IEF, the apices were thoroughly macerated in a solution of 1% glycine. The volume of 1% glycine added was adjusted for a uniform application of samples on the gel. Using a 4x3 mm piece of Whatman no. 1 chromatography paper, approximately 4μl of the 1% glycine-tissue slurry was absorbed and placed on the gel at the cathode end.

Isoelectric focusing was performed employing a Model 1415 electrophoresis cell and a Model 1420 power supply (Bio-Rad instruments, Richmond, CA 94804). The voltages were applied in the following sequence: 100 V 0.5 hr, 200 V 1 hr, 300 V 0.5 hr, 500 V 0.5 hr. After the 200 V interval 2 µl of the 1% glycine was pipetted on each sample blotter.

Immediately after IEF, the gel slab was rinsed with glass-distilled water and immersed in a fixing solution of 5% trichloroacetic acid, 1% 5' sulfosalicylic acid, and 10% methanol for 10 minutes. The gel was rinsed with glass-distilled water and oven dried prior to staining. The dried gel was placed in a staining solution of 0.1% Coomasie brilliant blue R-250 in 25% ethanol for 20 minutes at room temperature. Destaining was accomplished in a 25% ethanol solution.

RESULTS AND DISCUSSION

Survey of Field Grown Plants. A preliminary survey of floral initiation in hard red winter wheat was undertaken on field grown plants in the spring of 1976. At weekly intervals, three plants per cultivar were harvested from the winter wheat nursery in Brookings. The shoot apex of the main culm was evaluated for floral apex development. An early (cv. Sage) and a late-heading (YTO-117, experimental line) type were used in this study. At the first sampling date, May 5, both cultivars had initiated floral apices. Sage was further advanced in spike development, indicating an earlier time in floral apex initiation compared to YTO-117.

The survey of floral apex initiation for the succeeding four years was extended to several sites in the state. Several winter wheat cultivars, including three hardy types, were chosen for the study.

The data indicated that shoot apices did not show initial stages of floral development before mid-April. In some years, no floral apices were found on the April 15 sampling date. In other instances, only a few cultivars had floral apices by this date, indicating that induction occurs only during the spring period in South Dakota. This is in contrast to George's data on soft winter wheat grown in a milder winter climate (14), where some cultivars contained

floral primordia before the onset of winter, while others initiated floral primordia between late January and early March.

Daylength Extension in Controlled Environments. Two hard red winter wheat cultivars were chosen for controlled environment studies of floral apex initiation. Both are commercially grown in South Dakota; "Centurk" has a lower level of winter hardiness compared to "Winoka" and has an earlier heading date by about six days. Pilot tests were conducted to determine the minimal duration of red light needed beyond an 11.5-hour photoperiod to cause floral initiation. The results showed that for the cultivar Centurk, 4.5 hours of red light (650 nm) extension per day, when applied on four consecutive days after 6 weeks of vernalization, resulted in double-ridge apices within 7 days. No double-ridge apices were observed for the cultivar Winoka following this light treatment. A similar pilot test was conducted with the objective of determining the minimum post-treatment response time for double-ridge formation. Seven days following the end of treatment, the primary culm of 6 out of 8 Centurk plants were in the double-ridge stage. In contrast, no floral primordia were observed in the primary culm of Winoka plants.

This study was repeated with a second group of plants grown under identical conditions, with apices excised on days

7, 8, and 9 after treatment. Eighteen plants of each cultivar were used at each of the three post-treatment intervals. From this group of 108 dissected plants, no double-ridge apices were found.

The procedure, using 4.5 hours of red light to extend the daylength, was used in three tests. Ten plants per cultivar were kept at the entrainment conditions during the course of each experiment and were considered as controls. Their shoot apices were characterized together with the treated plants and showed no double-ridge formation.

In Test 1 (Table 1) the entrainment and induction periods for the three groups of 14 to 18 plants were the same, but each group was sampled at different post-treatment response periods. No double-ridge apices were observed for each group at the different post-treatment response time. In Test 2 (Table 1) four or five weeks of entrainment were used in conjunction with either four or six induction cycles,, again involving 14 to 18 plants per variable. The use of older plants was prompted by Evans' data which suggest that plant age influences the ability of the plants to respond to the photoinduction treatment (9). However, a combination of older plants, treatment durations of four and six days with red light, and a longer post-treatment response time of three weeks were all without effect.

Experiments by McKinney and Sando (22) and Hurd-Karrer (16) showed that entrainment of winter wheat at

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photoperiods near 12 hours, during the initial phases of growth, interferes with early initiation of the floral apex. A pilot test with 4 plants per cultivar grown under a shorter day-length of 8 hours, prior to the 4 long days with red light, agreed with their results. However, in a subsequent experiment under the same conditions and treatment procedure, but with a larger number of samples (14 to 18 plants per cultivar), no shoot apices were found in the double-ridge stage (Test 3 in Table 1).

<u>Effect of Light Quality</u>. Table 1 shows that combinations of different entrainment conditions, together with varied number of long days under red light, did not initiate floral development. Next, consideration was given to the quality of light used during the long-day photoperiod. An increase in red light (650 nm) prior to sunset is known to occur and Salisbury has suggested that the floral response may be influenced by this shift in the spectrum quality (24).

The effects of varied amounts of red light at the end of an 8-hour photoperiod were designed to simulate increasing increments of red light during sunset. In each experiment, ten plants per cultivar were entrained at an eight-hour photoperiod for four weeks prior to the four long days. The post-treatment response period was two weeks, at which time the shoot apices were examined.

Table 2 shows results where light quality was varied during the 3.5 hours after the eight-hour day. In Treatment 1, the photoperiod was extended to 11.5 hours with a light combination of fluorescent, incandescent, and red light. It was anticipated that this treatment would result in the highest level of Pfr at the end of the 11.5-hour photoperiod, due to the high proportion of red light during the 3.5-hour daylength extension. Only the fluorescent and incandescent lamps were used for Treatment 2, resulting in a lower level of tissue Pfr than provided in Treatment 1. In Treatment 3, only incandescent lamps were used and due to the higher proportion of far-red from the light source, it was expected that Pfr levels would be lower than either Treatment 1 or 2. Treatment 4 consisted of a period of no light after the 8-hour day and therefore it was expected that this treatment would have the lowest level of tissue Pfr at the end of the 3.5-hour dark period. The influence of the assumed variable levels of Pfr, created by the different combinations of light sources provided by the four treatments, was evaluated by supplementing 4.5 hours of red light to all treatments after the 11.5-hour period.

From the four experimental conditions shown in Table 2, Treatment 2 elicited the highest number of floral apices in Centurk. This procedure is the same treatment used in a previous experiment (Test 3, Table 1) but which showed no floral apices. This difference in the response of the

plants, presumably grown and treated under the same conditions, prompted us to repeat the experiment. In six experiments, with 240 plants under identical conditions (data not shown), not one apex was observed to be in double ridge for either cultivar. Results of these tests demonstrated no influence of entrainment condition or light quality on floral development. Possible explanations for the failure to repeat the results, using 240 plants, will be discussed later.

Light Interruption of the Dark Interval. In contrast to the procedure of extending daylength as a means of promoting the vegetative reproductive transition, interruption of the dark period with light has also been effective. Evans showed that periods of red-light illumination caused optimum flowering, when a certain dark period elapsed following the short daylength (10, 11). Experiments by Lane et al. showed that light sources which transformed large amounts of phytochrome to the P_{fr} form, were inhibitory to flowering in five long-day plants, when given during the four hours immediately following the entrainment period or even as brief light additions at that time (18). Evans concluded from his extensive work with Lolium sp., a long-day plant, that the action spectrum for flowering depends on the entrainment conditions (9, 11). For photoperiods of near critical length (i.e. 11.5 hours) short periods of light in the middle of

the night are necessary. A shorter photoperiod during entrainment (i.e. eight-hour days) requires a longer red-light period, also in the middle of the night. Lane's model of phytochrome action is in accord with Evans' action spectrum for floral induction (18). This conclusion was also reached by Vince (28).

This model suggests that the amounts of P_{fr} needed for optimal floral initiation varies during the course of each long day. The sequence of light conditions imposed on the plants of the previous experiments (Tables 1, 2) did not provide for this possibility. Perhaps, success of the fixed duration of red-light treatment used depends on a favorable level of P_{fr} in the tissue for an optimal flowering response, a situation which may not have existed as a result of the entrainment conditions and/or subsequent treatment (Table 1 and 2).

The next series of experiments, with 12 plants per cultivar, involved an 8-hour photoperiod (0800 to 1600 hr) in a 24-hour day during the 4 weeks of entrainment. At the fifth week of growth, the samples were given four hours of red light on four consecutive days. To determine the most effective period for the red light irradiation, the remaining sixteen hours of darkness were divided into four different time frames. Treatments with dark periods prior to red light would provide for a lowering of P_{fr} levels. Such treatments may indicate at which time the low

P_{fr} process is completed and when the higher P_{fr} level is favorable, according to Lane's model of phytochrome action and Evans' action spectrum for floral initiation. Each set of plants received only one period of red-light at the designated time frame of the dark interval on four consecutive nights. Figure 1 shows the time of red-light irradiation and the observed results.

The 1600- to 2000-hr and the 2400- to 0400-hr 12 Centurk apices, respectively, in the double-ridge stage two weeks from the last dark interval with red light. The other two treatment periods with red light (0400- to 0800- and 2000- to 2400-hr, Figure 1) were not as effective in promoting Centurk to flower. Only 7 of 12 Centurk samples had floral apices from 0400- to 0800-hr red light period and with the 2000- to 2400-hr red light period, 8 of 12 Centurk samples had floral apices. Winoka had vegetative apices after all red-light treatments; however when given a longer response time of three weeks, two floral apices were initaited from ten samples for the 2400- to 0400-hr period of red light.

The 2400- to 0400-hour treatment was used in a subsequent experiment with an extra set of plants receiving eight cycles of red-light instead of the four cycles as shown in Figure 1. The significance of the differences between the red-light periods in Figure 1 may not be clear for

Centurk, however, the 2400- to 0400-hour treatment was the only interval which initiated floral apices in Winoka. In addition, Evans' action spectrum in floral induction suggests that optimum periods for red-light illumination occurs in the middle of the night (9, 11). The 2400- to 0400-hour period meets this requirement. All plants were sampled three weeks from the last dark interval interrupted with red light. The results, shown in Table 3, indicate that all Centurk samples had floral apices when treated with red light at the 2400- to 0400-hr after four or eight consecutive treatment intervals. After eight cycles with red light, some of the apices of Centurk were beyond the double-ridge stage of development, compared to those which received only four cycles of red light. Four night treatments with red light, resulted in only three of the twelve Winoka samples being transformed to floral development. However, with eight cycles of red light, six Winoka samples showed double-ridge development.

The results in Table 3 and Figure 1 (2400 to 0400 hr) indicate the possible light sequence for variable P_{fr} amounts required in floral initiation in winter wheat. An eight-hour period of darkness followed by four hours of red light appears to have fulfilled the sequential requirement for a low amount of P_{fr} followed by a high amount of P_{fr}. This light treatment initiated floral development in 100% of the Centurk apices, and appeared to meet the optimum

requirement of induction. In the case for Winoka, such light treatment given for four cycles did not completely stimulate the floral initiation mechanism, as shown by only 16% of samples at double-ridge. When light treatment was applied for eight cycles, 50% of the samples were induced to flower, suggesting a more complete stimulation of the initiation mechanism. Compared to results in Table 2, the observed response in Table 3 was the first light treatment attempted which initiated floral apices in Winoka.

A treatment procedure with four hours darkness followed by four hours red light (2000 to 2400 hr) caused only one half of Centurk samples to be initiated (Figure 1). Four hours darkness followed by four hours red light may not satisfy the low/high sequential requirement of P_{fr} to initiate floral development.

Evans has presented evidence showing red-light treatment after four hours darkness to be only slightly effective for floral initiation in a long-day plant (11). The reversion of P_{fr} to P_r is believed to occur at a much slower rate than the conversion of P_r to P_{fr} (25, 28). One can hypothesize that the influence of four and eight hours of darkness prior to the red-light irradiation becomes significant, based on the extent to which the low P_{fr} levels are attained during such dark periods. When a low level of P_{fr} is reached, it seems necessary that the four-hours red light follows immediately for effective floral induction.

The light condition shown in Table 2 [Treatments 1 and 2] and Figure 1 (1600 to 2000 hr) presumably did not allow for a complete lowering of P_{fr} amounts, and yet floral apices were initiated. Other light treatments in Figure 1 represent a range of conditions which initiated floral apices for Centurk. Data provided by Martinic (21) on the floral initiation of various winter wheat cultivars by different photoperiods, led him to conclude that cultivars which are of the early heading types do not have a very precise long-day requirement for floral initiation. Centurk seems to be of the same type.

In contrast to Centurk, Winoka exhibited floral apex development only at certain photoperiod conditions (Table 3). Furthermore, the treatment in Table 3 may be suboptimal to initiate floral apices, as shown by the small number of shoot apices in Winoka at the double-ridge stage. This observation and the persistent negative response of Winoka from the other conditions imposed (Table 2) suggest a more stringent biological control of floral initiation than for Centurk.

The requirements for the vegetative/reproductive transition in winter wheat appears complex. This conclusion is substantiated by the wide seasonal variation in floral induction in field grown plants shown by others (14, 15, 21) and results of my growth chamber study. Undoubtedly, when selection for yield is the predominant consideration, pheno-

types which tend toward earliness are favored. However, when freeze stress is a factor, such as after the spring equinox, selection may favor populations that are quiescent until such time that the probability of stress is low.

Effect of Temperature. Inconsistency in demonstrating floral induction in Centurk plants grown and treated at a 25/15 C temperature regime was initially attributed to the possibility of devernalization. It was seldom possible to show any floral initiation with Winoka. Japanese workers (5) showed that loss of the vernalized state is possible around 28 C. Since temperatures used in all previous experiments of this study approached this range, it was possible that devernalization could have occurred. In particular it was thought that Winoka was most seriously affected, explaining the failure of this cultivar to respond to the treatments provided. Yet, when light experiments were repeated at a 20/10 C, results from treatments which previously showed floral initiation in Centurk could not be repeated. This is shown in Test 2, Table 4, where four hours of red light was given at 2400 to 0400 hr. However, when a portion of these treated plants were transferred to the greenhouse, 100% of Centurk plants and 50% of the Winoka plants were in the double-ridge stage three weeks after the transfer. From this result, it was concluded that difficulty in consistently demonstrating floral development in

both cultivars could not be explained by the loss of the vernalized state.

The interaction between temperature and photoperiod may not be excluded as an explanation for the results in Test 2, Table 4. The reversion of P_{fr} to P_r is influenced by low temperatures (25, 28). Temperature effects on photoinduction were not further evaluated due to limited facilities for conducting light-temperature studies in the time available.

Plant Density and Floral Response. The experimental protocol perhaps deserve further consideration in an attempt to explain the variable effects of photoinduction conditions. Typically, pilot tests were conducted with a small number of plants as a means to ascertain the treatment parameters resulting in floral induction. Once treatment parameters were established, an adequate number of plants were started to provide large plant populations per treatment, as well as for the planned sequence of related experiments. In these instances, the controlled environment chamber used for plant entrainment and the post-treatment response period frequently was filled to capacity. In most cases, it was during such experimental periods that difficulty was experienced in repeating the floral initiation results (Tables 1, 2 and 4), previously obtained in pilot tests.

One reason for changing from the 25/15 C to 20/10 C

temperature regime was that air flow may have been restricted by high plant densities, causing a slight elevation of temperature into a critical range, which reversed the effects of vernalization. This possibility was eliminated at the lower growth temperature. Yet, consistent results could not be shown (Table 4). Another possibility was that under high plant populations a limiting factor may have been created. For example, a chamber full of plants may have needed more CO_2 than could be supplied by the air volume in the chamber, causing the CO_2 to drop below 300 ppm. Air turnover occurred at least once per day because of opening the doors during plant watering and observation.

Competition for this limiting factor could affect the floral initiation process of the plants. This led me to estimate the daily available CO_2 in the chamber in comparison to the daily CO_2 requirements of mature plants when the chamber was filled to capacity. The calculated ambient CO_2 concentration in one chamber volume of air was 0.098 moles. The calculated CHO product per day of 288 plants at 7 weeks growth was 0.27 moles CO_2. The calculation showed that the estimated CO_2 requirement of 288 plants was almost three-fold greater than the CO_2 available in one chamber volume of air. Although the chambers were light proof, air leaks still were present which would provide a certain degree of air exchange in the chamber. Therefore the supply of CO_2 available to the plants was expected to be higher

than calculated. However, it was possible that CO_2 levels occasionally may have dropped below 300 ppm.

Starting schedules for plants used in photoinduction experiments and their effects on flowering response were reviewed. My aim was to determine whether there was a relationship between plants propagated at maximum chamber capacity and their response to the long-day treatments. Figure 2a and 2b shows the planting schedule and the resulting plant densities in the entrainment chamber for three groups of Winoka and Centurk plants used to obtain the data in Table 4.

In Test 1, Figure 2a, the samples were grown at high plant population three weeks prior to the long-day treatment. Results of this test showed 30% of Centurk apices in vegetative growth two weeks from treatment (Figure 2b). Test 2, Figure 2, underwent all four weeks of growth in a chamber full of plants prior to treatment. All shoot apices showed vegetative development at two weeks following treatment (Figure 2b). Growth conditions requiring maximum chamber space for plants of Test 3 (Figure 2a) was only for two weeks after transplanting. After treatment with long days, 15% of the shoot apices were vegetative (Figure 2b). By contrast, Winoka showed all vegetative apices in these three tests, except for 2 of 20 plants in Test 1.

A consistent pattern was found between a high number of plants maintained in the entrainment chamber and the failure to show floral initiation, both for earlier studies

conducted at 25/15 C and those performed at 20/10 C.

If floral induction is sensitive to slightly lower levels than 300 ppm, CO_2 limitation could be a possibility. In contrast to this possibility, an environment chamber may also be prone to the accumulation of volatile substances evolved by plants. In situations where a large number of plants are grown in the chamber, accumulation of such substances may approach a level which affects the floral induction mechanism. For example, wheat plants have been shown to evolve ethylene (26). Under high plant populations in the chamber, ethylene may accumulate to levels which influence the floral induction mechanism. Additional research is needed to investigate this possibility, since the literature has not reported effects of ethylene on floral induction.

Isoelectric Focusing of Floral Apices. Protein synthesis has been shown as a requirement in the transition from the vegetative to a double-ridge apex in *Lolium* sp., (9). Isoelectric focusing of extracts from macerated vegetative apices from Centurk and Winoka revealed no significant differences in the positions of protein bands in gel slabs. Centurk had 11 protein bands and Winoka had 10. Comparison of the iso-electric points of proteins and number of protein bands from a vegetative and double-ridge apices of Centurk also showed no differences. Considering the small size of

the vegetative apex (avg 0.5 mm) and double-ridge apex (avg 0.9 mm), the staining method used may not be sensitive enough to detect the small sample differences in protein composition consisting of 10 or fewer apices. The transition of a vegetative apex to a double-ridge one involves specific "target cells"; extremely minute areas within the shoot apex. Changes in the protein composition in these few cells may not be detected by the methods used.

When using apices beyond the double-ridge stage, some changes in protein composition were recorded as shown in Figure 3. A protein band at pH 4.5 appeared for a floral apex at stage F (spikelet in elongation phase) which was not observed at stage D (double-ridge stage). This protein band was also found at later stages of floral development. From stage F to stage G (generation of floret parts on the spikelet) two distinct changes were observed; 1) A protein band at pH 6.6 from stage F apices was not found at stage G. 2) A protein band at pH 4.3 appeared in stage G and is not present for stage F. Apices at stage G to H showed one difference in their protein bands. A protein band at pH 5.1 appeared only in stage H.

A floral apex at stage F showed an additional protein band (pH 4.5). Appearance of this protein, at this advanced stage of floral development, was the only difference observed between vegetative and floral apices. The development of the floral apex from stage D to F may be a signifi-

cant change. George has shown apices at stage F in development are most susceptible to freeze injury (14).

Proteins in the higher pH range seem to be of less importance with the progression of floral development, since they were not as abundant at later stages of floral ontogeny. By contrast, proteins in the lower pH range seem to be necessary for floral development, as more bands in that range were recorded for more developed apices.

SUMMARY

A survey of floral initiation in hard red winter wheat cultivars grown in South Dakota was undertaken. The data showed that these cultivars initiated floral primordia as early as mid-April, a period at which freezing temperatures are common in South Dakota. As pointed out by George (14), early floral initiation increases the plant's susceptibility to freeze damage. Distinctions in flower development were not consistently observed between late and early cultivars in field grown plants. However, since freezing conditions did prevail during the years when no distinction between apices of early and late cultivars were observed, it is possible that plants with early development may have been damaged by low temperature prior to sampling time.

Floral initiation of two cultivars of winter wheat was also studied under controlled environmental conditions. It was observed that Centurk is less sensitive to long-day conditions for floral initiation as compared to Winoka. This appears consistent with the early heading traits of less-hardy cultivars. In the case for Winoka, only limited photoperiod treatments were effective for induction of reproductive growth. More often a negative response was observed suggesting a more stringent biological control of floral initiation in Winoka, hence, a probable cause for the late floral development among hardy types.

Variation in the floral initiation of plants presumably grown and treated under the same conditions were encountered. Possible causes for the variation in response were discussed.

Changes in the soluble proteins from the vegetative stage to floral apex development were examined by isoelectric focusing. The results showed no difference in the protein complement of vegetative apices from Centurk and Winoka. Also, no difference was found comparing vegetative and double-ridge developmental stages of Centurk. Isoelectric focusing of more developed floral apices showed some changes in protein composition compared to double-ridge apices. Later stages of floral development showed protein bands at the lower pH and a disappearance of protein bands in the higher pH range.

LITERATURE CITED

1. Bingham, J. 1967. Investigations on the physiology of yield in winter wheat by comparison of varieties and by artificial variation in grain number per ear. J. Agr. Soc. Cambridge 68:411-422.

2. Bonnett, O. T. 1966. Development of the wheat spike. In O. T. Bonnett (ed.) Inflorescence of maize, wheat, rye, barley and oats: Their initiation and development. Illinois Univ. Agr. Expt. Sta. Bull. 721.

3. Borthwick, H. A., S. B. Hendricks, and M. W. Parker. 1948. Action spectrum for photoperiodic control of floral initiation of a long day plant, Wintex Barley, *Hordeum vuglare*. Bot. Gaz. 110-118.

4. Borthwick, H. A., S. B. Hendricks, M. J. Schneider, R. B. Raylorson, and V. K. Toole. 1969. The high energy light action controlling plant responses and development. Proc. Nat'l. Acad. Sci. 64:479-486.

5. Chujo, H. 1966. The effect of diurnal variation of temperature on vernalization in wheat. Proc. Crop Sci. Soc. Japan. 35:187-194.

6. Dantuma, G. 1973. Rates of photosynthesis in leaves of wheat and barley varieties. Neth. J. Agr. Sci. 21:181-187.

7. Duncan, W. G., and J. D. Hesketh. 1968. Net photosynthetic rates, relative leaf growth rates, and leaf number of 22 races of maize grown at eight temperatures. Crop Sci. 8:670-674.

8. Evans, L. T. 1965. Inflorescence initiation in *Lolium temulentum* L. VII. Spectral dependence of induction. Aust. J. Biol. Sci. 18:745-762.

9. Id. 1969. *Lolium temulentum*. pp. 328-349. In L. T. Evans (ed.) Induction of flowering; some case histories. Cornell University Press, Ithaca, New York.

10. Id. 1971. Flower induction and the florigen concept. Ann. Rev. Plant Physiol. 22:365-394.

11. Id. 1975. A transducer for the light-dark cycle. pp. 31-45. In L. T. Evans (ed.) Daylength and the flowering of plants. W. A. Benjamin, Inc., Menlo Park, California.

12. Id. 1975. The physiological basis of crop yield. pp. 327-355. In L. T. Evans (ed.) Crop physiology. Cambridge Univ. Press, London.

13. Id. 1975. Wheat. Pp. 101-150. In L. T. Evans (ed.) Crop physiology. Cambridge Univ. Press, London.

14. George, D. W. 1982. The growing point of fall-sown wheat: A useful measure of physiologic development. Crop Sci. 22:235-239.

15. Hunt, L. A. 1979. Photoperiodic responses of winter wheats from different climatic regions. Z. Pflanzenzuchtg. 82:70-80.

16. Hurd-Karrer, A. M. 1933. Comparative responses of a spring and a winter wheat to daylength and temperature. J. Agr. Research 46(10):867-888.

17. King, R. W., I. F. Wardlaw, and L. T. Evans. 1967. Effect of assimilate utilization on photosynthesic rate in wheat. Planta (berl.) 77:261-276.

18. Lane, H. C., H. M. Cathey, and L. T. Evans. 1965. Spectral dependence of flowering in several long-day plants to extend the photoperiod. Am. J. Botany 52(10):1006-1014.

19. Lucas, D. 1972. The effect of daylength on primordia production of the wheat apex. Aust. J. Biol. Sci. 25:649-656.

20. Mohapatra, P. K., D. Aspinall, and C. F. Jensen. 1981. The growth and development of the wheat apex: The effects of photoperiod on spikelet production and sucrose concentration in the apex. Ann. Bot. 49:619-626.

21. Martinic, Z. 1966. Response of various genotypes of common wheat to shortened photoperiods in the natural environment of spring sowing. Savremena Poljaoprivreda 14(11-12): 585-600.

22. Mckinney, H.H., and W. J. Sando. 1933. Earliness and seasonal growth habit in wheat. J. Heredity 24:160-179.

23. Rawson, H. M. 1970. Spikelet number, its control and relation to yield per ear in wheat. Aust. J. Biol. Sci. 23:1-15.

24. Salisbury, F. B. 1981. The twilight effects initiating dark measurement in photoperiodism of Xanthium. Plant Physiol. 67:1230-1238.

25. Smith, H. 1975. The photomorphogenic response system and their photoreceptors. pp. 25-52. In H. Smith (ed.) Phytochrome and photomorphogenesis. McGraw-Hill Book Co., London.

26. Steucek, G. L. and L. K. Gordon. 1975. Response of wheat (Triticum aestivum) seedlings to mechanical stress. Bot. Gaz. 136(1):17-19.

27. Thorne, G. N., M. A. Ford, and D. J. Watson. 1968. Growth, development and yield of spring wheat in artificial climates. Ann. Botany 32:425-426.

28. Vince, D. 1975. The measurement of time. pp. 155-195. In D. Vince (ed.) Photoperiodism in plants. McGraw-Hill Book Co., London.

29. Williams, R. F. and C. N. Willams. 1968. The physiology of growth in the wheat plant. IV. Effects of daylength and light energy levels. Aust. J. Biol. Sci. 21:835-854.

Table 1. Experimental protocol used to evaluate the minimum requirements for floral induction of hard red winter wheat cultivars Centurk and Winoka using daylength extension.

Entrainment[a] Photoperiod (Hrs)	Entrainment[a] Duration (Wks)	Induction Cycles[b] (Days)	Post-induction[c] (Weeks)	Response[d]
Test 1[a]:				
11.5	4	4	1	-
11.5	4	4	2	-
11.5	4	4	3	-
Test 2[a]:				
11.5	4	6	3	-
11.5	5	4	3	-
11.5	5	6	3	-
Test 3[a]:				
8	4	4	2	-
8	4	8	2	-

[a]A 24-hr light cycle and 25/15 C (day/night) temperatures. Test 1 indicates related experiments with constant entrainment and induction periods with variable post-induction response. Test 2 contains experiments with constant entrainment daylength and post-induction response with variable entrainment duration and induction period. Test 3 experiments show constant entrainment conditions and post-induction response with variable induction periods.

[b]Daylength was held constant at 11.5 hrs (white light) and was extended 4.5 hrs with red light (650 nm).

[c]After treatment, plants were returned to entrainment conditions.

[d]Combined floral response of Centurk and Winoka. Fourteen to eighteen plants were evaluated for floral apex development at each treatment (- indicates no floral apices).

Table 2. The floral response of Centurk and Winoka as influenced by variation in light quality when extending the 8-hour entainment photoperiod prior to red-light treatment.

Treatment 3.5 hrs before red-light[a]	Number of floral apices[b]	
	Centurk	Winoka
1. fluorescent, incandescent and red-light	3	0
2. fluorescent and incandescent	8	0
3. incandescent	5	0
4. no light	0	0

[a]See Materials and Methods for % of total illumination provided by each light source. Cool white fluorescent light has a predominant light band at 600 nm. Incadescent light provides spectral energy at longer wavelengths between 600 nm and 700 nm. Treatment interval was 4 hours each of four consecutive 24-hour intervals.

[b]The primary culm of twelve plants was sampled for each cultivar per treatment.

Table 3. The influence of a red-light treatment during the dark period on the floral response of Centurk and Winoka.

Days of red-light treatment[a]	Number of plants with floral apices[b]	
	Centurk	Winoka
4	12	3
8	12	6

[a]Red light was provided between 2400 and 0400 hrs each 24-hour period.

[b]The primary culm of twelve plants was sampled two weeks after induction treatment. Plants were entrained at 8-hour light and 16-hour dark cycles for 4 weeks. Starting on the fifth week, 8 hours of darkness elapsed each 24 hours before the red-light treatment was initiated.

Table 4. The effect of 20/10C (day/night) during entrainment, photoinduction and post-treatment on the floral response of Centurk and Winoka.

Entrainment Conditions[a] Photoperiod/Duration (Hrs)/(Wks)		Induction Period[b] (24-hr intervals)	Number of Floral Apices[c]	
			Centurk	Winoka
Test 1	8/4	4	14/20	2/20
Test 2	8/4	4	0/20	0/20
Test 3	8/4	4	17/20	0/20

[a]Induction variations were as follows: Test 1 and 3 - The 8-hour entrainment photoperiod was extended to 11.5 hours with white light followed by 4.5 hours of red light alone. In Test 2, the 8-hour entrainment was followed by 8 hours of darkness and subsequently 4 hours of red light between 2400 to 0400 hr.

[b]After treatment plants were returned to entrainment conditions.

[c]Twenty plants per cultivar were sampled two weeks after treatment. The number shown first indicates number of plants with floral development.

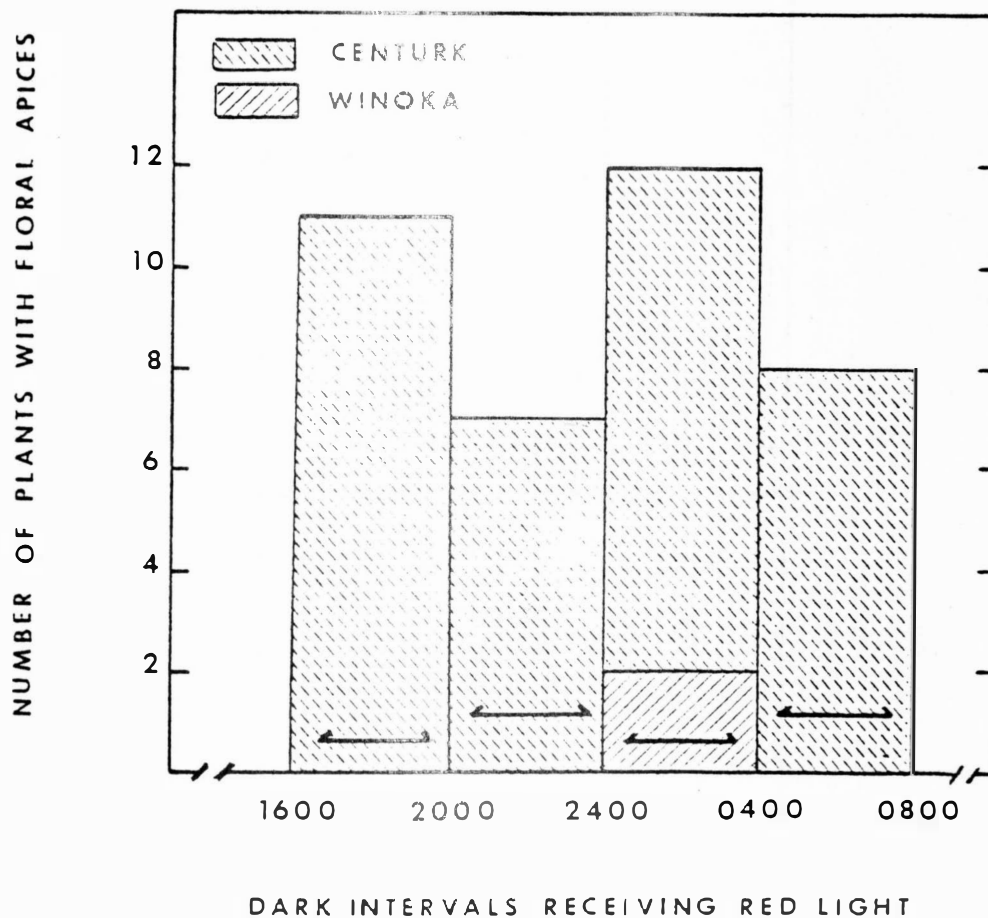
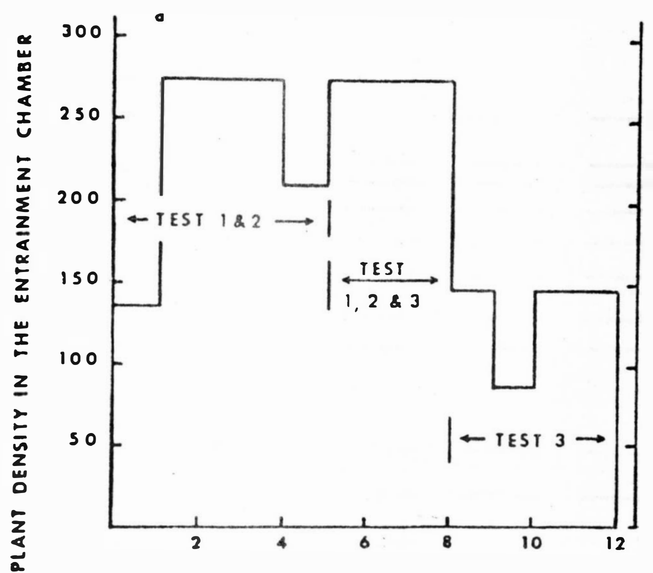


Figure 1. The effect of dark interruption, by four hours of red light during various periods of the night, on the floral response of Centurk and Winoka. The entrainment, post-treatment, and floral induction interval are described in Table 3. The primary culm of 12 plants per treatment were dissected for floral apex evaluation.



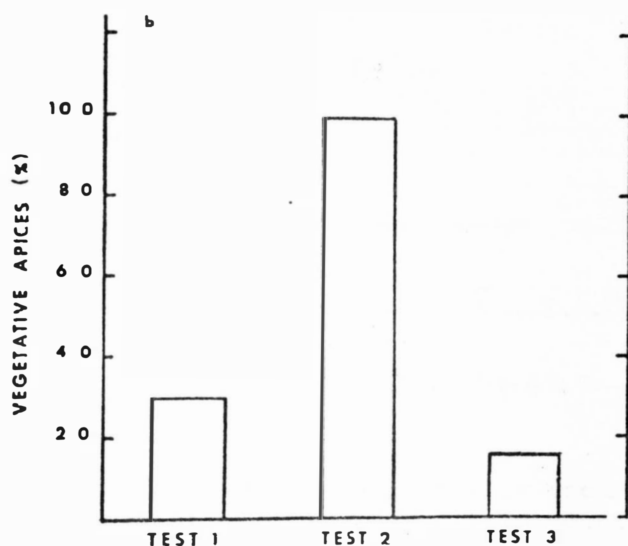


Figure 2a. The accumulative plant density in the entrainment chamber showing the relationship between planting, transfer to and return from the induction chamber, and harvest schedule of three simultaneous tests. The calculated ambient CO_2 concentration in one chamber volume of air was 0.098 moles. The calculated CHO product per day of 288 plants at 7 weeks growth was 0.27 moles CO_2. Therefore, approximately a 3-fold turnover of CO_2 per day was necessary to support such a population at 20/10 C (D/N) and 7 weeks growth.

Figure 2b. The percent of vegetative apices from Centurk samples of each test shown in Figure 2a, as an indicator of the inhibitory effect for floral initiation of plants at a high plant population.

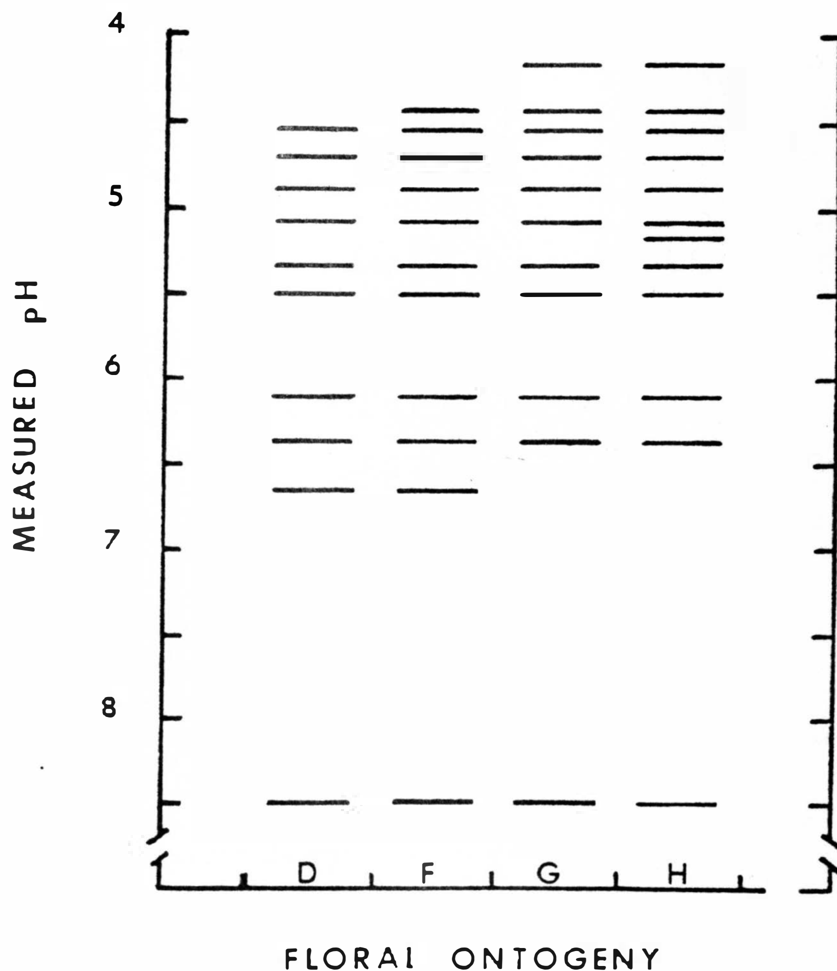


Figure 3. Distribution of isoelectrically focused proteins from floral apices at various stages of development. Stage D (5 apices) denotes a floral apex at double-ridge. Stage F (5 apices) indicates the elongation phase of the spikelets. Stage G (4 apices) represents a floral apex with floret initiation and Stage H (3 apices) is for floral apices with a terminal spikelet.