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Vernalization Response and its Implication in Wheat (Triticum Aestivum L.)

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VERNALIZATION RESPONSE AND ITS IMPLICATION IN WHEAT (*TRITICUM AESTIVUM* L.)

By

ShiYing Wang

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ABSTRACT

VERNALIZATION RESPONSE AND ITS IMPLICATION

IN WHEAT (*TRITICUM AESTIVUM* L.)

BY

ShiYing Wang

Vernalization response in wheat so far has been characterized poorly, and less well quantified. The discrepancies and inconsistencies in the literature regarding terminology, measure of response, classification of response types, operative temperatures, etc. stem in part from the lack of a general conceptual model of vernalization phenomena. A fundamental technique in wheat vernalization study is to count the number of leaves emerged before, during, and after vernalization treatment, rather than to calculate calendar days or thermal time after the end of vernalization treatment. Final leaf number on the main stem for vernalization-sensitive cultivars in general decreased until reaching a plateau as days of vernalization treatment increased. There is not an absolute "vernalization requirement" for wheat. Accumulated plant age, expressed as leaf stage, enables attainment of vernalization insensitivity, independent of, or in combination with vernalization treatment. There is an interchangeability between plant age and the duration of vernalization treatment. After the onset of vernalization insensitivity, a plant will emerge six more leaves under long

photoperiod conditions. The quantitative features of this vernalization response, up to the point of insensitivity, were characterized with a linear regression: $(F_l - 6) = \alpha - \beta T_v$, where F_l is the number of leaves observed for a particular vernalization treatment, T_v is the time in days of that vernalization treatment, and α and β are the Y-intercept and the slope of the regression, respectively. The parameters α and β varied among cultivars, and are useful for quantifying vernalization response in wheat. The implication of each parameter can be interpreted biologically: α is the "changeable number of leaves", i.e., how many leaves can be potentially decreased by vernalization treatment; and β represents the "exchange rate" between leaf numbers and vernalization days, i.e., how many leaves can be reduced by one day of vernalization treatment. A novel conceptual framework was proposed for characterizing and quantifying wheat vernalization response.

Affectionately Dedicated to

my Loving

Father, Zhan-ao Wang

and

Mother, Zheng-ai Shen

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GENERAL INTRODUCTION

Wheat originated in the so-called Fertile Crescent of southwestern Iran, northeastern Iraq, and southeastern Turkey some 8 to 10 thousand years ago, and spread to India, China, and even England by about 5000 B.C. (Harlan and Zohary, 1966; Bell, 1987; Tahir and Valkoun, 1994). Today, it is grown across a wide range of agrogeographical regions from the equator to greater than 60° latitude (Briggle and Curtis, 1987). Among all the world's major food crops, wheat is ranked number one based on either the harvest area (about 221.7 million hectares in 1993), or total production (about 564.5 million tonnes in 1993, 95% being bread wheat) (FAO, 1994). Wheat represents almost 30% of world's grain production, with nearly 40% of the population utilizing it as a staple (Hancock, 1992).

The native home of wheat is basically characterized by long, hot, dry summers and short, mild, wet winters. Wheat is planted soon after the first fall rains and it undergoes vegetative growth in winter. It switches to reproductive growth as temperatures and photoperiods increase in spring, and matures in early summer (Loss and Siddique, 1994). Wheat thus evolved as a cool-season annual. After several thousand years, both natural and human selection have resulted in different wheat genotypes adapted to specific environments and cropping practices.

A principal farming objective and a major contribution of the plant breeder to it is to ensure that the life cycles of particular genotypes fit the constraints of the local (or target) environment (Summerfield et al., 1991). Timely anthesis and maturity in relation to the growing season available in a particular location are also essential for large potential yields from annual crops (Bunting, 1975). Crop phenological development is a result of interaction between genotype and environmental factors. Marcellos and Single (1970) envisaged the length of any developmental period (D) to be a function of a number variables such that

$$D = f\,(G, V, T, P_p, M_i)$$

where G symbolizes genetic factors, V those for vernalization, T those for temperature P_p for photoperiod, and M_i miscellaneous factors such as plant nutrition and plant water status. Evidence indicated that vernalization, photoperiod and temperature are the main and almost exclusive determinants for wheat's phenological development (Pinthus, 1985).

Although vernalization response in wheat was studied extensively from 1930s to 1950s (Whyte, 1948; Chouard, 1960), it so far has been characterized poorly, and less well quantified as compared with the photoperiod and general thermal responses (Ellis et al., 1989). There is not consistency in the literature regarding a variety of key issues, such as how to express changes in development induced by vernalization, how to characterize and quantify the genetic variability for vernalization response, which temperatures are the effective temperature for vernalization response, whether vernalization-sensitive wheats have an absolute requirement for vernalization. Those were the primary reasons for initiation of the present studies.

The starting point was to investigate the relationship between plant age at the onset of vernalization treatment and the duration of vernalization treatment. The parameters of vernalization responsiveness were determined and quantified in a set of wheat cultivars and lines from diverse geographical origins. The main objective was to outline a novel conceptual framework that integrates both the recent advances in literature and the enlightenment from current studies for wheat vernalization.

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Section I

Vernalization In Wheat

I. A Model Based On The Interchangeability Of Plant Age And Vernalization Duration

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Vernalization in wheat
I. A model based on the interchangeability of plant age and vernalization duration

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Abstract

Vernalization treatments of 0 to 70 d initiated when 0 to 8 leaf tips were visible were applied to plants of the winter wheat (*Triticum aestivum* L.) cultivars Pioneer 2548 and Augusta. All plants headed irrespective of duration of vernalization. Unvernalized plants of Pioneer 2548 and Augusta had mean final leaf numbers (FLN) of 20.8 ± 1.3 and 21.7 ± 1.0, respectively. Increased duration of vernalization generally reduced FLN within an age treatment until an age-dependent point of vernalization insensitivity was reached. Estimates of the minimum days of vernalization required to reach vernalization insensitivity decreased in a linear fashion as plant age at the onset of vernalization treatment increased. The number of leaves appearing after the onset of vernalization insensitivity averaged 6.3 ± 0.5. FLN minus six appears to be a valid estimate in our experimental conditions for the onset of vernalization insensitivity, at least for plants that had six or more leaves appearing after the end of vernalization treatment. Linear regressions of FLN minus six against days of vernalization were significant for both cultivars (for treatments with six or more leaves emerging after vernalization). The Y-intercepts of the fitted regressions were close to values obtained by subtracting six from FLN of unvernalized plants. Both intercept and slope were controlled genetically. Accumulated plant age, expressed as leaf stage, enables attainment of vernalization insensitivity, independent of, or in combination with vernalization treatment.

Keywords: Leaf number; Modelling; Plant age; *Triticum*; Vernalization; Wheat

1. Introduction

The well-established concept of using thermal unit as a measure of physiological time derives from the fact that duration in calendar days of any phenological phase of plant development is generally increased as temperatures are lowered (Ritchie and NeSmith, 1991). On the other hand, the duration of the vegetative phase of many species is reduced by exposure to low temperatures (Purvis, 1961; Lang, 1965). Lysenko (1928, see Whyte, 1948 and Chouard, 1960) coined the term vernalization (to make spring like) to refer to this phenomenon in wheat (*Triticum aestivum* L.). One of the great challenges to wheat physiologists and modelers continues to be the accurate prediction of wheat's developmental response to low temperatures, primarily

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because a good conceptual model of vernalization phenomena does not exist.

Research has shown that several factors influence wheat's vernalization response, including temperature and duration of low temperature conditions, photoperiod, and genotype (Evans et al., 1975; Pinthus, 1985). There is little unity among researchers on a variety of key issues, however, including means of expressing vernalization response, whether vernalization is required for some wheats to flower, and how genetic variability for vernalization response should be characterized. This is partially reflected in the range of terminology that is used to describe vernalization, beginning with use of the word "vernalization" itself, which is used in reference to both a plant's physiological state and the state of the environment in which it is grown. Plants that no longer respond to vernalization have been described as "fully vernalized", or "vernalized", which practice suggests that vernalization refers to a plant's physiological status. On the other hand, it is common to refer to the process of subjecting imbibed seeds or young plants to low temperatures as "vernalization", or to say that plants were "vernalized" for a certain number of days. These two usages of forms of the word vernalization lead to problems interpreting a simple statement such as "the plants were not vernalized", because that could mean either that no low temperature conditions were imposed, or that the plants had not yet reached a particular state of physiological development, or both.

Here, vernalization is used to describe environmental circumstances rather than a plant's physiological state, and vernalization response is used to refer to a plant's developmental response to exposure to low, nonfreezing temperatures. Consequently, a plant that has been vernalized will not necessarily show any response, while an unvernalized plant is one that was not exposed to vernalizing conditions. Likewise, a vernalization treatment is one that exposes plants to low temperature and will not necessarily elicit vernalization responses from wheat plants.

There is not consistency in the literature regarding how to express changes in development induced by vernalization. Some authors use calendar days or thermal time as their primary unit of measure, while others use the final number of leaves on main stems (Pugsley, 1971; Levy and Peterson, 1972; Berry et al., 1980; Flood and Halloran, 1984; Miao et al., 1992). Neither calendar day nor thermal time approaches are likely to reveal clear biological principles because in both cases a plant's response results from the sum of both the accelerating and retarding effects of low temperatures. The leaf number approach is more appropriate, because it directly reflects differences in the timing of the transition from vegetative to floral apex development (Hay and Kirby, 1991).

Another aspect of vernalization that seems poorly resolved is whether vernalization-responsive wheats have an absolute requirement for vernalization. Genetic lines are often assessed for their "vernalization requirements", i.e., how many days of low temperature they require in order to undergo floral initiation and flowering (Krekule, 1987). On the other hand, there are reports where all tested wheats, even those adapted to autumn planting at high latitudes, will eventually flower without exposure to low temperatures (Ahrens and Loomis, 1963; Chujo, 1966; Martinic, 1973; Gotoh, 1976; Ledent, 1980; Miao et al., 1992). Studies involving low-temperature treatments of varying duration almost always yield quantitative response curves (Ledent, 1980; Miao et al., 1992), which also seems inconsistent with the notion of absolute vernalization requirements, at least in artificial conditions where season length is not limiting.

Techniques for the characterization of genetic variation in vernalization response are also not well developed. Various approaches have revealed that each of wheat's three genomes has one or two loci whose allelic variants influence vernalization response in a qualitative fashion, but minor genes are also reported (see Flood and Halloran, 1986, for review). This picture of the genetic control of vernalization response logically leads to a continuum of phenotypic classes, and that expectation is confirmed by many studies (Flood and Halloran, 1986). To this day, however, there is no formal system for classifying wheats beyond use of the terms "spring" and "winter" in combination with modifiers such as "strong" and "weak". That system fundamentally refers to a genotype's adaptation to spring- or fall-sown systems and does little to characterize variability in vernalization response. For instance, it is well established that many spring wheats exhibit some response to vernalization (Levy and Peterson, 1972; Jedel et al., 1986).

Several reports demonstrate that wheat's vernalization response is influenced by stage of development (e.g., developing embryos, germinating seeds, and growing plants) (Riddell and Gries, 1958; Pugsley, 1971; Hoogendoorn, 1984; Sharma and Mascia, 1987; Kato and Yamashita, 1991; Whelan and Schaalje, 1992), and plant age (Gott, 1957; Ahrens and Loomis, 1963; Chujo, 1966; Jedel et al., 1986). Those reports all concluded that wheat gradually loses its sensitivity to vernalization as it grows older. However, that phenomenon was not considered in most of the studies that employed more than one vernalization duration. The reports that addressed plant age generally used only one low temperature treatment of fixed duration, or a single vernalization duration plus an unvernalized control. The work of Jedel et al. (1986) addressed both plant age and duration of vernalization treatment with spring wheats. The experiments reported here were designed to extend the work of Jedel et al. to winter wheats. A rich array of treatment combinations provided data used in the derivation of a generalized conceptual model for wheat vernalization. The applicability of that model to wheats from a wide range of adaptation zones is reported in a companion paper (Wang et al., 1995, in press).

2. Materials and methods

The study was carried out in two greenhouses and a growth chamber in 1992 at Michigan State University (42°N). Greenhouse A (20 ± 5°C) was used for both pre- and post-vernalization growth. Greenhouse B (15 ± 2°C) was used to acclimate plants to higher temperature conditions after vernalization. Photoperiods in both greenhouses were extended to 20 h with high-pressure sodium lamps providing a photosynthetic photon flux density of approximately 200 μmol m^{-2} s^{-1} at pot level. Vernalization treatments were applied in a growth chamber that had a photoperiod of 8 h and a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1} from fluorescent and incandescent lamps. The short photoperiod during vernalization treatment was used in order to mimic natural conditions. Temperatures in the growth chamber were 5°C and 2°C during the light and dark periods, respectively.

Two winter wheat cultivars adapted to Michigan, Pioneer 2548 and Augusta, were used in this study. The genotype of these cultivars at known vernalization loci is unknown. Seeds soaked for 24 h at 20 to 25°C were sown in greenhouse A in 15-cm diameter plastic pots in a soil mixture of 5 loam:2 peat:3 sand (v/v/v). There were four plants per pot. All pots remained in greenhouse A until initiation of the vernalization treatments. Plant age treatments were based on the number of leaf tips visible on the main shoot at the onset of vernalization treatment. Nine plant ages, from leaf tip stage 0 (LTS0, germinated seed) through LTS8 (eighth true leaf tip visible), were evaluated. For the LTS0 age treatments, pots were transferred to the vernalization chamber immediately upon sowing. The days from sowing to the onset of vernalization treatment for LTS0 through LTS8 were 0, 5, 8, 13, 17, 23, 30, 39, and 46 d, respectively. Plants were subjected to vernalizing conditions in the growth chamber for 7, 14, 21, 28, 35, 42, 49, or 70 d. The 49- and 70-d vernalization treatments were omitted for the LTS4 through LTS8 treatments. The 42-d vernalization treatment was also omitted for the LTS8 treatment. After the end of each vernalization treatment, pots were moved to greenhouse B for 3 d to stabilize vernalization effects (Chouard, 1960). Pots were then returned to greenhouse A. Unvernalized control plants were grown continuously in greenhouse A. The experiment was terminated after all plants reached maturity.

A completely randomized design with two replications was used. Each replication consisted of one pot of each treatment. Pot positions were randomized weekly to minimize possible position effects. The number of emerged leaves at the end of each vernalization treatment and the final leaf number (FLN, i.e. total number of leaves on the main stems at heading) were determined for the main stems of four plants in each pot. Data were analyzed with the GLM procedure of SAS (SAS Institute, 1991). Where appropriate, differences among treatment means were examined using the Duncan *t*-test. FLN data were transformed to logarithmic values for means comparison tests.

3. Results

3.1. Pioneer 2548

Plants in all treatments, including unvernalized controls, produced flag leaves and headed. Average final leaf number (FLN) for controls was 20.8 ± 1.3. FLN averages for the vernalized treatments were always

Table 1
Effect of vernalization duration and leaf tip stage (LTS) at the onset of vernalization treatment on the total number of leaves on the main shoot at heading (final leaf number, FLN), for cultivar Pioneer 2548

Vernal. duration (days)	Leaf tip stage (LTS)								
	0	1	2	3	4	5	6	7	8
0	20.8 a*	20.8 a	20.8 a	20.8 a	20.8 a	20.8 a	20.8 a	20.8 a	20.8 a
7	20.3 ab	20.0 a	19.5 a	20.8 a	20.3 a	20.8 a	19.8 a	20.0 a	21.0 a
14	19.8 ab	19.0 a	18.3 b	17.8 b	17.8 b	18.3 b	18.3 b	18.0 b	18.8 b
21	18.8 b	16.3 b	14.3 c	15.0 c	16.0 c	16.0 c	16.3 c	17.0 c	17.0 c
28	17.3 c	13.5 c	13.3 d	12.8 d	15.0 d	15.0 c	15.3 d	16.0 d	16.8 c
35	14.5 d	11.3 d	10.8 e	12.0 d	13.3 e	13.8 d	**13.5 e**	**14.0 e**	15.0 d
42	10.3 e	10.5 de	10.3 ef	**11.0 e**	**12.0 f**	**12.3 e**	13.0 e	14.3 e	-
49	9.0 f	9.8 ef	**10.0 f**	10.8 e	11.8 f	12.0 e	12.8 e	14.0 e	-
70	8.0 g	9.0 f	9.8 f	11.0 e	-	-	-	-	-

Cells with bold FLN values are the points where a response plateau became evident as vernalization duration was extended within an age treatment.
- No treatments were applied.
*Values within a column not followed by letters in common are significantly different at the 5% level of probability.

smaller or equal to the mean FLN for unvernalized controls (Table 1). Average FLNs for the 7-d vernalization treatments were not significantly different from the average of unvernalized controls in any of the age treatments. All vernalization treatments equal to or longer than 14 d reduced FLN relative to unvernalized controls in one or more age treatments.

Response to vernalization reached a plateau in the LTS2 to LTS7 treatments. Plants at and after the stage where the vernalization response began to plateau were vernalization insensitive because additional vernalization did not reduce FLN. Cells in Table 1 with bold FLN values are the points where a response plateau became evident as vernalization duration was extended within an age treatment. The number of leaves remaining to emerge after vernalization was remarkably consistent (6.3 ± 0.5) among plants from those treatments.

The minimum vernalization duration required to reach a stage of vernalization insensitivity decreased as plant age at the onset of vernalization increased. Linear regression of actual leaf stage (including leaves emerging during vernalization) versus days of vernalization for plants from treatments that brought the plants to the onsets of the response plateaus was significant ($r^2 = 0.73$). Whether that linearity also applied to plants that were not yet vernalization insensitive was answered by assuming, for conditions of nonlimiting photoperiod, that a plant that had flowered became insensitive to vernalization when it had six leaves left to emerge. That point is estimated for our treatments by average FLN minus 6, so average values of FLN − 6 were plotted versus days of vernalization (Fig. 1). Treatments with fewer than six leaves emerging after vernalization were assumed to have passed the point of vernalization insensitivity during treatment and were excluded from the plot. Linear regression (slope = −0.244 leaves/day, Y-intercept = 15.7 leaves) of the observed values was significant ($r^2 = 0.89$).

The Y-intercept of the regression in Fig. 1 is the predicted age of an unvernalized plant when it reaches a developmental state equivalent to that of plants at the vernalization insensitive points, i.e., bold values in Table 1. It was expected that plants at that developmental stage would have approximately six more leaves appear prior to heading, because that was a common attribute of those that just became vernalization insensitive. In fact, FLN of unvernalized plants was 20.8, which is very close to the predicted value of 21.7 (derived from Fig. 1's regression equation).

The slope of the regression in Fig. 1 can be interpreted to mean that the leaf stage at the onset of vernalization insensitivity is reduced by 0.244 leaves for each additional day of vernalization exposure. Put another way, the duration of vernalization required to reach vernalization insensitivity is reduced by 4.1 days when plant age is increased by one leaf. This linear relationship implies that accumulated age and vernalization days independently contribute to a plant's attain-

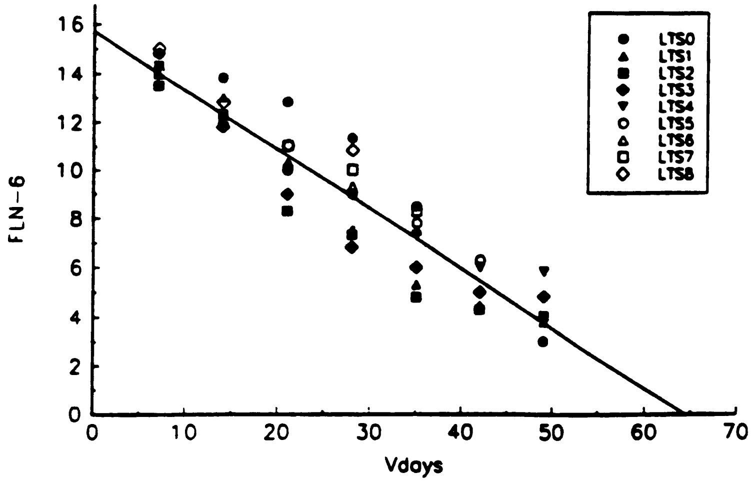

Fig. 1. Relationship between FLN − 6 and days of vernalization duration for Pioneer 2548. The data include all age treatments except those with fewer than six leaves emerging after vernalization. The linear regression line is fitted as: $(FLN-6) = 15.7 - 0.244\, V_{days}$. The r^2 is 0.89.

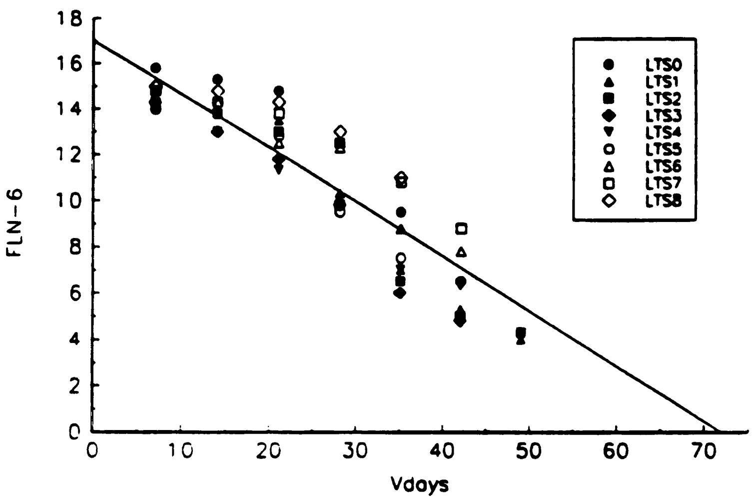

Fig. 2. Relationship between FLN − 6 and days of vernalization duration for Augusta. The data include all age treatments except those with fewer than six leaves emerging after vernalization. The linear regression line is fitted as: $(FLN-6) = 17.0 - 0.236\, V_{days}$. The r^2 is 0.85.

ment of vernalization insensitivity. A series of combinations of vernalization days and plant ages can result in vernalization insensitivity.

Assuming that the Y-intercept is estimated by the FLN of unvernalized plants minus six, then a generalization of the relationship portrayed in Fig. 1 is:

$$L_i = (F_0 - 6) - \beta T_v \tag{1}$$

or on rearrangement,

$$(F_0 - 6) = L_i + \beta T_v \tag{2}$$

where L_i is the leaf stage at the onset of vernalization insensitivity, F_0 is the final leaf number with no vernalization, T_v is the days of vernalization, and β, which represents the "exchange rate" between leaf numbers and vernalization days, is the absolute value of the slope of the linear regression line in Fig. 1.

Eq. 2 indicates that a plant becomes vernalization insensitive when the sum of (1) the current leaf stage and (2) the leaf equivalents gained by vernalization days (i.e., the product of the days of vernalization and the leaf number/vernalization days exchange rate) is equal to the FLN of unvernalized plants minus six. One should bear in mind that leaves continue to grow and develop even during vernalization treatment. The leaf emergence rate was 0.029 to 0.036 leaves per day in our growth chamber.

If the relationship between vernalization days and leaf stage at vernalization insensitivity is linear, then plant age does not alter the effect of vernalization as long as the plant is still responsive to vernalization. The wide range of plant ages at the onset of vernalization employed in this study allows confirmation of that assumption. Regressions of vernalization days versus FLN − 6 were conducted within each of the nine age treatments. That analysis compares the effects of 7 to 70 d of vernalization applied at leaf tip stages ranging from zero to eight. In all age groups, r^2 values were greater than 0.91 (data not shown), confirming that plant age during vernalization had little effect on the interrelationships implied in Eq. 2.

3.2. *Augusta*

Average FLN for unvernalized plants was 21.7 ± 1.0. Trends in vernalization effects were similar to those for Pioneer 2548 except that distinctive plateaus were observed only for age treatments LTS3, LTS5 and LTS6. The relationship between FLN − 6 and vernalization days for treatments which had six or more leaves appearing after vernalization seems nonlinear (Fig. 2). The linear regression of the points in Fig. 2, however, was significant ($r^2 = 0.85$). The fitted line had a Y-intercept of 17.0 leaves and slope of − 0.236 leaves/day. The expected Y-intercept, derived from the FLN of unvernalized plants, was 15.7 leaves.

4. Discussion

Vernalization insensitivity, evidenced by plateaus in the response of plants to increased duration of vernalization, was observed in a number of other studies (Gott, 1957; Ahrens and Loomis, 1963; Halloran, 1977; Berry et al., 1980; Flood and Halloran, 1984; Jedel et al., 1986; Kato and Yamagata, 1988). The number of days of vernalization required to achieve vernalization insensitivity has sometimes been considered a genotype's "vernalization requirement" (Kato and Yamashita, 1991). Halloran (1977) referred to the minimum duration of vernalization needed to attain vernalization insensitivity as the amount of vernalization required to satisfy a plant's vernalization response. Our finding is that the number of days of vernalization needed to reach insensitivity changes with plant age, expressed as leaf stage, as well as with genotype.

Several studies can be interpreted to indicate that the accumulated thermal time between floral initiation and flowering is constant (Halloran and Pennell, 1982; Flood and Halloran, 1984; Griffiths and Lyndon, 1985). It is reasonable to assume that other phenological events coupled to floral initiation, such as the proposed state of vernalization insensitivity, would also show consistency as to its timing relative to flowering. The number of leaves remaining to emerge in plants that had just become vernalization insensitive was about six for Pioneer 2548. The good fit of the Augusta data to Eq. 2 indirectly confirms that Augusta also exhibits a constant number of leaves emerging after attainment of vernalization insensitivity. Support for this concept can be found through reinterpretation of several other published reports. Hoogendoorn (1985) reported that the average final leaf number for a range of wheat lines subjected to 8 weeks of imbibed seed vernalization at 5°C was 6 to 10 leaves. Two cultivars classified as "super-winter" wheats had mean FLNs of 8.7 and 9.5. By our model, those plants became vernalization insensitive at about the two- to three-leaf stage, which is a reasonable approximation of the age they would have attained at the end of vernalization at that temperature. Similar analysis of data from Griffiths and Lyndon (1985) and Miao et al. (1992) also tends to confirm that the number of leaves emerging after vernalization insensitivity under long-day conditions is six.

It is likely that photoperiod can influence the number of leaves emerging between vernalization insensitivity and flowering. In the data of Levy and Peterson (1972), the average FLN of the winter wheat Triumph given a 56 days of vernalization treatment changed from 7.0 to 13.7 when the post-vernalization photoperiod was decreased from 17 to 9 h. The plants with seven leaves were vernalization insensitive, because further reduction in their leaf number was unlikely, so transfer to a shorter daylength must have increased the number of leaves emerging after vernalization insensitivity. This means that vernalization insensitivity is not equivalent to floral initiation. The period between vernalization insensitivity and floral initiation is very probably stable for a given genotype grown in constant post-vernalization conditions, however, because that is the most likely explanation of the constancy of the number of leaves emerging after vernalization insensitivity.

The rate of apical primordia production is usually greater than the rate of leaf appearance during vegetative growth (Kirby, 1990). As a result, the number of primordia acropetal to the emerging leaf increases as leaf number increases. The mechanism by which a plant fixes the number of leaves emerging after vernalization insensitivity must therefore involve a zone of determination where the emerging leaf and the five to six primordia and leaves immediately younger than it are committed to becoming leaves, while younger primordia are labile and will become either spikelets or leaves depending on when floral initiation takes place. In fact, Griffiths and Lyndon (1985) and Rawson and Zajac (1993) showed that primordia can be labile. The constancy of the number of leaves emerging after vernalization insensitivity or floral initiation could be of adaptive significance, because the additional acropetal primordia of plants with more emerged leaves would increase the duration of the period from floral initiation to flowering if all primordia did become leaves. That strategy would make the time from floral initiation to flowering vary with the number of leaves at floral initiation.

It is interesting to note that our estimate of number of leaves emerging at vernalization insensitivity under long-day conditions is close to five to seven leaves, which is also the number of leaves either postulated or observed to be the minimum number of leaves possible in wheat (Purvis, 1934; Aitken, 1966; Miao et al., 1992). Is this accidental or is there some basic underlying mechanism linking these phenomena? Minimum leaf number is probably related to the number of internodes that elongate in wheat. This value stays very constant at five (only a few could be four or six), irrespective of the number of leaf nodes actually present on the stem. In order to construct a stem with four to six internodes, the plant must develop at least that many leaves.

It therefore appears that wheat has adopted developmental strategies that enable it to avoid having fewer than four to six primordia committed to becoming leaves, and to maintain a constant number of leaves emerging after floral initiation. Perhaps the postulated zone of determination from the emerging leaf to the 5th or 6th younger primordium serves both to prevent younger primordia from becoming leaves upon floral initiation, and to prevent primordia within the zone from becoming spikelets. The result would be the observed similarity between minimum leaf number and the number of leaves emerging after floral initiation under long photoperiods. How the plant identifies the emerging leaf or maintains the postulated zone of determination is not clear. A similar and possibly related feat is accomplished when the wheat plant accurately identifies the 4th or 5th internode below the lowest spikelet as the first internode to elongate, irrespective of the total number of nodes on the stem at that time.

Neither of the winter wheats studied here required vernalization in order for flowering to take place. Similar results can be found in numerous reports (Gott, 1957; Ahrens and Loomis, 1963; Ledent, 1980; Davidson et al., 1985; Masle et al., 1989; Hay and Kirby, 1991). Final leaf numbers of unvernalized plants grown under long photoperiods were consistent for individual lines and ranged from 7 to 23 in a set of spring and winter wheats adapted to a wide range of conditions (Wang et al., 1995, in press). Final leaf numbers in the range of 20 to 21 have been reported for unvernalized plants of other winter wheats (Cooper, 1956; Riddell and Gries, 1958; Ahrens and Loomis, 1963; Aitken, 1966). The ability of winter wheats to flower without vernalization is evidence that plant age can substitute for vernalization. A logical corollary of that view is that vernalization can substitute for plant age as a determinant of time of flowering. A state of vernalization insensitivity is also probably reached in unvernalized winter wheats at or prior to the onset of floral initiation, when the plant has only six more leaves left to emerge (under long photoperiods). That means that unvernalized plants would become insensitive to vernalization when they had a leaf number equal to their maximum minus six. That hypothesis was not directly tested, but evidence to support it can be found in the literature. Chujo (1966) presented data that can be interpreted to show that plants with more leaves than the FLN for unvernalized plants minus six could not respond to vernalization, while younger plants could. A similar interpretation can be applied to data from Gott (1957).

The relative linearity of the relationship between FLN − 6 and vernalization days, and the good fit between the predicted and actual Y-intercepts of the fitted regression lines for those data indirectly confirm that about six leaves remain to emerge at the stage of vernalization insensitivity, irrespective of the plant age at which that state is attained. Similarly, the number of vernalization days experienced by a plant does not influence the number of leaves remaining to emerge once vernalization insensitivity is attained. Figs. 1 and 2 demonstrate that the interchangeability of vernalization days and plant age is effective across the range of plant ages and vernalization durations, at least within the boundaries set by the X- and Y-intercepts. Ledent (1980) proposed a linear relationship between age and vernalization days. Expressing the time dimension in days, he concluded that one day of cold exposure of "incompletely vernalized plants", i.e., plants not yet insensitive to vernalization, would reduce the sowing to anthesis time by 2.6 days. No leaf-number data were presented so a direct comparison with our results is not possible. However, a linear relationship between vernalization days and plant age is a clear conclusion of Ledent's work. Halloran (1977) also found an almost linear reduction in leaf number for spring wheats with increased duration of vernalization.

The biological implication of Eq. 2 is that accumulation (or depletion) of the products of a process whose rate is tied to the rate of leaf emergence is accelerated by low temperatures. The products of that process eventually reach a level that enables floral initiation. The fact that vernalization insensitivity is probably not equivalent to floral initiation suggests either that a second process is initiated at the onset of vernalization insensitivity or that the contributions from vernalization are suppressed beyond that point. The second process clearly can involve photoperiod, although photoperiod effects during the period of vernalization response should not be ruled out. Plants of different age and vernalization duration treatments in the present studies had varying photoperiod regimes because of the difference in daylength between the growth chamber and the greenhouses. That probably did not influence the results greatly, because there was no increase in FLN from additional vernalization days in those cases where response plateaus were evident.

As indicated in the introduction, the literature is consistent that older plants are less responsive to vernalization than younger plants. That may seem a contradiction to our conclusion that the interchangeability of vernalization and plant age is independent of the age of plants during vernalization (up to the onset of vernalization insensitivity). The apparent conflict is resolved, however, by application of a common vocabulary to results of those studies. In all the cases where older plants were less responsive, "less responsive" was used to mean either requiring fewer vernalization days in order to flower in a predetermined time period, or showing less total reduction in either final leaf number or days to flower. That is exactly the relationship portrayed by Eq. 2, so there is no conflict between our view and the literature on plant age and vernalization.

Departure from the linearity between vernalization days and FLN−6 is apparent for both Pioneer 2548 and Augusta where plants were vernalized for only 7 days. Average FLNs for those treatments were often not significantly different from those for the unvernalized controls. Jedel et al. (1986) also noted a lag period prior to the initial response to vernalization with some spring wheats. Ahrens and Loomis (1963), Halloran (1977), Ledent (1980), Davidson et al. (1985), and Griffiths and Lyndon (1985) all found similar results. This lag phase would be most critical in situation where total vernalization time was short, or where the total vernalization days were large but individual episodes of vernalization were brief.

The key experimental techniques that enabled development of these concepts were use of leaf numbers as the measure of vernalization response and employment of plant age at the onset of vernalization as a treatment factor. Analysis of our data in terms of calendar days reveals no biologically meaningful trends such as the FLN vernalization response plateaus (and consequently vernalization insensitivity), or the remaining leaf number at vernalization insensitivity. Omission of the age component of the treatment combinations would have also obscured what appear to be significant relationships.

The concepts developed here have utility in wheat modeling. The key genetic coefficients for vernalization response would be the Y-intercept of Eqs. 1 and 2, and the slope of the line. The onset of vernalization insensitivity during crop simulation can be determined by maintaining a running total for both accumulated vernalization days (T_v) and accumulated phyllochrons. When the sum of the phyllochrons and vernalization day leaf equivalents (βT_v) equals that genotype's Y-intercept, vernalization insensitivity has been reached.

Further research is needed to characterize the effects of photoperiod and other factors on the duration of the period between vernalization insensitivity and flowering.

The constancy of the number of leaves remaining at attainment of vernalization insensitivity within an environment enables study of apexes as they change from a state of vernalization sensitivity to a state of vernalization insensitivity. Comparative biochemical and ultrastructural characterization of vernalization sensitive and insensitive apexes with varying numbers of total primordia could lead to a deeper understanding of the underlying processes leading up to floral initiation.

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Section II

Vernalization In Wheat

II. Genetic Variability For The Interchangeability Of Plant Age And Vernalization Duration

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Vernalization in wheat
II. Genetic variability for the interchangeability of plant age and vernalization duration

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Abstract

Differences in response to vernalization in wheat (*Triticum aestivum* L.) were quantified through controlled environment experiments with 26 lines with diverse geographical origins. Vernalization treatments of 0 to 56 d were applied to plants at their first leaf stage. All plants headed irrespective of duration of vernalization treatment. Vernalization response was assessed through the change of final leaf number (FLN) on the main stem at heading. Five lines did not respond to vernalization. FLN for vernalization-sensitive lines generally decreased to a minimum as days of vernalization treatment increased. Plants at and after the stage where additional vernalization did not reduce FLN were vernalization insensitive. The quantitative features of this vernalization response, up to the point of insensitivity, were characterized with a linear regression: $(F_i - 6) = \alpha - \beta T_v$, where F_i is FLN observed for a particular vernalization treatment, T_v is the time in days of that vernalization treatment, and α and β are the Y-intercept and the slope of the regression, respectively. This model fitted the experimental results well. The parameters α and β varied among lines, and are useful for quantifying vernalization response in wheat. The implication of each parameter can be interpreted biologically: α is the "changeable number of leaves", i.e., how many leaves can be potentially decreased by vernalization treatment, and β represents the "exchange rate" between leaf numbers and vernalization days, i.e., how many leaves can be reduced by one day of vernalization treatment.

Keywords: Leaf number; Modelling; *Triticum*; Vernalization; Wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is grown across a wide range of agrogeographical regions from the equator to greater than 60° latitude (Briggle and Curtis, 1987). Wheat phenology varies widely depending upon the genotype, location, and date of sowing. Response to vernalization is one of the most important factors affecting wheat's environmental adaptation. At least five loci involved in the control of the response to vernalization have been identified (Pugsley, 1971, 1972; Law and Scarth, 1984). Some studies have reported that vernalization response is polygenically controlled (as reviewed by Flood and Halloran, 1986). Despite this rather detailed genetic knowledge, there is no system in general use for the quantification of a

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wheat line's vernalization response. The universally used spring/winter classification system relates more to the sowing system to which a wheat line is adapted than to the specific nature of a line's response to vernalization. For instance, many wheats that are adapted to spring sowing (so-called spring wheat) can respond to vernalization (Levy and Peterson, 1972; Wall and Cartwright, 1974; Halloran, 1977; Jedel et al., 1986), and wheats adapted to fall sowing (so-called winter wheat) vary markedly in their response to vernalization (Gotoh, 1976; Ledent, 1980; Miao et al., 1992). Moreover, some wheats included in the International Winter Wheat Performance Nursery had little or no response to vernalization (Gotoh, 1975). The lack of a workable system for quantifying wheats for vernalization response stems in part from the lack of a general conceptual model of this complex phenomenon.

We recently reported that plant age and vernalization duration are related as follows (Wang et al., 1995):

$$(F_0 - 6) = L_i + \beta T_v \quad (1)$$

where F_0 is the final leaf number of unvernalized plants, L_i is the leaf stage at the onset of vernalization insensitivity, T_v is the days of vernalization treatment, and β represents the "exchange rate" between leaf numbers and vernalization days. Eq. 1 indicates that a wheat plant becomes vernalization insensitive when the sum of the current leaf stage and the leaf equivalents gained by vernalization days (i.e., the product of the days of vernalization and the leaf number/vernalization days exchange rate) is equal to the FLN of unvernalized plants minus six.

A key premise underlying Eq. 1 is that plants will emerge six more leaves after the onset of vernalization insensitivity. The leaf stage at which a plant with an emerged flag leaf reached vernalization insensitivity is consequently estimated by FLN minus six. If leaf stage at the onset of vernalization insensitivity (Y) is plotted against vernalization days (X), then Eq. 1 implies a linear relationship as follows:

$$(F_i - 6) = \alpha - \beta T_v \quad (2)$$

where F_i is the FLN observed for a particular vernalization treatment, α is the Y-intercept and is an estimate of F_0 minus six, and β and T_v are as described for Eq. 1.

Eq. 2 applies only up to the point of vernalization insensitivity, as judged retrospectively by the attainment of a minimum in FLN. This report explores the utility of Eq. 2 in development of vernalization response parameters for a set of wheat lines with diverse geographical origins.

Table 1
Wheat lines used in the experiments with their countries and latitude of origin

Series	Line	Country of origin	Latitude (°N)	Type
1	Seri 82	Mexico (CIMMYT)	25	Spring
	Pitic 62	Mexico (CIMMYT)	25	Spring
	Yecora Rojo	Mexico (CIMMYT)	25	Spring
	Sonora 64	Mexico (CIMMYT)	25	Spring
	Glennson	Mexico (CIMMYT)	25	Spring
	FL 303	USA (Florida)	27	Winter
	Phoenix	USA (California)	30	Winter
	Anza	USA (California)	30	Spring
	MO 298	USA (Missouri)	37	Winter
	Wakefield	USA (Virginia)	37	Winter
	MD 286-21	USA (Maryland)	38	Winter
	CA 841	China (Beijing)	39	Winter
	Excel	USA (Ohio)	39	Winter
	Clark	USA (Indiana)	40	Winter
	Pioneer 2548	USA (Indiana)	40	Winter
	Augusta	USA (Michigan)	42	Winter
	NY 73116-4w	USA (New York)	42	Winter
	Thatcher	Canada	52	Spring
2	Xiangmai	China (Hunan)	28	Winter
	Shumai	China (Jiangshu)	33	Winter
	215953	China (Shandong)	36	Winter
	Ji 84-5418	China (Hebei)	38	Winter
	CA 8686	China (Beijing)	39	Winter
	CA 8646	China (Beijing)	39	Winter
	Jing 411	China (Beijing)	39	Winter
	Jingnong 86-74	China (Beijing)	39	Winter

2. Material and methods

The 26 wheat (*Triticum aestivum* L.) lines used in this study are listed in Table 1 along with their country and latitude of origin. Seeds were sown in 10-cm diameter clay pots in a greenhouse soil mixture of 5 loam : 2 peat : 3 sand (v/v/v). Plants were grown in a greenhouse except during the vernalization treatments, which were applied to seedlings at the first leaf stage (7 d after sowing). The greenhouse was maintained at about 20°C and the natural photoperiod was extended to 20 h by high-pressure sodium lamps that delivered a photosynthetic photon flux of approximately 200 μmol m^{-1} s^{-1} at pot level. The vernalization chamber had an 8-h photoperiod with a photon flux of 200 μmol m^{-1} s^{-1} provided by fluorescent and incandescent lamps. Temperatures were 5°C and 2°C during the light and dark periods, respectively.

In experiment 1, seedlings of 18 lines (series 1 in Table 1) were vernalized for 0, 7, 21, 28, or 42 d. In experiment 2, seedlings of eight lines (series 2 in Table 1) were vernalized for 0, 16, 28, 42, or 56 d. The experiments were terminated when all plants reached maturity.

Five seedlings were kept in each pot after plant emergence. After appearance of the fifth leaf tip, plants were thinned again, and only two well-established seedlings were left in each pot. Measurements were made on the main stems of the plants in each of two pots for each treatment, except for the zero vernalization treatment which had four pots. Final leaf number (FLN) was recorded as the total number of leaves on the main stem at heading.

A separate linear regression analysis was performed for each wheat line. Values of FLN minus six were used as dependent variables and days of vernalization treatment were used as independent variables. Plants at and after the stage that additional vernalization did not reduce FLN were vernalization insensitive. The regressions were made after discarding points in the region of insensitivity.

3. Results and discussion

All 26 wheat lines headed even in the absence of low temperature vernalization. This confirms that there is not an absolute vernalization requirement in wheat's life cycle (Martinic, 1973; Ledent, 1980; Pinthus, 1985; Miao et al., 1988; Wang et al., 1995). Two quantitative features of the vernalization response were evident in terms of FLN change (Table 2). Firstly, FLNs of a line generally decreased to a minimum as days of vernalization treatment increased. Secondly, mean FLNs differed markedly among the lines under the same vernalization treatments. This was especially true in the unvernalized controls, where mean FLN was as small as 7.0 for Yecora Rojo and as large as 22.7 for NY 73116-4w, a range of 15.7 leaves. That range diminished as vernalization duration increased. After a 42-d vernalization treatment, the range of FLNs among all 26 lines became 7.7 leaves.

The regression analysis was not performed for five lines whose FLNs changed only about one leaf among all treatments. Three of these lines, Yecora Rojo (Davidson et al., 1985; Jedel et al., 1986), Sonora 64 (Levy and Peterson, 1972; Wall and Cartwright, 1974) and Thatcher (McIntosh, 1973; Syme, 1973; Flood and Halloran, 1984; Davidson et al., 1985; Penrose et al., 1991) have been studied extensively, and were reported not to respond to vernalization treatment. Our results coincide with the previous conclusions. The other two lines, Anza and Seri 82, were developed in California and Mexico, respectively and both have been described as not responding to vernalization. FLN of Sonora 64 changed little with treatment whereas that of Thatcher increased from 7.3 to 8.8 as days of vernalization treatment increased from 0 to 42 d. This may be because Sonora 64 is insensitive to photoperiod (Levy and Peterson, 1972) whereas Thatcher is sensitive (Halloran and Pennell, 1982; Crofts et al., 1984). In the current study, photoperiod during the vernalization treatment was only 8 h.

Mean FLNs of the remaining 21 lines were subjected to separate linear regression analyses as described. The coefficients of determination (r^2) of this model were greater than 0.80 for most lines (Table 3). The resultant estimates of the parameters α and β are also listed in Table 3.

Both α and β are useful parameters for quantifying vernalization response in wheat. The implication of each parameter can be interpreted biologically. The slope, β, represents the "exchange rate" between leaf numbers and vernalization days, i.e., how many leaves can be reduced by one day of vernalization treatment. The meaning of β is clarified by $1/\beta$, which represents how many days of vernalization treatment are necessary to reduce the leaf number by one. The Y-intercept, α, is the "changeable number of leaves", i.e., how many leaves can be potentially decreased by vernalization treatment. Alpha is also biologically equivalent to the leaf number of an unvernalized plant at the onset of vernalization insensitivity. Accordingly, the mean

Table 2
Mean FLN in various lines at different vernalization treatments

	Line[a]	Days of vernalization treatment						
		0	7	16	21	28	42	56
1	Yecora Rojo	7.0a[b]	7.0a	–[c]	7.3a	7.5a	7.8a	–
2	Sonora 64	7.3a	7.0a	–	7.0a	7.3a	7.3a	–
3	Thatcher	7.3c	8.0b	–	8.0a	8.0a	8.8a	–
4	Anza	8.0b	8.3b	–	8.0b	8.0b	9.0a	–
5	Seri 82	8.8a	8.0b	–	8.0b	8.0b	8.3ab	–
6	Glennson	10.8a	9.0b	–	8.5bc	8.0bc	9.0b	–
7	Shumai	11.3a	–	9.0b	–	8.8b	9.0b	9.3b
8	Pitic 62	11.5a	10.5b	–	9.0c	9.0c	8.8c	–
9	FL 303	11.5a	10.8b	–	10.0c	9.3d	8.5e	–
10	Xiangmai	15.3a	–	11.0b	–	10.0c	8.8d	9.0d
11	215953	16.0a	–	11.0b	–	9.8c	9.0c	9.0c
12	CA 8646	17.8a	–	15.0b	–	9.5c	8.5d	9.0cd
13	Ji 84-5418	18.8a	–	18.0a	–	11.3b	10.0b	10.0b
14	Phoenix	19.0a	18.5a	–	11.5b	10.0c	9.0d	–
15	CA 8686	19.2a	–	18.0a	–	10.8b	9.0c	9.3bc
16	Wakefield	19.2a	18.5a	–	13.5b	12.0c	9.3d	–
17	Jing 411	19.3a	–	16.3b	–	11.0c	9.8d	10.0d
18	Jingnong 86-74	19.4a	–	17.8b	–	11.5c	10.0d	10.0d
19	Clark	19.7a	18.8b	–	17.5c	16.0d	12.4e	–
20	Pioneer 2548	20.3a	19.8a	–	18.0b	14.8c	11.0d	–
21	CA 841	20.3a	–	–	19.0b	13.8c	11.5d	–
22	Augusta	20.8a	20.5a	–	18.8b	16.5c	12.5d	–
23	Excel	21.0a	19.8ab	–	18.0bc	16.8c	13.8d	–
24	MD 286-21	21.3a	20.3a	–	19.8a	16.9b	11.8c	–
25	MO 298	21.3a	19.5b	–	18.5b	15.8c	12.0d	–
26	NY 73116-4w	22.7a	22.3a	–	20.5b	18.0c	15.0d	–
	Range	15.7				10.7	7.7	

[a]The lines have been arranged in order of their FLNs for unvernalized plants.
[b]Values within a row not followed by letters in common are significantly different at the 5% level of probability in using the Duncan t-test:
[c]No treatment is represented by –.

FLN minus six for plants in the zero vernalization treatments was close to the α value (Table 3).

The values of α and β in general are lower in so-called spring wheats than in so-called winter wheats. Their values can be equal to or close to zero in some wheats such as the five lines identified above, which did not respond to vernalization. However, some spring-sown wheats may have the same α or β values as fall-sown wheats. For instance, a spring wheat, Pitic 62, which was reported to respond to vernalization (Levy and Peterson, 1972; Syme, 1973; Wall and Cartwright, 1974; Davidson et al., 1985; Jedel et al., 1986), had the same α value as a winter wheat, FL 303. The β value of Pitic 62 was greater than that of FL 303, which means that FL 303 needs more days of vernalization or accumulated leaves to reach vernalization insensitivity. Some lines had the same α value but different β values (Fig. 1), or the same β but different α (Fig. 2). Therefore, the vernalization responsiveness of a line is described by both the "changeable number of leaves" due to vernalization, α, and the "exchange rate" between vernalization days and plant age, β.

The extrapolated regression lines in Fig. 1 and Fig. 2 indicate that FLN for a particular vernalization treatment is also related to temperatures applied in that vernalization treatment. The FLN will increase as tem-

Table 3
The Y-intercept (α) and the slope (β) derived from linear regression analysis of F_i minus six and the days of vernalization treatment for a range of wheat lines, and resultant estimates of substituted days of vernalization treatment by one leaf's growth, $1/\beta$. The r^2 is coefficient of determination of the linear regression. F_0 is the mean FLN of unvernalized plants

	Line[a]	F_0-6	α	β	$1/\beta$	r^2
1	Yecora Rojo[b]	1.0				
2	Sonora 64[b]	1.3				
3	Thatcher[b]	1.3				
4	Anza[b]	2.0				
5	Seri 82[b]	2.8				
6	FL 303	5.5	5.4	0.0750	13.3	0.98
7	Shumai	5.3	5.0	0.0921	10.9	0.88
8	Glennson	4.8	4.3	0.0969	10.3	0.77
9	Pitic 62	5.5	5.4	0.1174	8.5	0.99
10	Xiangmai	9.3	8.5	0.1508	6.6	0.91
11	215953	10.0	9.0	0.1640	6.1	0.86
12	Clark	13.7	14.1	0.1668	6.0	0.95
13	Excel	15.0	15.1	0.1670	6.0	0.99
14	NY 73116-4w	16.7	17.4	0.1880	5.3	0.95
15	Augusta	14.8	15.7	0.1971	5.1	0.93
16	MO 298	15.3	15.5	0.2105	4.8	0.95
17	MD 286-21	15.3	16.2	0.2138	4.7	0.87
18	CA 841	14.3	15.1	0.2181	4.6	0.84
19	Pioneer 2548	14.3	15.1	0.2240	4.5	0.94
20	Ji 84-5418	12.8	13.5	0.2346	4.3	0.86
21	CA 8646	11.8	11.8	0.2389	4.2	0.93
22	Jing 411	13.3	13.3	0.2428	4.1	0.94
23	Jingnong 86-74	13.4	14.0	0.2461	4.1	0.91
24	Wakefield	13.2	13.4	0.2529	4.0	0.98
25	CA 8686	13.2	14.0	0.2700	3.7	0.89
26	Phoenix	13.0	12.9	0.2719	3.7	0.90

[a]The lines have been arranged in order of their β values.
[b]The regression analysis was not performed because the FLNs of those lines changed only about one leaf among all treatments.

perature of vernalization treatment increases. However, the values of α and β were relatively constant for a given cultivar. For instance, in the present study, Pioneer 2548 had α and β values of 15.1 and 0.224, respectively. In a previous study (Wang et al., 1995), they were 15.7 and 0.244, respectively. At 20°C and with a 16-h photoperiod, unvernalized plants of Pioneer 2548 had a mean FLN of 19.7 (Fowler et al., 1995). That FLN is close to 20.3 that was observed in this reported experiment.

These results confirm the general linearity of the relationship between plant age and vernalization days as reported by Wang et al. (1995). FLN data of plants not yet vernalization insensitive were used here as dependent variables in linear regressions with days of vernalization as the independent variables. Values of α, indicating the "changeable number of leaves", exhibited pronounced variation both among and within the lines previously classified as winter or spring types. Beta, or the "exchange rate" between leaf number and vernalization days also varied between and within the winter and spring types.

The vernalization response of wheat can be experimentally quantified by using the response parameters derived from Eq. 2. Differences among lines for the parameters α and β are presumably caused by different allelic configurations at genes influencing response to vernalization. Alpha and beta, although loosely related, appear to vary independently, indicating that some of genes conditioning variation in the vernalization days

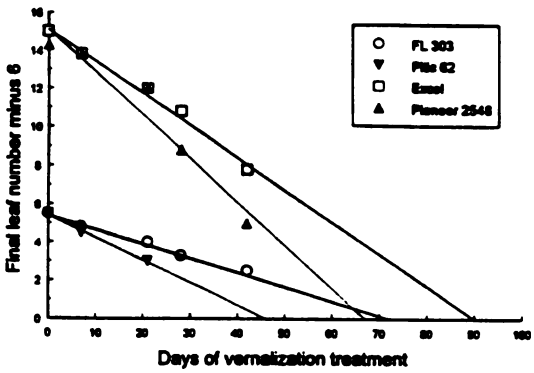

Fig. 1. Vernalization response for wheat lines with a similar Y-intercept (α) but different slopes (β). The parameters of the lines are listed in Table 3. The symbols represent the observed values after discarding points in the region of vernalization insensitivity.

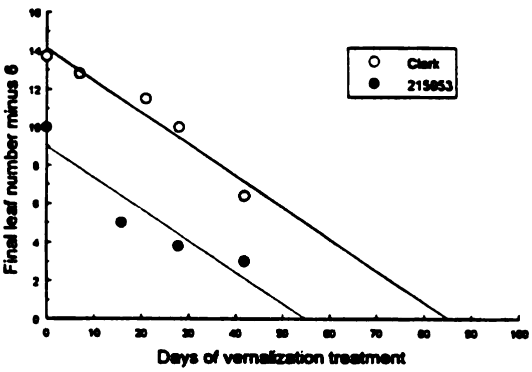

Fig. 2. Vernalization response for wheat lines with a similar slope (β) but different Y-intercepts (α). The parameters of the lines are listed in Table 3. The symbols represent the observed values after discarding points in the region of vernalization insensitivity.

vs. plant age exchange rate (β) are probably distinct from those influencing the changeable number of leaves (α). The continuous nature of variation in both α and β indicates a large number of possible genetic states for each of the physiological mechanisms. Genetically, that could be caused by allelic variation at a few loci or by allelic variation at several to many loci.

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Section III

A Novel Conceptual Framework For Wheat Vernalization

A novel conceptual framework for wheat vernalization*

Abstract

Vernalization response in wheat so far has been characterized poorly, and less well quantified. The discrepancies and inconsistencies in the literature regarding terminology, measure of response, classification of response types, operative temperatures, etc. stem in part from the lack of a general conceptual model of vernalization phenomena. Low temperature during the early development stage of wheat reduces the number of leaves emerged during that period and results in a lower final leaf number. A plant's response to temperature during that physiological phase is its vernalization response. Temperature as an unity affects both growth and development. There is not a so-called operative temperature for wheat vernalization. The key technique for measure of vernalization response is to count the number of leaves emerged before, during, and after vernalization treatment, rather than only to calculate

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* The paper format was adopted for this section in accordance with "Field Crops Research". Dr. R.A. Fischer of International Maize and Wheat Improvement Center (CIMMYT) in Mexico, Drs. R.W. Ward and J.T. Ritchie of Michigan State University in the United States, Professor G.-Y. Miao of Shanxi Agricultural University in People's Republic of China, and Dr. E.J.M. Kirby of West Australia University in Australia involved in this study.

calendar days or thermal time after the end of vernalization treatment. Plants become vernalization insensitive through accumulating leaf number, the leaf equivalents gained by vernalization days, or both. After vernalization insensitivity, a plant will emerge six more leaves before heading under long photoperiods. Vernalization response of wheat, up to the point of insensitivity, can be quantified experimentally by using the response parameters, α and β, derived from a linear regression: $(F_l - 6) = \alpha - \beta T_v$, where F_l is final leaf number observed for a particular vernalization treatment, T_v is the time in days of that vernalization treatment.

Key words: Leaf number; Model; *Triticum*; Vernalization; Wheat.

1. Introduction

The three main factors which modulate the development of wheat are photoperiod, temperature and vernalization. Although vernalization in wheat was studied extensively from 1930s to 1950s (Whyte, 1948; Chouard, 1960), vernalization response so far has been characterized poorly, and less well quantified as compared with the photoperiod and the general thermal responses (Ellis et al., 1989). Except the greatest advances in understanding the genetic background (as reviewed by Flood and Halloran, 1986), the progress in this subject has been slow at least in last two decades. Physiological investigations are marked by the accumulation of phenomena (Krekule, 1987). Most of the recent work on wheat vernalization is done in the field of agronomy. Over the years crop physiologists and modellers attempt to make generalizations for wheat's vernalization response, but they found that it is extremely difficult. This is not only because the results obtained are from a wide range of tests on different cultivars in various environments, but also because there is not a well established conceptual model. The objective here is to outline a novel conceptual framework that integrates both the recent advances and our knowledge on wheat vernalization.

2. Terminology

Since Lysenko (1928, see Whyte, 1948) coined the term "vernalization" to describe the phenomena that development of winter wheat is hastened by chilling germinated seeds, this term had been used with various meanings. A furthermost derived meaning is that any physiological action stimulating the capacity for flowering, whatever the agent (Chouard, 1960). In this sense, vernalization could be obtained by heat or cold, by long days or short days, by light or dark, by nutrition or chemical. In order to make clarification, Chouard (1960) gave the restricted definition for vernalization as: "the acquisition or acceleration of the ability to flower by a chilling treatment." A similar definition, suggested by Vince-Prue (1975), is that vernalization is the specific promotion of flower initiation by a previous cold treatment given to the imbibed seed or young plant. Although this definition has been well accepted, the confusion in terminology of vernalization seems never-ending.

Chouard's definition clearly means that vernalization is the physiological or biochemical processes leading to flowering. In other words, vernalization is plant's flowering response to a cold environment. The term "vernalization" in the recent literature is, however, used at least in reference to both a plant's physiological state and the state of the environment in which it is grown (Napp-Zinn, 1987). Plants that no longer respond to vernalization have been described as "fully vernalized", or "vernalized", which suggests that vernalization refers to a plant's physiological status. On the other hand, it is common to refer to the process of subjecting imbibed seeds or young plants to low temperatures as "vernalization", or to say that plants were "vernalized" for a certain number of days. These two meanings of vernalization lead to problems interpreting a simple statement such as "the plants were not vernalized", because that could mean either that no low temperature conditions were imposed, or that the plants had not yet reached a particular state of physiological development, or both.

Wang et al. (1995a) proposed using vernalization to describe environmental circumstances rather than a plant's physiological state, and using vernalization response to refer to a plant's developmental response to exposure to low, nonfreezing temperatures. Therefore, a plant that has been vernalized will not necessarily show any response, while an unvernalized plant is one that was not exposed to vernalizing conditions. Likewise, a vernalization treatment is one that exposes plants to low temperature and will not necessarily elicit vernalization responses from wheat plants. The terminology of vernalization in this paper will follow the definition of Wang et al. (1995a).

Another misused term is "short day vernalization". Without experience of any low temperatures, ear emergence is fastest when long days are preceded by short days (McKinney and Sando, 1935; Cooper, 1960; Krekule, 1964; Davidson et al., 1985). The phenomenon, that short days can substitute for low temperature vernalization to promote flowering in winter cereals, was termed as "short day vernalization" by Purvis and Gregory (1937). However, evidence indicated that the mechanisms of winter wheat plants response to short day and low temperature are different and independent of each other: 1) all winter wheat cultivars respond to vernalization, only some of them respond to short days (Krekule, 1964; Miao et al. 1993); 2) the shoot apices of plants vernalized for eight weeks at 2-3°C reveal no progress towards inflorescence initiation, double ridges are apparent in the apices of plants given short days for eight weeks (Evans, 1987); 3) exposure of the developing grains in ear to short days and low temperatures is less effective than to long days and low temperatures (Evans, 1987); 4) the shoot apical meristem responds to vernalization (Ishihara, 1961), and the leaves perceive the short days (Gott et al., 1955). Although the effect of short day will not be discussed further in this paper, we suggest use "short day response" instead of "short day vernalization".

3. Measure of response

Vernalization response of wheat is usually evaluated by the degree of acceleration, due to the cold treatment, of floral initiation, stem elongation, flag leaf unfolding, heading, and/or flowering under a long photoperiod (>15-h) and high temperature (>15°C) regime in terms of calendar days or thermal time from the end of vernalization treatment. The minimum length of cold treatment which maximized vernalization response was defined as the "vernalization requirement" of a cultivar (Martinic, 1973). Gotoh (1976) used a criterion that flag leaf was able to unfold within 34 days after the termination of cold treatment to determine a cultivar's "vernalization requirement". A similar way, but 40 days for head emergence, was used by Hunt (1979). This criterion is associated with some distortion resulting from the varietal difference in narrow-sense earliness (Takahashi and Yasuda, 1971; also called "intrinsic earliness" by Hoogendoorn, 1985; and "flowering tendency" by

Wallace, 1985; defined as the time to reach anthesis when vernalization and photoperiod do not constrain development).

The results presented in calendar days provide little information about the exact response and has little basis in morphophysiology, and are hardly to be compared among different experiments which different temperatures are applied at post-vernalization growth. When the duration of cold treatment is not long enough to saturate plant's vernalization response (i.e., so-called partial vernalization), neither calendar day nor thermal time are likely to reveal clear biological principles because in both approaches a plant's response results from the sum of both the accelerating and retarding effects of low temperatures used for post-vernalization growth. The confusions encountered in explaining vernalization effects for the field data can also be attributed to using calendar day or thermal time as the primary unit of measure.

While being cold treated, wheat continues to grow and develop. The higher the temperature used in vernalization treatment, the more growth for treated plant (Chujo, 1966). Therefore, increasing the vernalization duration, even when a plant has become insensitive to vernalization, the calendar days or thermal time from the end of cold treatment to heading has a tendency to be decreased (Fig. 1). In order to measure vernalization response *per se*, several ingenious vernalization techniques and data-analysis methods have been developed, such as: 1) vernalizing developing grains in the ear (Hoogendoorn, 1984); 2) reducing the amount of growth during the vernalization treatment through using lower

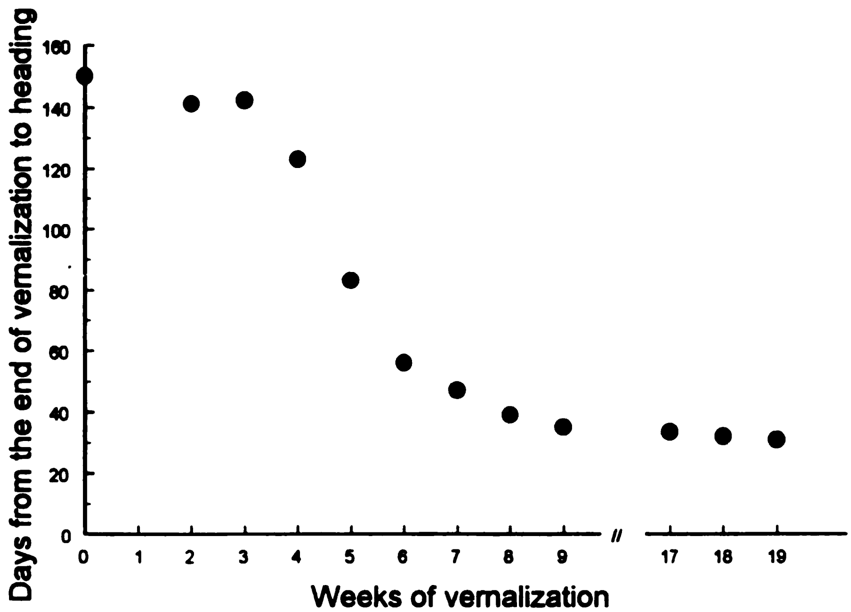

Fig. 1. Days from the end of vernalization treatment to heading in response to weeks of vernalization treatment. Adapted from Ahrens and Loomis (1963), imbibed seeds of winter wheat Minter were vernalized at 1°C for varying periods, then transferred to a warm greenhouse (24°C, 18/6-h), and observed for heading date.

temperatures (Riddell and Gries, 1958; Pirasteh and Welsh, 1980), or less moisture (Hoogendoorn, 1984); 3) growing control plants at a nonvernalizing temperature to the same size as the vernalized seedlings (McKinney and Sando, 1933; Syme, 1968); 4) estimating growth during vernalization treatment in terms of days of growth at high temperature through the linear regression of primordia (Hoogendoorn, 1984), or growth increment (Kato and Yamagata, 1988), or regression on the leaf number at transfer against final leaf number (Halloran, 1975). However, no one of those approaches has been incorporated into a general model.

The coleoptile and early leaves are shortened and hair development on the leaf sheath is suppressed in wheat plant due to vernalization treatment (Purvis and Hatcher, 1959), but those are not related to flower initiation and hardly have meaning in evaluation of vernalization response. The morphology of main shoot apices should be a good indicator which shows the transition from vegetative to reproductive stage. However, in a detailed study for the effects of vernalization on shoot apices, Griffiths et al. (1985) were unable to detect any major changes before the appearance of double ridges. Plant become vernalization insensitive before floral initials are present (Thomas and Vince-Prue, 1984). It is no question that wheat has lost its vernalization responsiveness at the double ridge stage (Halse and Weir, 1970; Weir et al., 1984; Flood and Halloran, 1986). The question is how long the lag period between the onset of vernalization insensitivity and double ridge stage could be.

Many reports showed that the effect of vernalization can be interpreted more clearly from the results of experiments in which final leaf number is recorded (Levy and Peterson, 1972; Berry et al., 1980; Hay and Kirby, 1991). Although final leaf number is an indirect indicator, it does give the unambiguous information about the transition of plant from a state of vernalization sensitivity to vernalization insensitivity. Final leaf number decreases with increase of vernalization duration until reaching a plateau (Fig. 2). Plants at and after the stage where final leaf number begins to plateau is vernalization insensitive because additional vernalization did not reduce final leaf number. In addition, final leaf number is a non-destructive observation and easy to be recorded. As discussed later on, the different effects of temperature on vernalization response and on general growth can be analyzed through counting the leaf number change. Before other more reliable physiological or phenological marker(s) is established, using final leaf number as a measure in vernalization research is suggested.

4. Response types

Wheat is a world-wide distribution crop, and characterized by marked variability in its vernalization pattern which is associated with the geographical origin and the cultivation season of a specific cultivar (Hunt, 1979; Ford et al., 1981; Hoogendoorn, 1985). Various approaches have revealed that each of wheat's three genomes has one or two loci whose allelic variants influence vernalization

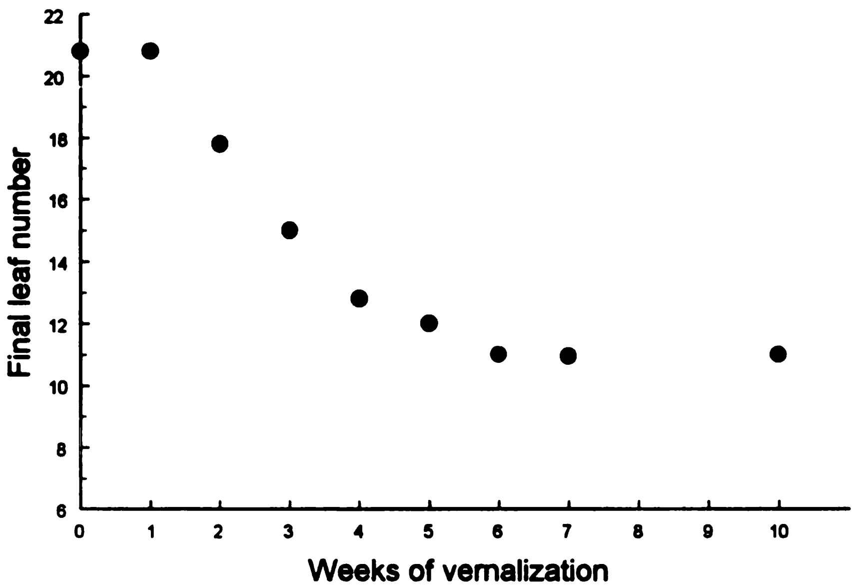

Fig. 2. Final leaf number on the main stem in response to weeks of vernalization treatment. Adapted from Wang et al. (1995a), seedlings of winter wheat Pioneer 2548, aged at the third leaf tip visible, were vernalized at the growth chamber (5/3°C, 8/16-h) for varying periods, then transferred to a warm greenhouse (20°C, 20/4-h), and observed for final leaf number on the main stem.

response in a qualitative fashion, but minor genes are also reported (see Flood and Halloran, 1986 for review). This picture of the genetic control of vernalization response logically leads to a continuum of phenotypic classes, and that expectation is confirmed by many studies (Flood and Halloran, 1986). However, the vernalization responsiveness of wheat (including all species) in general is only classified into three types (Pinthus, 1985), i.e., 1) distinct winter wheat (true winter wheat, winter wheat): cultivars of this type require vernalization to reach the stage of spike differentiation within a normal season of growth; 2) intermediate wheat (semi-winter wheat, facultative wheat): cultivars of this type do not require vernalization for normal floral initiation but will respond to it by accelerated progress towards this stage; 3) spring wheat: cultivars of this type do not respond at all to vernalization. The terms "spring" and "winter" in combination with modifiers such as "strong" and "weak" are also commonly used. Furthermore, the terms "spring' and "winter" are held to be synonymous with "early" and "late" in some papers (e.g., Aitken, 1966). In North America the terms "winter" and "spring" are also used to categorize certain market grades (Pugsley, 1983).

The universally used spring/winter classification system relates more to the sowing system to which a wheat cultivar is adapted rather than to the specific nature of a cultivar's response to vernalization. For instance, many wheats that are adapted to spring sowing (so-called spring wheat) can respond to vernalization (Levy and Peterson, 1972; Wall and Cartwright, 1974; Halloran, 1977; Jedel et al., 1986), and wheats adapted to fall sowing (so-called winter wheat) vary markedly in their response to vernalization (Gotoh, 1976; Ledent, 1980; Miao et al., 1992). Moreover, some wheats included in the International Winter Wheat Performance Nursery had little or no response to vernalization (Gotoh, 1975).

In order to reveal major genotype by site interactions in vernalization response of different cultivars, more groups were divided. For example, Kakizaki and Suzuki (1937, cited by Gotoh, 1976) grouped Japanese cultivars into the classes I (extreme spring habit) to VII (extreme winter habit). Miao et al. (1988) classified 40 cultivars into six groups, i.e., from strong springness cultivar which vernalization response was zero to ultra-strong winterness cultivar which responded to over 70 days of vernalization treatment. Those arbitrary groups may work better than only describing cultivar as spring or winter type. This kind of classification was, however, based either on the difference of days from the end of vernalization treatment to heading (or flowering) or on the difference of heading date among tested cultivars at the special vernalization experiment. There was no unambiguous parameter(s) for quantifying vernalization responsiveness of each group.

Pugsley (1983) suggested that the classification of cultivars for their vernalization response should be based on genotype. He proposed three genetic types as follows: 1) spring wheats: bearing the major gene Vrn1; 2) semi-winter wheats: lacking Vrn1, but carrying Vrn2, Vrn3, Vrn4, or a combination of those; 3) winter wheats: bearing the recessive alleles vrn1, vrn2, vrn3 and vrn4. This classification system is hardly to be generally accepted. At first, it was only based on four loci (Vrn1 to Vrn4). The genetic background of vernalization in fact is very complicated and by no means clear (Flood and Halloran, 1986). Except the nuclear genes, there was also some evidence that cytoplasm can influence vernalization response (e.g., Ward, 1983). In addition, it is impossible to know each cultivar's genetic background due to technical reason and economic reason.

5. Operative temperatures

There is not consistency in the literature regarding effective vernalization temperatures. Vernalization response is generally believed to take place at 2 to 8°C (Fischer, 1984). Ahrens and Loomis (1963), working with a winter wheat cultivar, found the vernalization effect at 1°C and 3°C, but no effect at -2°C. The effectiveness of the cold treatment in wheat seedlings was maximum at 7°C and decreased very rapidly if temperature was raised to 9°C or lowered to 3°C (Trione and Metzger, 1970). Chujo (1966) demonstrated that vernalization response progressed more rapidly at 4, 8, or 11°C than at 1°C. Many efforts have been made to integrate results of such studies into simulation models. However, the lower limit, the range of optimum, and the upper limit for vernalization temperatures are discrepant among different studies (Fig. 3).

Some researchers believed that the operative temperatures for vernalization vary among cultivars. Higher temperatures (e.g., 8 and 11°C) were more favorable for vernalizing semi-winter wheats, but winter wheats were more sensitive to lower

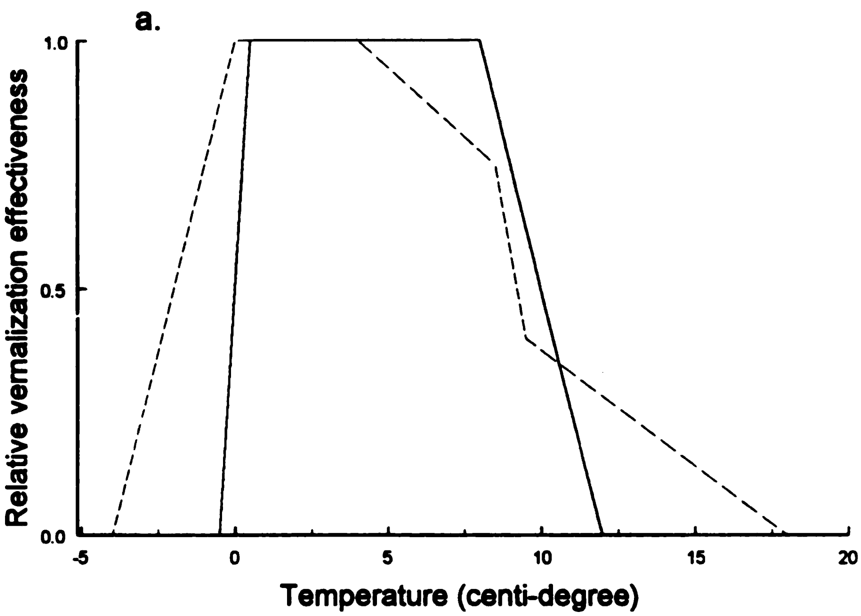

Fig. 3. The effectiveness of temperature (°C) on vernalization response in the literature. Adapted from (a) Kirby (1992, solid line) and Maas and Arkin (1980, dashed line), (b) Hansel (1953, solid line) and Reinink et al. (1986 dashed line), (c) Ritchie (1991, solid line) and Weir et al. (1984, dashed line).

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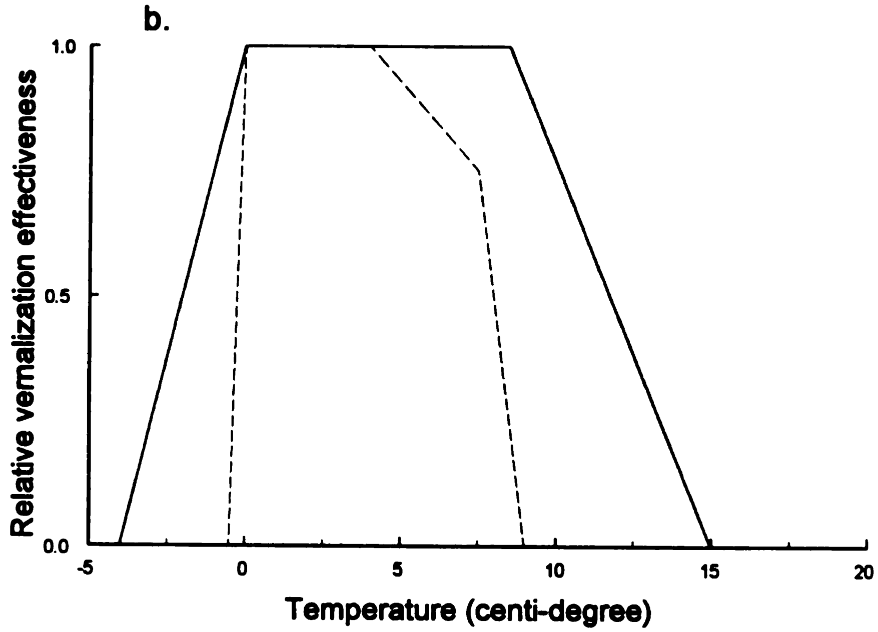

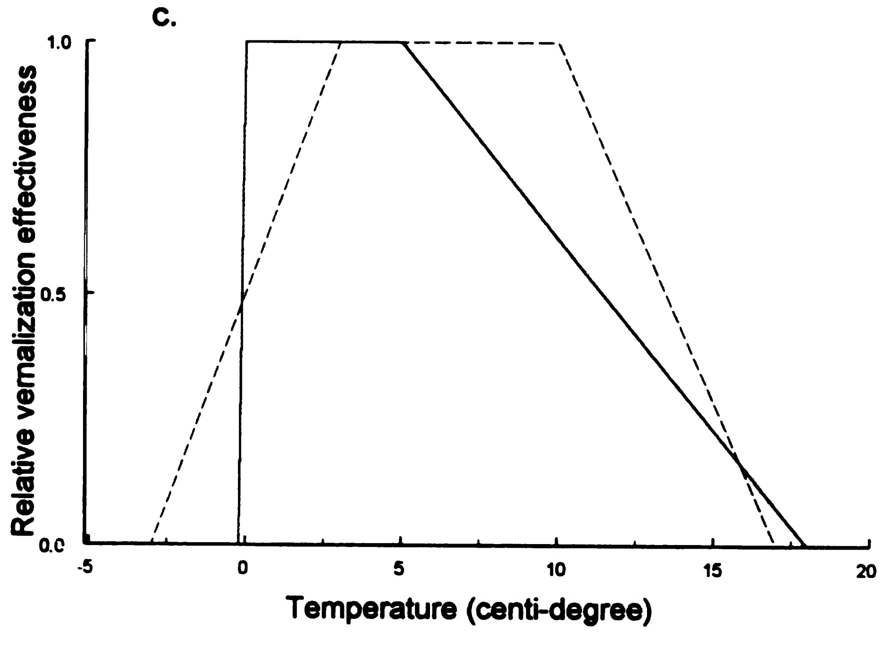

Fig. 3. –Continued.

temperatures (e.g., 4 and 8°C) (Chujo, 1966). Vavilov (1951) showed that the most effective vernalization temperatures for different types of cultivars were as follows: 10 to 12°C for soft-grained spring, 2 to 5°C for hard-grained spring, 5 to 10°C for semi-winter, and 0 to 5°C for winter cultivars.

Almost all studies on the operative vernalization temperatures was based on the measure of calendar days or thermal time after the end of vernalization treatment. The magnitude of growth under different vernalization temperatures was not taken into account. I.R. Brooking (pers. comm.) suggested to separate the temperature effects on vernalization response and on vegetative growth by using isogenic lines or monitoring the floral transition through recording the primordia at the end of treatment and final leaf number. Emphasis on final leaf number rather than calendar time should make interpretation more relevant.

6. Relationship between leaf number and vernalization responsiveness

Final leaf number on the main stem can be affected by vernalization treatment. To explore the relationship between final leaf number and the number of leaves emerged at the onset of vernalization insensitivity should be of great importance in wheat vernalization study. Wang et al. (1995a) estimated that the number of leaves emerging at vernalization insensitivity under long day conditions is about six leaves. In other words, the leaf stage at which a plant with an emerged flag leaf reached vernalization insensitivity is estimated through final leaf number minus six. Evidence to support this hypothesis can be found in the literature. Chujo (1966) presented data that can be interpreted to show that plants with more leaves than final leaf number for unvernalized plants minus six could not respond to vernalization, while younger plants could. A similar interpretation can be applied to Gott's (1957) data. Data from Hoogendoorn (1985), Griffiths and Lyndon (1985), and Miao et al. (1992) also tend to confirm that the number of leaves emerging after vernalization insensitivity under long day conditions is about six.

It is interesting to note that after the onset of vernalization insensitivity, plants will emerge six more leaves, which is also the number of leaves either postulated or observed to be the minimum number of leaves possible in wheat (Purvis, 1934; Aitken, 1966; Miao et al., 1992; Brooking et al., 1995), and the number of leaves emerged in spring for most normal fall-sowing wheats in commercial production. Minimum leaf number is probably related to the number of internodes that elongate in wheat. This value stays very constant at five (only a few could be four or six), irrespective of the number of leaf nodes actually present on the stem. Final leaf number can be reduced by the vernalization treatment. However, in order to construct a stem with four to six internodes, the plant must develop at least that many leaves.

It is no question that photoperiod has an effect on the number of leaves emerging after the onset of vernalization insensitivity (Wang et al., 1995c; Brooking et al., 1995). In the data of Levy and Peterson (1972), the average final leaf number of the winter wheat Triumph given a 56 days of vernalization treatment changed from 7.0 to 13.7 when the post-vernalization photoperiod was decreased from 17- to 9-h. The similar results were reported by Miao et al. (1992). Anyhow, the number of leaves emerging between the onset of vernalization insensitivity and flowering is stable for a given cultivar grown in constant post-vernalization conditions. When the photoperiod used in post-vernalization is long enough, the number of leaves emerging after the onset of vernalization sensitivity will be minimized, i.e., six.

The concepts based on the above discussion are presented in Fig. 4. Under long day conditions, the number of leaves below the sixth leaf from the flag leaf is determined by a cultivar's vernalization responsiveness. That value can be as low as 0 (i.e., the cultivar which does not respond to vernalization at all and has a final leaf number of six), or as large as more than 15 (i.e., the cultivar which is very sensitive to vernalization treatment and has a final leaf number of great than 21). Although the photoperiod effect will not be discussed in detail in this paper, plant is very probably sensitive to photoperiod until the fourth leaf stage that is counted from the flag leaf, because final leaf number is determined at about the leaf stage of the fourth leaf from the flag leaf (Aitken, 1974; Zhang et al., 1986).

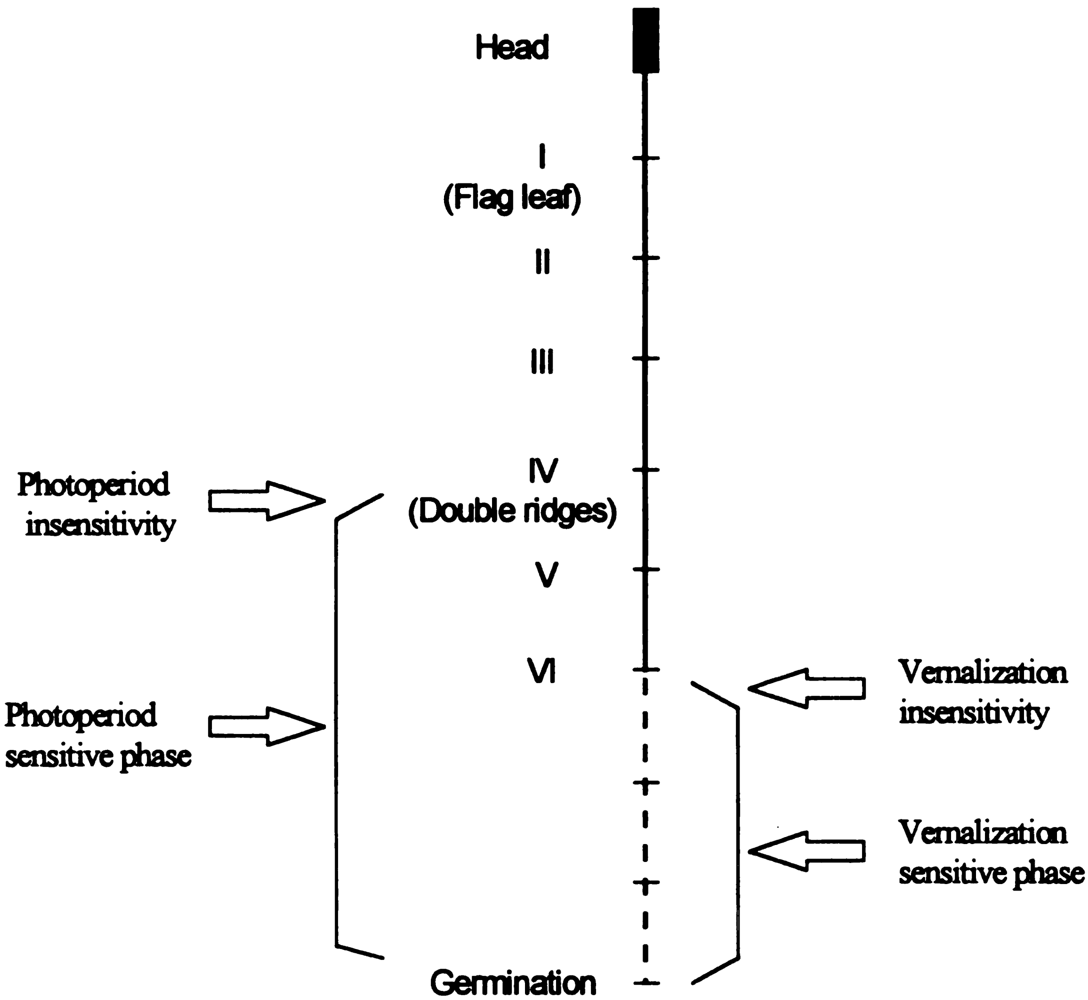

Fig. 4. Schematic representation of the effects of vernalization and photoperiod on the number of leaves in wheat. The capital Roman numerals represent the ordinal leaf number from the flag leaf.

7. Interchangeability between plant age and vernalization duration

Wheat does not have an absolute cold requirement for flowering. Numerous reports demonstrated that all tested wheats, including those adapted to fall sowing system at high latitudes, will eventually flower even without exposure to low temperatures (Purvis, 1961; Pauli et al., 1962; Ahrens and Loomis, 1963; Chujo, 1966; Martinic, 1973; Gotoh, 1976; Ledent, 1980; Rahman, 1980; Miao et al., 1992; Wang et al., 1995a, b). It is also clear that there is not a so-called juvenile stage in wheat in terms of vernalization response. The developing grain, even still attached on the mother ear, or embryogenic callus from immature embryos, can respond to vernalization (Purvis, 1961; Pugsley and Warrington, 1979; Hoogendoorn, 1984; Sharma and Mascia, 1987; Whelan and Schaalje, 1992). The vernalization response of the developing grain, anyhow, is unlikely to be of major significance in most commercial wheat growing areas (Hay and Kirby, 1991). In the conventional vernalization studies, imbibed seeds or young seedlings are usually treated with low temperature in refrigerator or growth chamber for a certain number of days, and then transferred to a high temperature environment. Several reports noted that the young seedlings are more sensitive to vernalization treatments (Gotoh, 1976; Salisbury et al., 1979). Most fall-sowing wheats are subject to low temperatures as seedlings during late fall, winter, and even early spring. Therefore, understanding vernalization response of the growing plant is of great importance.

Wheat plant loses its sensitivity to vernalization as it grows older (Ahrens and Loomis,1963). The upper age limit for vernalization response varies with cultivars.

The cultivar "Winter Minflor" was responsive to vernalization at any stage from just-germinated seed up to 42 days' old plant with six to seven leaves on the main shoot (Gott, 1957). Another winter cultivar "Norin No. 27" had no vernalization response in the plants aged 90 days (Chujo, 1966). Spring wheat "Pitic 62" responded to vernalization only at ages less than 14 days (Jedel et al., 1986).

With a rich array of treatment combinations of plant age and vernalization duration, Wang et al. (1995a) demonstrated that the vernalization effect in terms of the change of final leaf number on the main stem decreased linearly as the plant age at the onset of vernalization increased. The age effect of vernalization response was quantified as follows:

$$(F_0 - 6) = L_i + \beta T_v \qquad (F_0 > 6) \qquad (1)$$

where F_0 is final leaf number with no vernalization, L_i is the leaf stage at the onset of vernalization insensitivity, T_v is the days of vernalization treatment, and β is the absolute value of the slope in the linear regression between average values of final leaf number minus six and days of vernalization. The β represents the "exchange rate" between leaf numbers and vernalization days.

Eq. 1 indicates that a plant becomes vernalization insensitivity when the sum of the current leaf stage and the leaf equivalents gained by vernalization days (i.e., the product of the days of vernalization and the leaf number/vernalization days exchange rate) is equal to final leaf number of unvernalized plants minus six. In other words, vernalization can substitute for plant age as a determinant of time of flowering. Chujo (1966) found that the vernalization effect is large and vernalization response is rapid when the growth of plants is possible during the vernalization treatment. The weaker the vernalization responsiveness of a cultivar, the higher the vernalizing temperature necessary for maximum rate of vernalization response (Flood and Halloran, 1986). That is because the spring wheat in general has a small "exchange rate" (Wang et al. 1995b).

8. A novel conceptual framework

It is a well-established concept that the base temperature for wheat, at least at wheat's early developmental stage, is 0°C (Ritchie, 1991; Kirby, 1992). Any vernalization treatment which is above the base temperature will allow plant to accumulate vernalization response and thermal time simultaneously. The higher the temperature used in vernalization treatment, the higher the rate of primodium initiation and leaf emergence during that period. The interchangeability between plant age and vernalization duration indicates that the effect of vernalization days can be expressed as the leaf equivalents gained by vernalization days. Another important assumption is that plant has a constant number of leaves, which is six, to emerge after the onset of vernalization insensitivity. Therefore, it is possible to estimate the general thermal response and vernalization response through counting the change of leaf number.

As mentioned above, plants at and after the stage where additional vernalization does not reduce final leaf number are vernalization insensitive. The quantitative features of vernalization response, up to the point of insensitivity, can be characterized with a linear regression (Wang et al., 1995b):

$$(F_l - 6) = \alpha - \beta T_v \qquad (F_l \geq 6) \qquad (2)$$

where F_l is final leaf number observed for a particular vernalization treatment, α is the Y-intercept and is an estimate of average final leaf number of unvernalized plants (F_0) minus six, and β and T_v are the same as described for Eq. 1.

The fundamental concept essential to generalize this linear regression equation as a novel development model for wheat vernalization response is to consider the number of leaves produced before, during, and after vernalization treatment, rather than only final leaf number, or calendar days (or thermal time) after the end of vernalization treatment. Eq. 2 is further described as:

$$((F_b + F_d + F_a) - 6) = \alpha - \beta T_v \qquad (F_b + F_d + F_a \geq 6) \qquad (3)$$

where F_b, F_d, and F_a is the number of leaves emerged before, during, and after vernalization treatment, respectively. If the same high temperature (e.g., 20°C) is used before and after vernalization treatment, the relationship represented in Eq. 2 or Eq. 3 is not affected by the temperatures applied at vernalization treatment, or by the plant age expressed as leaf stage at the onset of vernalization treatment. Plants perceive leaf number (F_l) (via thermal time and phyllochron) and calendar day (T_v) (via clock time) as its physiological time. The state of vernalization insensitivity is reached at $F_l \geq \alpha$, $T_v \geq (\alpha/\beta)$, or $(F_l + \beta T_v) \geq \alpha$.

The parameters α and β varied among cultivars (Fig. 5), and are useful for quantifying vernalization response in wheat. The implication of each parameter can be interpreted biologically: α is the "changeable number of leaves", i.e., how many leaves can be potentially decreased by vernalization treatment; and β represents the "exchange rate" between leaf numbers and vernalization days, i.e., how many leaves can be reduced by one day of vernalization treatment. The values of α and β in general are lower in so-called spring wheat than in so-called winter wheat. However, α and β appear to vary independently. For instance, the winter wheat FL 303 has the same α value as the spring wheat Pitic 62 (Fig. 5).

Eq. 3 reveals that plants can be induced at an early leaf stage because of lower temperatures. A logical corollary of that view is that there are no so-called operative temperatures for wheat vernalization. The ability of unvernalized winter wheats to flower under a high temperature (e.g., 20°C) environment is evidence that plant is able to be induced at high temperature. However, different temperatures result in different final leaf numbers for the same cultivar, and different cultivars have different temperature responses. For instance, Chujo (1966) grew four winter wheats under constant temperature of 15 and 20°C, and all plants headed, but plants from 20°C environment had a greater final leaf number than that from 15°C. Under higher temperature condition in the field, plants which respond to vernalization produced more leaves and had a higher total number of leaves at heading (Ford et al., 1981; Midmore et al., 1982).

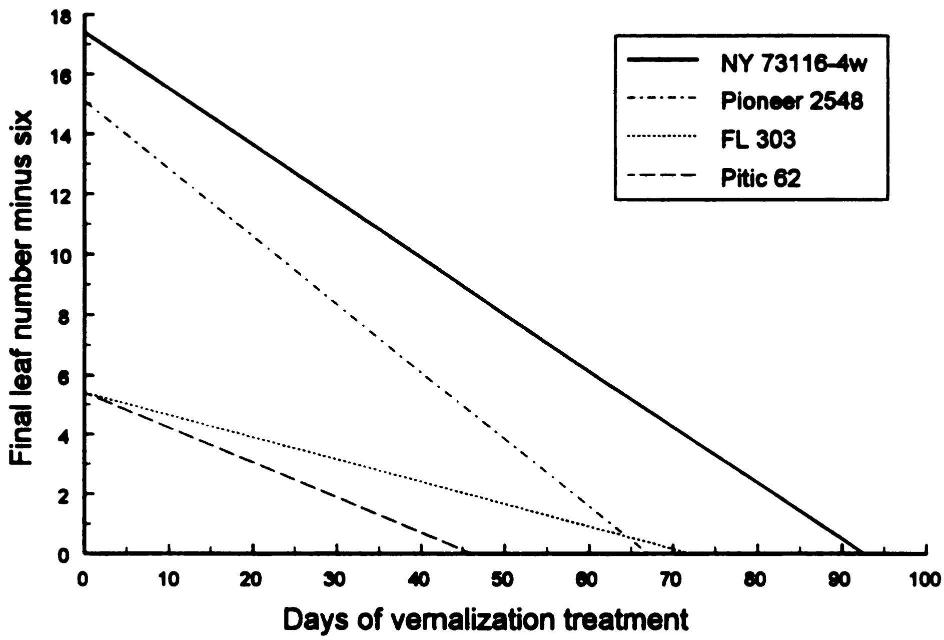

Fig. 5. Vernalization response quantified with a linear regression: $(F_l - 6) = \alpha - \beta T_v$ for different cultivars. The parameters are as follows: $\alpha = 5.4$, $\beta = 0.1174$ for Pitic 62; $\alpha = 5.4$, $\beta = 0.0750$ for FL 303; $\alpha = 15.1$, $\beta = 0.2240$ for Pioneer 2548; $\alpha = 17.4$, $\beta = 0.1880$ for NY 73116-4w. Data taken from Wang et al. (1995b).

Our concept for wheat vernalization is that in wheat life cycle, there is a special physiological phase that low temperature can reduce the number of leaves emerging during that period and result in a low final leaf number. A plant's response to temperature during that phase is its vernalization response. This concept clarifies that temperature as an unity affects both growth and development.

Vernalization response of wheat can be experimentally quantified by using the response parameters, α and β, derived from Eq. 2 or Eq. 3. Two key premises underlying a simple scheme to get the parameters in a comprehensive screening protocol suitable for all wheats are: 1) the same temperature of about 20°C should be used in pre- and post-vernalization growth, because 20°C is the possible high temperature encountered before the onset of vernalization insensitivity in the field for normal fall-sowing wheats; 2) long photoperiods should be applied.

A simplified model relating leaf number and vernalization responsiveness for wheat can be constructed as Fig 6. Line AD is a cultivar's vernalization response curve determined by Eq. 2 or Eq. 3. Point A, B, C or D represents vernalization insensitivity under different temperature regimes. The slope of the dotted line increases as temperature increases. Plant becomes vernalization insensitive after $T_v \geq (\alpha / \beta)$ days at temperature about 0°C (point A in Fig. 6). At that special case, plants of vernalization-sensitive wheats will have a final leaf number of six.

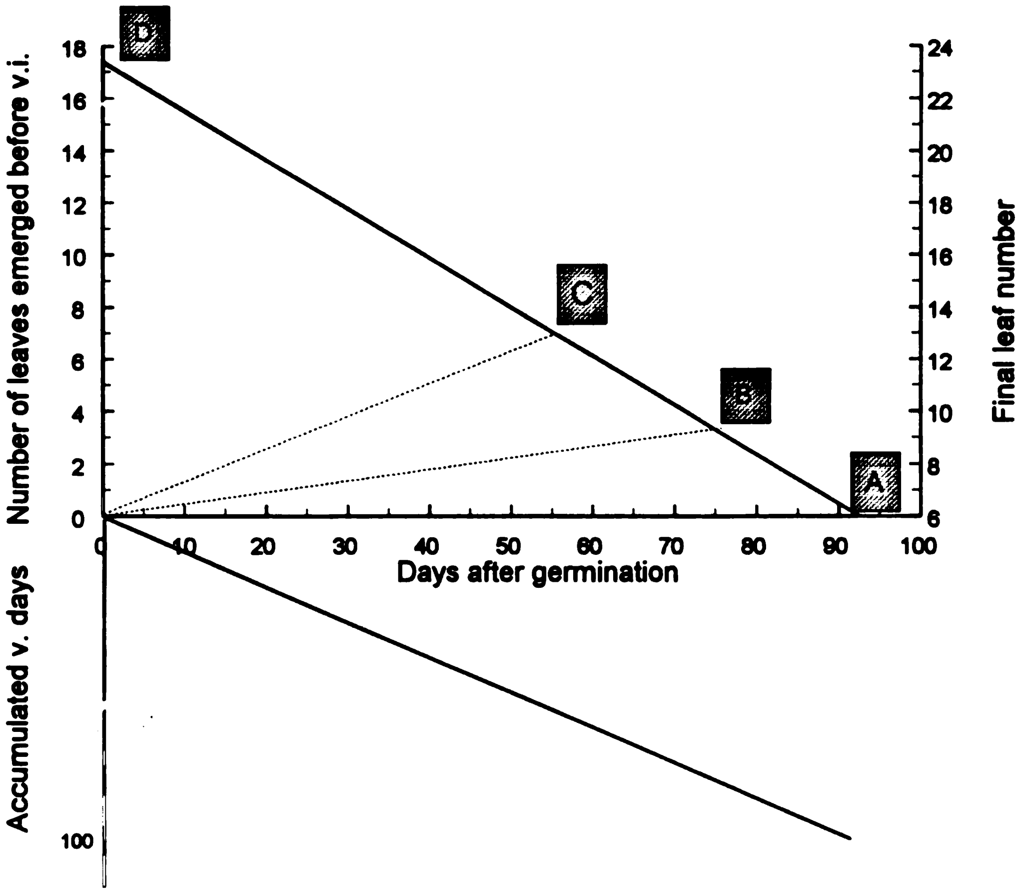

Fig. 6. Model of relation between leaf number and vernalization responsiveness. The vernalization response line (line AD) used as an example is: $F_i - 6 = 17.4 - 0.188T_v$, i.e., NY 73116-4w in Fig. 5. Point A, B, C or D represents vernalization insensitivity under different temperature regimes. The abbreviation "v." stands for "vernalization" and "i." for "insensitivity".

If either F_l or T_v can make plant insensitive to vernalization, the calendar days from seed germination to flag leaf unfolding should be very close between vernalized and unvernalized plants if days of vernalization treatment is taken into account. However, many experiments with winter wheats did not come to that conclusion. The winter wheat Pioneer 2548 is used here as an example (unpublished date from authors). The calendar days from seed germination to flag leaf unfolding were 102 days for plants which experienced 70 days cold treatment (5/3°C, 8/16-h) started from seed germination and then transferred to a high temperature condition (20°C, 20/4-h), and 147 days for plants which were grown under the high temperature condition (20°C, 20/4-h) with no pre-treatment of low temperature.

The apparent conflict is resolved, however, by taking the difference of phyllochron into account. Although considerable debate exists on how constant the phyllochron is among leaves during a growing season, there does seem to be variation among leaves (McMaster and Wilhelm, 1995). Constant phyllochrons can only occur if the rate of extension of each subsequent leaf increases enough to counterbalance the increasing distance each leaf primordia has to cover from apex, where it is initiated, to the point of emergence. Otherwise, there will be a constant decline in the rate of appearance of subsequent leaves (Miglietta, 1991). The increase of phyllochron for subsequent leaves may be not obvious in the field or in the controlled condition where the vernalized plant is used, so that a constant phyllochron is observed.

However, the difference of phyllochron between early emerging and late emerging leaves in a plant of unvernalized winter wheat which has a final leaf number of 20 or more could be significant. In the experiment mentioned above, plants vernalized for 70 days had a final leaf number of eight, and unvernalized plants had 21. That is one of the reasons why flowering of winter wheat can be promoted by low temperature applied in the vernalization sensitive phase.

9. Concluding remarks

The conceptual framework proposed in this paper provides some new clues for understanding vernalization response in wheat. However, some well designed experiments are needed to further develop a general vernalization model. An important element of the present model is the concept that plant will emerge six new leaves after the onset of vernalization insensitivity, but more than six leaves might be emerged under shorter photoperiod conditions. The interactions between temperature and photoperiod before and after vernalization insensitivity need to be clarified. Temperature ranged from 0 to 20°C is used as the effective temperature for wheat development in this paper. A more accurate temperature function should be worked out. It would be interested to look at apex condition in relation to final leaf number minus six. Comparative biochemical and ultrastructural characterization of vernalization sensitive and insensitive apexes with varying numbers of total primordia could lead to a deeper understanding of the underlying processes leading up tu floral initiation.

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