This is to certify that the

dissertation entitled

THE INHERITANCE OF BOLT RESISTANCE IN AN INTERSPECIFIC CROSS SIBERIAN KALE (BRASSICA NAPUS) X CHINESE CABBAGE (B. CAMPESTRIS L. SSP. PEKINENSIS) AND AN INTRASPECIFIC CROSS CHINESE CABBAGE x TURNIP (B. CAMPESTRIS L. SSP. RAPIFERA).

presented by

Carl E. Mero

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Horticulture

Shigemi Honma
Major professor

Date Feb 2 1983

THE INHERITANCE OF BOLT RESISTANCE IN AN INTERSPECIFIC CROSS SIBERIAN KALE (*BRASSICA NAPUS*) X CHINESE CABBAGE (*B. CAMPESTRIS* L. SSP. *PEKINENSIS*) AND AN INTRA-SPECIFIC CROSS CHINESE CABBAGE X TURNIP (*B. CAMPESTRIS* L. SSP. *RAPIFERA*)

By

Carl E. Mero

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1983

ABSTRACT

THE INHERITANCE OF BOLT RESISTANCE IN AN INTERSPECIFIC CROSS SIBERIAN KALE (BRASSICA NAPUS) X CHINESE CABBAGE (B. CAMPESTRIS L. SSP. PEKINENSIS) AND AN INTRA-SPECIFIC CROSS CHINESE CABBAGE X TURNIP (B. CAMPESTRIS L. SPP. RAPIFERA)

By

Carl E. Mero

The inheritance of bolting in Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) was investigated by hybridizing Chinese cabbage with Siberian kale (*B. napus*), Chikale (*B. campestris* L. ssp. *pekinensis* x *B. napus*), and turnip (*B. campestris* L. ssp. *rapifera*). The inheritance model was developed from ratios observed in segregating populations from these crosses after various durations of vernalization at 5°C and 16 hour daylength. Percent of bolters was determined by the sum of visible bolters and longitudinally cut plants with pointed apices, observed when the bolt-resistant parent either visibly bolted or reached a marketable size.

Differences in bolting between Chinese cabbage, Chikale, Siberaian kale, and an F_2 population (Siberian kale x Chee Hoo) under natural field vernalization suggested (1) differences in bolting habit observed after artificial vernalization at a constant temperature (5°C) were similar to those under natural vernalization with fluctuating temperatures, and (2) bolt resistance from

Siberian kale was transferred to Chikale and the F_2 population.

Segregation for bolting response in the progeny from the crosses Siberian kale x Chee Hoo, Siberian kale x Nozaki Early, and Madarin x Siberian kale suggested that bolting response was conditioned by a few major additive genes and that percent of bolters was dependent on the Chinese cabbage cultivar used. Differences in chromosome number between Chinese cabbage (n=10) and Siberian kale (n=19) produced varying degrees of fertility in the progeny. The observed segregations appear to have resulted from variable ploidy levels, random assortment, and probable crossing over of chromosomes. Segregation in F_3 families from these crosses suggested one or more major additive genes.

Based on these results and the observed segregations for bolting response in the crosses Chinese cabbage x Chikale and Chinese cabbage x turnip, it appears that 4 major genes with modifiers conditioned bolting response. Genotypes for Chinese cabbage, Chikale, Siberain kale, and turnip were proposed. Cytoplasmic effects on the response to vernalization were also noted.

DEDICATION

To my Wife, Debbie,

and My Parents

ACKNOWLEDGMENTS

The author wishes to express his special appreciation to Dr. S. Honma for his guidance and advice during the preparation of this thesis. Special thanks are also extended to Dr. H. Price, Dr. J. Hancock, Dr. W. Adams, Dr. J. Hanover, and Dr. J. Whalton for their recommendations in the preparation of this manuscript.

Special thinks are also extended to Mr. Fred Richy, Mr. Marshal "Lou" Pollack, and Mr. Bill Preist for their help and cooperation in maintaining my field plots.

Appreciation and special thanks are also expressed to my wife, Debbie, and to my parents and family for their immeasurable encouragement and support.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	viii
INTRODUCTION	1
LITERATURE REVIEW	3
Vernalization	3
Interspecific Hybridization	14
MATERIALS AND METHODS	20
Parent Material	20
Chinese Cabbage	20
Kale	20
Chikale	25
Turnip	27
Hybridization	28
Vernalization	30
Determination of Plant Age Effect on Vernalization Response	33
Establishing Bolting Criteria	35
Vernalization Experiments	42
Experiment I: Natural Vernalization of Siberian Kale, Chinese Cabbage, an F_2 Population (Siberian x Chee Hoo) and Chikale	42
Experiment II: Artificial Vernalization Studies Siberian Kale x Chinese Cabbage	43
Experiment III: Artificial Vernalization Studies of Siberian x Nozaki Early F_3 Families	44
Experiment IV: Artificial Vernalization Studies of Chinese Cabbage x Turnip	44
Experiment V: Artificial Vernalization Studies of Chinese Cabbage x Turnip	45

Page

RESULTS AND DISCUSSION 46

Experiment I: Natural Vernalization Studies of Siberian Kale, Chinese Cabbage, an F_2 Population (Siberian x Chee Hoo) and Chikale 46
Experiment II: Artificial Vernalization Studies of Siberian Kale x Chinese Cabbage 51
Experiment III: Artificial Vernalization Studies of Siberian x Nozaki Early F_3 Families 64
Experiment IV: Artificial Vernalization of Chikale 67
Chikale LB-7 x Chikale QB-2 68
Chikale LB-7 x Wong Bok 73
Wong Bok x Chikale QB-2 74
Experiment V: Artificial Vernalization of Chinese Cabbage x Turnip Populations 80

SUMMARY AND CONCLUSIONS 85

BIBLIOGRAPHY 89

LIST OF TABLES

Table		Page
1.	Effect of plant size and duration of exposure to 5°C on the percent bolting in Siberian kale	36
2.	Percentage of bolted plants at weekly intervals after vernalization	42
3.	Percentage of bolters under natural field conditions .	47
4.	Bolting percentages after 5 durations of vernalization for the various populations for the interspecific cross Siberian kale x Chee Hoo	52
5.	Bolting percentages after five duration of vernalization for the various populations from the interspecific cross Siberian kale x Nozaki Early	54
6.	Bolting percentages after five durations of vernalization for the various populations from the interspecific cross Mandarin x Siberian kale	56
7.	Percent bolters after five durations of vernalization in two F_3 families from the cross Siberian kale x Chee Hoo	63
8.	Percent bolters after three durations of vernalization of F_3 families from the cross Siberian x Nozaki Early	65
9.	Chi-square test for goodness of fit to a single additive gene model for bolting after three durations of vernalization of F_3 families from the cross Siberian x Nozaki Early	66
10.	Bolting percentages after five durations of vernalization for the various populations from the cross LB-7 x QB-2	69
11.	Chi-square tests for goodness of fit to a two additive gene model for bolting after four durations of vernalization for the cross LB-7 x QB-2	72

Table		Page

12. Percentage of bolters after five durations of vernalization in the various populations from the cross LB-7 x Wong Bok 73

13. Chi-square tests for goodness of fit to a one additive gene model for bolting after 3 durations of vernalization for the cross LB-7 x Wong Bok . . . 75

14. Percentage of bolters after five durations of vernalization in the various populations from the cross Wong Bok x QB-2 76

15. Chi-square tests for goodness of fit to a one additive gene model for bolting after three durations of vernalization for the cross Wong Bok x QB-2 . . 77

16. Proposed genotypes and phenotypes for bolting response produced from the cross Siberian kale x Chinese cabbage 80

17. Percentage of bolters after four durations of vernalization in the various populations from the cross Mandarin x Milan White 81

18. Chi-square tests for goodness of fit to a two additive gene model for bolting after four durations of vernalization for the cross Mandarin x Milan White . 84

LIST OF FIGURES

Figure | Page

1. Leaves of Chinese cabbage, hybrid, and Siberian kale; Seedling of Siberian kale, hybrid, and Chinese cabbage; Inflorescence of Chinese cabbage, hybrid, and Siberian kale 21

2. Metaphase I in Pollen Mother Cells of Siberian Kale, Chinese Cabbage, the F_1 Hybrid, Siberian Kale x Chee Hoo and Chikale 23

3. Leaf and taproots of Chinese cabbage, hybrid, and turnip 31

4. Stages in seedstalk development 40

5. Minimum-Maximum air temperature chart for the period May 4 to June 21, 1982 48

INTRODUCTION

Exposure for a period of low temperature is necessary for flower initiation in many biennial species while it hastens flowering in some annuals. This phenomenon, vernalization, is defined as the change from vegetative growth to reproductive growth in response to low temperatures. Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) develops flowering shoots prematurely (bolting) if exposed to low temperatures, thereby reducing the market value. The degree of bolting increases with duration of the cold period (temperatures below 13°C) and flower stalk elongation is hastened by subsequent warm temperatures and long days. Some species of crop plants are responsive to vernalizing temperatures only after a juvenile phase, however, the Chinese cabbage is sensitive with the onset of germination.

Several cultural practices have been developed to reduce the losses from bolting of spring grown crops. The use of protective row coverings is a common practice in early season celery production, but this is expensive and unsatisfactory during prolonged cold periods. Adjusting planting dates and the use of larger transplants can also reduce losses to bolting. However, a more satisfactory solution to this problem is through the use of bolt resistant cultivars. In China, leafy nonheading Chinese cabbage cultivars which have some bolt-resistance are grown in the spring season. Evaluation of Chinese cabbage cultivars for bolting under natural field conditions showed varying degrees of bolting but none of the nappa cultivars had the desired level of resistance.

The genus Brassica encompasses very diverse species which vary in their response to vernalizing temperatures. Interspecific crossing between Brassica spp. has been used to introgress genes for disease resistance, incompatibility, as well as genes for quantitative traits such as yield. Therefore, it was hypothesized that genes for bolt resistance could be transferred to Chinese cabbage from species with greater vernalization requirments. An F_3 population from a cross between Siberian kale and Chinese cabbage was screened at 10°C for 3 weeks and differences in susceptibility to bolting were observed. Selections were made for quick and slow bolting types for use in inheritance studies for bolting. This material was also hybridized with Chinese cabbage. Additional interspecific crosses were attempted between Chinese cabbage and kale, as well as an intraspecific cross between Chinese cabbage and turnip. The purpose of this study was to determine the inheritance of vernalization response in crosses between (1) lines developed from an interspecific cross beween Siberian kale and Chinese cabbage, (2) Siberian kale and Chinese cabbage, (3) Chinese cabbage and turnip, and to develop a breeding system for obtaining bolt resistance Chinese cabbage cultivars.

LITERATURE REVIEW

Vernalization

Several reviews and books on the subject of vernalization and flower induction have been published (Curtis and Clark, 1956; Chouard, 1960; Lang, 1965; Wellensiek, 1965; Purvis, 1966; Chailakhyan, 1968; Street and Opik, 1973). Vernalization is commonly defined as the change from vegetative to reproductive growth in response to low temperatures. This definition will be used in this study. Gassner, in 1918, was the first to investigate the promotive effect of low temperatures on flowering (Purvis, 1966). He observed a definite relationship between duration of exposure to low temperatures and flowering in certain winter cereals. Purvis and Gregory (1937) determined that Petkus rye could be induced to flower by exposing ripening seeds to low temperatures, however, crops differ in the stage of development at which they become responsive to vernalization. Boswell (1929) observed that cabbage plants were responsive to low temperatures only after they had reached a certain minimum weight or size. According to Wellensiek (1965), many biennials have a juvenile stage during which they are not responsive to vernalizing temperatures. Chouard (1960) suggested that generally sensitivity to low temperatures increased with age.

In most cold-requiring plants the receptive site for vernalization is the apical meristem. Curtis and Chang (1930) demonstrated that providing warm temperatures to the shoot tips of young celery plants during vernalization inhibited flowering. Purvis (1940) excised the shoot region of Petkus winter rye embryos and observed that if proper nutrients were supplied for growth, these excised apices could be vernalized. Other portions of the plant (leaves, endosperm, etc.) are important as a source of nutrients needed in the vernalization process (Lang, 1965).

Vernalization is unusual among biological processes in that it is favored by low temperatures. The low temperature effect on flower formation in cold requiring plants is quantitative in that longer duration and lower temperature act to accelerate the flowering response. Purvis and Gregory (1937) observed that in winter rye the days to flowering were shortened by increasing the number of days at the low temperature and by lowering the treatment temperature. A similar situation has been observed in table beets (Chroboczek, 1931), and in all other plants that have been investigated in this respect (Lang, 1965). Thus the concept of a temperature optimum for vernalization appears to be relative to duration, degree, and plant age. Lang (1965) suggested that as long as the vernalization process is progressing, it progresses in the same manner only at different rates, resulting in the same maximal level of induction if enough time is allowed.

The effect of low temperature on flowering is reversible by high temperatures under certain circumstances. This phenomenon has been called "devernalization" and has been observed in many cold requiring plants including sugarbeets (Stout, 1946), table beets (Chroboczek, 1931), celery (Thompson, 1034) and others (Lang, 1965). The range of inductive and inhibitory temperatures can be very close to one another. Temperatures at which neither induction or inhibition occurs are called "neutral" temperatures. Stout (1946) observed that temperatures in the range of 17° - 18°C were neutral while temperatures above 23°C were devernalizing in sugarbeets. Weibe (1973) reported that temperatures above 18°C were inhibitory to bolting while temperatures below 13°C can induce bolting in table beets. The devernalizing temperatures are effective if given during the vernalization period or immediately following. If a period at "neutral" temperatures was given prior to exposure to high temperatures, the high temperatures were no longer inhibitory to bolting. This has been called stabilization of the vernalized state (Lang, 1965). In most plants examined, if thermo-induction has reached its maximal level, devernalization is no longer possible (Lang, 1965).

In some plants a combination of low temperatures and long days favor flowering. Chroboczek (1934) found that low temperature treatment followed by warmer temperatures and long days resulted in rapid flower stalk development in table beets. In spinach, flowering is optimum if long days are provided during or following the low temperature treatment, although prolonged exposure to low temperatures under short days or exposure to long days at intermediate temperatures were also effective in promoting bolting and flowering

(Curtis and Clark, 1956). Purvis (1966) suggest that the vernalization treatment itself does not induce flowering, but rather ensures that the plant is able to respond to optimal conditions of light and temperature.

Varietal differences in vernalization response have been noted in many crop species, however, the number of genetic investigations is limited (Boswell, 1929; Wester and Magruder, 1938; Lorenz, 1946; Moore, 1958; Thomas, 1980). Hall (1928) reported that bolting was not simply inherited and that nonbolting was dominant in sugarbeets, mangolds, and leeks. Van Heel (1927) observed that in sugarbeets bolting was recessive and controlled by a single gene. Sutton (1932) found that bolting in cabbage was conditioned by recessive factors. Several reports on the inheritance of winter and spring types of cereals have also been made (Cooper, 1927; Gaines, 1917; Purvis, 1939; Gotoh, 1979). These reports can be generalized by stating that winter habit is recessive to spring habit and the number of genes segregating, although generally low, depended on the varieties. Gotoh (1980) suggested that differences in degree of cold requirements in winter wheat varieties was conditioned by 2 genes. In celery bolting resistance was quantitative and recessive (Elmsweller, 1934). Honma (1959) developed an artificial cold induction method to establish a cold units system that could be used to separate bolt susceptible and bolt resistant celery lines. Using this method, Bouwkamp and Honma (1970) determined that easy bolting in celery was conditioned by a single dominant gene and that degree of resistance was determined by modifiers. A similar cold unit system was used by Dickson et al. (1961) to evaluate bolting resistance in carrots, however, no genetic study was reported.

Although the vernalization process is generally similar in cold requiring plants, there is variation between and within species for many of the aspects of vernalization. This portion of the literature reveiw will focus on vernalization responses of the Brassica species used in this study: B. oleracea, B. campestris, and B. napus a natural allotetraploid between oleracea and campestris. Brassica oleracea includes many vegetable crops: vars. italica (broccoli); gemmifera (Brussels sprouts); capitata (cabbage); botrytis (cauliflower); acephala (collards and kale); gongylodes (kohlrabi); as does B. campestris ssp.: rapifera (turnips), pekinensis (Chinese cabbage), and chinensis (pak choi). The majority of B. napus forms are used as fodder and oil crops with the exception of rutabaga, which is grown as a vegetable crop.

Shinohara (1959) investigated the variation within and between Brassica species for the phase of development in which vernalization had a role in bolting and flowering. He observed that members of the oleracea spp. were susceptible to vernalization only during the "green plant stage" while species campestris could be seed vernalized. Shinohara also reported that B. napus had the vernalization characteristics of B. campestris. He concluded that Brassica species with the same genomic constitution have similar requirements for vernalization, however, the degree of these requirements varied within species.

Several reports on the response of cabbage to low temperatures have been made (Boswell, 1929; Miller, 1929; Detjen and McCue, 1933; Ito and Saito, 1961; Heide, 1970; and Patil, 1981). Boswell (1929) observed that if fall grown cabbage plants were too large when exposed to low winter temperatures, the plants would form flower stalks instead of heads the following spring. After investigating this phenomenon, Boswell (1929) and Miller (1929) concluded that exposure to low temperatures is a requisite for flowering in cabbage, but only after plants had obtained a certain size. Detjen and McCue (1933) investigated the effect of nutrition, time, and temperature at planting and various growth checks on bolting of cabbage cultivars. They concluded that susceptibility to the various environmental conditions was dependent on hereditary factors which controlled bolting. The optimum plant size (5 to 6 weeks) and temperature (4° - 9°C) for vernalization of cabbage varied with cultivar (Ito and Saito, 1961); however, in general, longer durations (8 weeks) and lower temperatures (4°C) could induce bolting and flowering in younger plants and hastened flowering (Patil, 1981). Temperatures above 18°C could inhibit flowering in cabbage if plants were exposed to these high temperatures during the cold period or immediately afterwards. However, if a cold period of 8 weeks was used, plants were no longer devernalized by exposure to high temperatures (Heide, 1970).

Similar vernalization responses were reported in Brussels Sprouts (Verkerk, 1954; Thomas, 1980). Verkerk (1954) observed that Brussels sprouts seedlings were more responsive to low temperatures after they were 8 weeks old; longer durations of cold exposure could induce flowering in younger plants. The optimum temperature for vernalization of Brussels sprouts appeared to be 4° to 7°C, depending on the cultivar, with longer durations at temperatures up to 14°C producing flowering. Thomas (1980) compared early season and late season cultivars of Brussels sprouts and observed that the early season cultivars bolted and flower more rapidly in response to less low temperature than the late slow growing cultivars.

Information on bolting and flowering in kohlrabi is limited. Marrewijk (1976) observed that kohlrabi plants will flower if exposed to 8 to 10 weeks at 4°C followed by 2 weeks at 10°C.

Moore (1958) observed that collard varieties differed in bolting tolerance to low temperatures under field conditions. Parham and Moore (1959) exposed collard seelings, cultivar Georgia, with 5 mm stem diameters to 5°C for 0, 2, 4, or 6 weeks. No bolting was observed in the 0 or 2 weeks treatments, 50% of the plants bolted at 4 weeks and 100% bolted at 6 weeks. They suggested that 5 weeks at 5°C was the critical temperature and duration for floral development for this cultivar of collards. Cheng and Moore (1968) investigated the relationship between seeling size and length of cold exposure to flowering in collards, cultivar Vates. Stem diameters were used to group plants into classes then each class was exposed to 5, 6, or 7 weeks at 5°C. Results of this study suggested that plants of Vates must be at least 4 mm stem diameter to be induced to bolt and flower by low temperatures and the period for appearance of the first flower buds was shortened by longer exposure to 5°C. In their study, flowering was considered to be when the first flower bud was visible to the unaided eye and stem diameter was used as an indication of the termination of the juvenile phase (Cheng and Moore, 1968).

As mentioned, the members of B. campestris differ from those of B. oleracea in their vernalization response. Lorenz (1946) studied the effect of low temperatures and daylength on flowering in 3 cultivars of Chinese cabbage: Wong Bok, Pe-tsai, and Chili. Plantings were made during the cool part of the year when as few as 2 true leaves were present which resulted in varying degrees of bolting, depending on the cultivar. He observed that bolting increased in all these Chinese cabbage cultivars when grown at temperatures between 10° - 15°C as compared to 15° - 20°C. Lorenz (1946) suggested that optimum bolting in Chinese cabbage could be obtained by exposing 2 week old plants to 5°C for a period of 2 weeks. Nagagawa and Henmi (1955) and Permadi (1974) induced flowering in Chinese cabbage seedlings by exposing them to 0°C for 32 days immediately following germination. Kagawa (1966) observed that the longer the duration of low temperature, the greater the acceleration of bolting and flowering in Chinese cabbage. Yamazaki (1956) investigated the effect of low temperatures on flower bud formation in Chinese cabbage and developed the following formulae: (13°C - X) Y = 87°C, where X is the temperature below 13°C and Y is the number of days with minimum temperatures below 13°C. When the sum of this equation reached or exceeded 87°C flower induction occurred. Nakamura (1976) suggested that this equation is a general rule and that cultivars differ in the duration of low temperatures required for flower induction. It was suggested that many cultivars could be induced to flower with 30 days with minimum temperatures of 13°C, 15 days with minimum temperatures of 10°C, or 10 days with minimum temperatures of 5°C. Honma (1981) reported that 14 days at 5°C resulted in bolting of standard cultivars of Chinese cabbage and that no known slow bolting heading Chinese cabbage cultivars exist at present. However, in screening heat tolerant Chinese cabbage lines at the Asian Vegetable Research and Development Center (AVRDC, 1979) resistance to bolting was observed when vernalized for 10 to 30 days at 10°C.

In *B. campestris* ssp. *chinensis* cultivar To-pe-tsai, Wang (1969) observed that exposing 12 day old (1 true leaf) plants for 3 days at 4°C induced flower bud development. Insufficient low temperature accumulation or exposure to high temperatures after the cold period produced abnormal flowering in this cultivar (Wang, 1969).

Wester and Magruder (1938) investigated cultivar differences in bolting of turnips. They observed that a period of low temperatures followed by a period of warmer temperatures was required for seed stalk development in turnips, with longer durations of low temperatures reducing time to express bolting. A plant was considered a bolter when the central axis had elongated sufficiently to distinguish internodes. Cultivars differed for number of bolters with some strains bolting prior to reaching marketable size while the majority bolted only after reaching a marketable size (Wester and Magruder, 1938). Sakr (1944) observed that turnips were sensitive to low temperature induced bolting from the early stages of seedling development to the mature plant stage.

The species B. napus also requires cold for flower induction. Peto (1934) reported that exposing rutabaga plants to 12°C for a period of 30 to 50 days following sowing date resulted in bolting approximately 70 days later. It has been reported that B. napus can be seed vernalized (Shinohara, 1959), and Kagawa (1971) reported that the F_1 between cabbage and Chinese cabbage had the Chinese cabbage early flowering habit and could be vernalized during all stages of development. Honma and Heeckt (1960) reported that the F_1 between Siberian kale and Chinese cabbage did not flower when seedlings were vernalized 5 weeks, however, all the hybrids flowered when given an additional 4 weeks of low temperature.

The vernalization process can be considered to include two distinct phases. The first phase in which low temperatures are the critical environmental parameter, involves the transition from vegetative to reproductive development, while the second phase involves the expression of the reproductive phase in the form of flower stalk development and flowering (Curtis and Clark, 1956). Although this study is concerned with the first phase of vernalization, the relationship between the vernalized state and the environment during the second phase of the vernalization process is also important.

Purvis (1966) suggested that daylength and temperature during the post-vernalization period can effect the development of flowers. The majority of plants with a cold requirement flower more rapidly if long days and warm temperatures are present during the time period immediately following vernalization (Lang, 1965). Reports on vernalization of the Brassica species considered in this study are in agreement that long days and warm temperatures tend to hasten flowering of vernalized plants. Heide (1970) observed optimum flowering in cabbage if conditions following vernalization were long days at temperatures between 12° and 18°C. Thomas found a similar response in Brussels sprouts (1980). In Chinese cabbage, long days following the cold period also hasten flowering (Lorenz, 1946, and Kagawa, 1966). Mori et al. (1979) treated 15-day-old Chinese cabbage plants with 2° or 8°C for 5, 10, or 15 days and then placed the plants at 20°C under 8, 12, 16, 20, or 24 hour daylengths. They concluded from this study that longer duration of cold period and longer daylength following vernalization shortened the delay in flowering. A similar effect of long days was observed in B. chinensis (Wang, 1969). Purvis (1966) suggests that the promotive effect of long days on flowering in vernalized plants is quantitative in that with increased photosynthesis as a result of longer daylength allows for a more rapid transition to reproductive development. Presumably the increased temperatures are acting in a similar manner.

Interspecific Hybridization

Karpechenko (1922), Pearson (1928), Morinaga (1929), and U (1935) were responsible for developing the relationships between members of the genus *Brassica*. Cytological observations on chromosome associations during pollen development and success of attempted hybridizations between members of this genus were the basis for the relationships proposed by U (1935) which has become known as the "triangle of U." There are 3 primary species: *B. campestris* (n=10), *a* genome; *B. nigra* (n=8), *b* genome; and *B. oleracea* (n=9), *c* genome. Through natural hybridization between these primary species, 3 secondary species were formed: *B. carinata* (n=17), *bc* genome; *B. juncea* (n=18), *ab* genome; and *B. napus* (n=19), *ac* genome. The basic chromosome number of the genus *Brassica* as determined from chromosome morphology and secondary associations is x = 6 and the differences in chromosome number between primary species resulted from aneuploidy of different chromosomes (Richharia, 1937; Robbelen, 1960; Nwankiti, 1970; Kamala, 1978). Many studies have been reported in which various cross combinations within and between species were attempted. In general, crossing between individuals with a common primary genome resulted in at least partial fertility (Morinaga, 1929). Success in crossing between individuals with different genomic compliments was dependent on the cultivars or forms used, the environment, plant age, and number of pollinations (Yarnell, 1956). Due to the large amount of literature on inter-crossing in the genus *Brassica*, this review will be limited to crosses between *B. oleracea* and *B. campestris*, and between *B. campestris* and *B. napus*.

In 1920, Ragonieri reported on attempts to cross the Chinese cabbage cultivar Pe-tsai (B. campestris ssp. pekinensis) with B. oleracea, "Cows Fodder," B. oleracea var. acephala, green scotch kale, and B. oleracea var. botrytis, cauliflower. Using Chinese cabbage as the female a few seeds were obtained from crosses with Cows Fodder and cauliflower, however, the crosses with kale were unsuccessful. Morinaga (1929) demonstrated that B. napus was a naturally occurring amphidiploid between the a genome (B. campestris) and the c genome (B. oleracea). U (1935) reported success in crossing these two genomes using the B. oleracea vars. gemmifera and acephala. He produced 4 F_1 hybrids which differed from each other in chormosome number, having 19, 28, 29, and 38 chromosomes. The genomic constitutions of these F_1's were ac (sterile), acc and aac (partially fertile) and aacc (fertile) respectively. Frandsen (1947) succeeded in crossing tetraploid forms of B. oleracea var. capitata and B. campestris ssp. rapifera with the resulting F_1 having 2n = 38 chromosomes. Hosoda (1950) excised portions of the style to improve the crossability of B. oleracea var. capitata and B. campestris ssp. pekinensis. The F_1 hybrids were sterile; however, applications of colchicine restored fertility. By crossing B. campestris ssp. rapifera and ssp. oleifera with vars. acephala, capitata, gongylodes, and gemmifera of B. oleracea, Olsson (1960) obtained 16 hybrids. No morphological description was given, but karyological studies revealed that the majority of the F_1's had 29 chromosomes which formed 10 bivalents and 9 univalents during meiosis resulting in partial fertility. With the aid of n-meta-tolyphalamic acid applied to the base of flowers, Honma and Heeckt (1960) obtained F_1 hybrids between B. campestis ssp. pekinensis cv. Mandarin and B. oleracea var. acephala cv. Siberain kale. This interspecific hybrid was partially fertile and was intermediate in morphological characteristics, however, no cytological analysis was made. The F_2 population segregated for both parental types with the majority of the plant characters observed showing continuous variation (Honma and Heeckt, 1960). Nishi et al. (1962) crossed reciprocally, B. oleracea var. capitata and B. campestris ssp. pekinensis and the hybrid embryos were successfully cultured in vitro. In a further study, Nishi et al. (1970) attempted to transfer soft rot resistance from heading cabbage to Chinese cabbage. To develop head forming B. napus cultivars, they crossed B. campestris ssp. pekinensis, chinensis, oleifera, rapifera and narinosa with different varieties of the cabbage group. In most all cases the hybrids were sterile and failed to backcross with common cabbage and Chinese cabbage (Nishi, 1980). Doubling the chromosomes with colchicine restored fertility. The amphidiploid was selfed to develop "Hakuran" a heading B. napus and was backcrossed to Chinese cabbage to transfer soft rot resistance (Nishi, 1980). Sarashima (1964), with the objective of producing forage types, crossed B. campestris ssp. including pekinensis with B. oleracea vars. acephala, capitata, botrytis, italica, and gongylodes. The hybrids were treated with colchicine to restore fertility and selection was made for improved forage types. Namai (1971) grafted

B. campestris ssp. onto B. oleracea var. acephala and used this plant as the female in a cross with B. oleracea var. acephala. The graft combination improved the success of crossing these two species; however, applications of a sugar and agar mixture to the flower bases had no noticeable effect. Inomata (1980) conducted a cytological investigation of interspecific hybrids between B. campestris ssp. rapifera, oleifera, chinensis, and pekinensis with B. oleracea vars. acephala, capitata, gemmifera, gongylodes, and italica obtained by embryo culture. The F_1 hybrids with 19 chromosomes generally had 9 bivalents and 1 univalent with low fertility while the F_1's with 38 chromosomes had 19 bivalents and were fertile. Harberd and McArthur (1980) observed from 3 to 9 bivalents in an F_1 hybrid between B. campestris and B. oleracea. In a cross between B. campestris ssp. pekinensis cv. Granaat and B. oleracea var. acephala cv. Normal, Balicka et al. (1980) produced 607 F_1 hybrid of which one was an allohaploid, 2n = 19 chromosomes, but it was sterile. In a further study with this cross (Zwierzykowska, 1981) embryo culture was used to rescue the F_1's obtained from several cultivars of pekinensis and acephala. The number of attempts at crossing these two genomes is large and the success appears to be dependent to a large extent on the cultivars used. Tsunoda and Nishi (1968) suggested that the self-incompatibility of the parental strains may influence the production of F_1 embryos and their further growth. Moue (1979) in a series of studies on interspecific crosses between Chinese cabbage and cabbage reported that the endosperms disappeared and the embryo had ceased to develop 20 days after pollination when cabbage was used as the female and 25 days after pollination when Chinese cabbage was the female. These results may explain the difference in success between reciprocal crosses and the effectiveness of embryo culture in rescuing hybrid embryos (Moue, 1979).

Many attempted crosses between B. campestris and B. napus have been reported with variable success (Morinaga, 1929; Frandsen and Winge, 1932; U, 1935; Calder, 1937; Mizushima, 1950; Jahr, 1962; Nwankiti, 1970; Jelinkova, 1971; Lammerink, 1970; McNaughton, 1973; Mackay, 1977; Snell, 1977; Fantes and Mackay, 1979). Morinaga (1929) was the first to report successful crosses between B. campestris ssp. and B. napus. The hybrids obtained from reciprocal crosses had 29 chromosomes (aac) and formed 10 bivalents and 9 univalents during metaphase I of meiosis. The hybrids were partially self- and cross-compatible with both parental species. The distribution of the 9 univalents appeared random and the number of chromosomes in the daughter cells ranged from 10 to 19 with a mode of 14-15. The hybrids (B. napus x B. campestris) obtained by U (1935), Calder (1937), Nwankiti (1970), Mackay, (1977), and Fantes and Mackay (1979) also had 29 chromosomes and were partially fertile. The morphology of the F_1 hybrids was intermediate to the parent species (McNaughton, 1973; and Mizushima, 1950). Frandsen and Winge (1932) crossed B. napus, swede, and B. campestris ssp. rapifera. The selfed progeny from this interspecific hybrid had 2n = 58 chromosomes which they attributed to spontaneous doubling of the gametic chromosome compliment (2n = 29). It was proposed that this auto-allo-hexaploid was a new species which they called _B. napocampestris_. In later studies, Jahr (1962) and Nwankiti (1970) produced _B. napocampestris_ by doubling the chromosome number of the hybrid (_B. napus_ x _B. campestris_) with colchicine. Nwankiti (1970) also backcrossed the hybrid to the _B. campestris_ parent. The chromosome numbers of the hybrid gametes generally were 10, 13, 16, or 19, suggesting nonrandom distribution. They attributed this to bivalent formation between chromosomes of the _c_ genome. The resulting plants from backcrossing the F_1 with _B. campestris_ were aneuploids. Plants which were nearly euploid (2n = 20 or 2n = 38) were fertile and occurred in the highest frequency. The progeny from this backcross with 2n = 20 chromosomes formed 10 bivalents at metaphase I and had high pollen fertility, but poor seed set. Sikka (1940) observed that the _a_ genome of _B. napus_ had chromosomes lacking satellite structures characteristic of the _B. campestris_ _a_ genome. Nwankiti (1970) suggested that this may account for the observed sterility. On further observation of these backcross progeny, Nwankiti (1971) observed that after one generation of selfing, the fertility was restored and the chromosome numbers were stabilized. Fantes and Mackay (1979) also observed a higher than expected number of plants with extreme chromosome numbers (2n = 20 and 2n = 38) when they backcrossed the hybrid (_B. napus_ x _B. campestris_) to the parent species.

MATERIALS AND METHODS

Parent Material

Chinese Cabbage

Chinese cabbage (*B. campestris* L. spp. *pekinensis*) is a head forming annual. The leaves are large with a prominent midrib, light green to yellowish green, soft and veiny with undulating margins (Figure 1). The inflorescence is short with tightly clustered flowers. The following open-pollinated Chinese cabbage nappa cultivars were used in this study: Chee Hoo, a mid-season large headed cultivar; Wong Bok, a late-season standard cultivar; Nozaki Early, an extra-early spring or early summer cultivar; and Mandarin, an early summer, small headed cultivar. The first 3 cultivars were obtained from Takii Seed Co., Kyoto, Japan, while the later was obtained from the National Seed Storage, Fort Collins, Colo. These cultivars were induced to bolt and flower by exposing 2 week old plants (2 true leaves, 2 cm long) at 5°C for 2 weeks. Plants were selfed 4 generations to obtain homozygosity for bolting response. Cytological observations of pollen mother cells of Chinese cabbage showed 10 bivalents at metaphase I (Figure 2).

Kale

The kale cultivar, Siberian, is cold tolerant and long standing. It is a leafy biennial plant having blue green foliage

Figure 1:	Top:	Leaves of Chinese cabbage, hybrid, and Siberian kale (left to right)
	Center:	Seedling of Siberian kale, hybrid, and Chinese cabbage (left to right)
	Bottom:	Inflorescence of Chinese cabbage, hybrid, and Siberian kale (left to right)

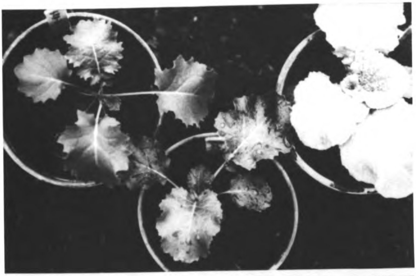
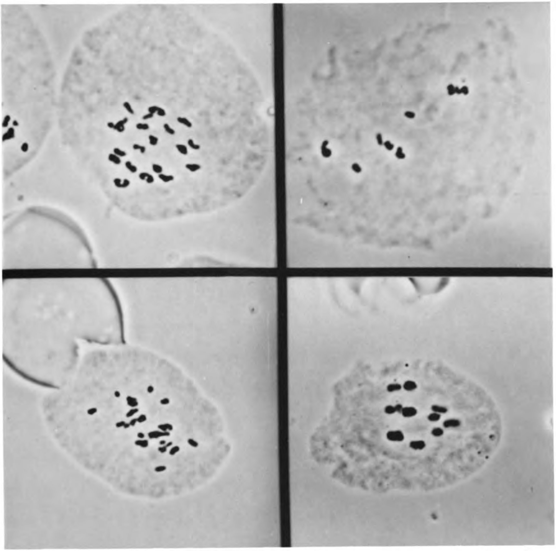


Figure 2: Metaphase I in Pollen Mother Cells of (Clockwise from upper left): Siberian Kale (n=19), Chinese Cabbage (n=10), the F_1 Hybrid, Siberian Kale x Chee Hoo (n=29), and Chikale (n=10).



which is coarse and crinkled with dentate margins (Figure 1). The inflorescence is elongated and the flowers become well spread along the stalk at anthesis. Seeds of Siberian kale were obtained from Harris Seed Co., Rochester, New York. Plants with 6 mm stem diameters were vernalized for 8 weeks at 5°C to promote flowering. After 3 generations of selfing, plants appeared homozygous for bolting response after 8 weeks of vernalization. Individual plants were hybridized with Chinese cabbage.

In 1960 Honma and Heeckt reported that Siberian kale used in their study to be *B. oleracea* var. *acephala*, a diploid (2n=18). These authors did not make a cytological study of Siberian kale and assumed it to have 9 chromosome pairs. Thomas and Crane (1942) reported that most kale strains belong to the *B. oleracea* var. *acephala* species, however, Late Rape kale, Hungary Gap kale, and Asparagus kale have 19 chromosome pairs and belong to the *B. napus* species. These amphidiploid kales are believed to have resulted from hybridization between *B. campestris* ssp. *rapifera*, turnip, (2n=20) and *B. oleracea* var. *acephala*, kale, (2n=18). In 1978, Borchers and Taylor reported that Siberian kale has 19 chromosome pairs which would suggest it to be *B. napus*. Cytological observations of pollen mother cells of Siberian kale showed 19 bivalents at metaphase I which suggest that Siberian kale belongs to *B. napus* (Figure 2)

Chikale

Quick-bolt and late-bolt lines were developed from an F_4 population derived from an interspecific cross between Siberian kale and Chinese cabbage (cv. Mandarin) made by Honma and Heeckt (1960). Although they reported that Siberian kale used in their study was B. oleracea var. acephala, it is possible that the Siberian kale strain used in their study was also B. napus. Cytological observations of the late-bolt segregant used in this study showed 10 bivalents (Figure 2) at metaphase I of pollen mother cells suggesting this Chikale line had 20 chromosome pairs.

The lines developed from this F_4 population will be referred to as Chikale (CK) lines. This F_4 population consisted of 9 sister lines which had not been screened for bolting response, but were selected for phenotypic similarity to Chinese cabbage. These lines were transplanted into a greenhouse ground bed in January, 1979. The temperature for the first 3 weeks following transplanting was 12°C at night and 17°C during the day. After 3 weeks, the temperature was raised to 20°C and observations were made on the time of bolting. Bolting was determined by the visible flower buds at the apex of the plant. The lines segregated for time of bolting with the majority of the lines bolting within 50 days after the temperature was raised to 20°C. Line CK-2 was the first to bolt and line CK-7 failed to bolt. Selections were made from these 2 lines for quick- and late-bolting types. The nonbolted plants were vernalized for 6 weeks at 5°C to induce flowering and were then self-pollinated. Progeny from these selections were vernalized for 2 weeks at 5°C when the second true leaves were 2 cm long. Lighting was provided by banks of 40 watt cool white fluorescent lamps (approximately 20 W m^{-2}) for 16 hours each day. The vernalized plants were transplanted into a greenhouse ground bed with a minimum night temperature of 20°C. The quick-bolt selections bolted by the 30th day after transplanting, however, none of the late-bolt selections bolted. Selections were again made for quick- and late-bolting types and the selfed progeny from these selections were screened for bolting response at the second true leaf stage at 5°C for 0, 1, 2, 3, 4, and 5 weeks. Staggered plantings were made so that all treatments were removed from the vernalization room on the same date. The treated plants were placed into a lath house for 1 week to stabilize vernalization prior to transplanting into the field. The quick-bolt selections appeared homozygous for bolting response and bolted in every treatment except the control (0 weeks). The late-bolt selections were also homozygous for bolting response and bolted after 5 weeks of vernalization. This experiment was repeated in the greenhouse during the fall (1980) and similar observations were noted. Individual plants selected from the field experiment were used in this study and will be referred to as QB-2 (quick-bolt) and LB-7 (late-bolt).

Turnip

The turnip (*B. campestris* L. ssp. *rapifera*) cultivar, Milan White, was obtained from Mikado Seed Co., Chiba-City, Japan. The root of Milan White is semi-globe shaped, medium sized, and white. The leaves are green, narrow, and coarse with entire margins. Vernalization was accomplished by exposing 2 week old plants to 5°C for 6 weeks. After 3 generations of selfing, individuals which were phenotypically homozygous for bolting response were hybridized with Chinese cabbage.

Hybridization

Inheritance studies on bolting in the interspecific cross, Siberian kale x Chinese cabbage were made from 2 separate hybridizations. One study involved Siberian kale and Chinese cabbage while the other study involved Chinese cabbage and Chikale. The Siberian kale x Chinese cabbage study included the following unilateral crosses:

Siberian x Chee Hoo

Siberian x Nozaki Early

Mandarin x Siberian

Approimately 500 recirocal pollinations between Siberian and 3 Chinese cabbage cultivars failed to produce viable seeds, suggesting interspecific incompatibility. Therefore, the flower setting hormone, n-meta tolyphalamic acid at 20 ppm was used to circumvent the incompatibility (Honma and Heeckt, 1960). A small piece of cotton was attached to the base of individual flowers and was soaked periodically with this hormone solution until the flowers abscissed or pods began to develop. Results of unilateral crosses produced the following number of viable seeds: 110 seeds of Siberain x Chee Hoo, 4 seeks of Siberian x Nozaki Early, and 2 seeds of Mandarin x Siberian. The F_1 hybrids were intermediate to the parents for leaf shape and leaf color (Figure 1). All of the F_1 plants appeared phenotypically similar regardless of the Chinese cabbage cultivar used as the parent. The F_1 hybrids with 6 mm stem diameters were vernalized for 8 weeks at 5°C. The inflorescence of the hybrid was also intermediate to the parents (Figure 1) for flower color, flower size, and flower spacing. The length of time for the appearance of flower buds after 8 weeks of vernalization was less for the hybrids (30 days) than for Siberian (50 days). The F_1 hybrids showed varying degrees of fertility and cross compatibility with Siberian and Chinese cabbage, although the anthers appeared plump and shed abundant pollen. Cytological observations of the hybrids were made by staining pollen mother cells with lacto-propionic orcein. Pollen viability was estimated by examining 500 pollen grains from each hybrid by staining with acetic carmine.

Three methods were used to obtain F_2 and backcross seeds: (A) the use of n-meta-tolyphalamic acid to obtain F_2 and backcross seeds resulted in limited success, (B) F_1 hybrids with similar phenotypes from a specific cross were placed in groups of 3 plants and sib-mated with the aid of bees; and (C) increasing the number of plants asexually of one Siberian x Chee Hoo hybrid which were then pollinated by bees. Due to the high degree of sterility only limited backcrosses were obtained. Two F_3 families from the cross Siberian x Chee Hoo and 6 F_3 families from the cross Siberian x Nozaki Early were obtained by selfing individual F_2 plants.

The second study involved Chikale and Chinese cabbage and included the following reciprocal crosses:

LB-7 x QB-2

LB-7 x Wong Bok

QB-2 x Wong Bok

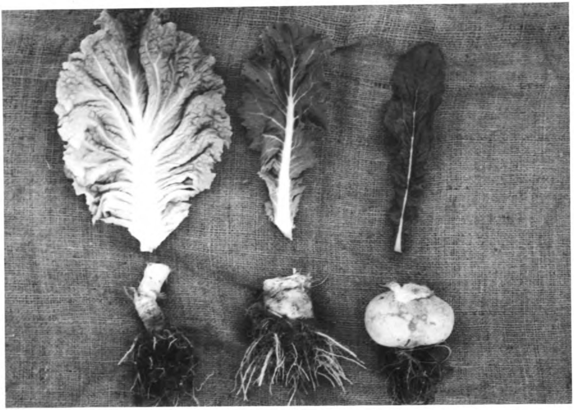
Hybridization was accomplished by bud emasculation followed by brushing pollen from the male parent onto the stigmatic surface of the female parent, then covering with a glassine bag until pods began to develop. All hybridizations were successful and the resulting F_1 hybrids were fertile and backcrossed successfully to both parents. The parents were maintained by leaf cuttings and were used as the female parent in backcrosses with the F_1 hybrids.

An intra-specific cross between Chinese cabbage and turnip was also made to study the inheritance of bolting. The cultivars used were Mandarin (Chinese cabbage) and Milan White (turnip). Hybridization was accomplished by bud pollination and the F_1 hybrid was selfed and backcrossed to Chinese cabbage only since flowering plants of Milan White were not available at the time the F_1 was in bloom. The F_1 was intermediate to the parents for both leaf and root morphology (Figure 3).

Vernalization

All artificial vernalization in this study was conducted in a cold room at a temperature of 5°C ± 1°. The plants were provided with a 16 hour day using banks of fluorescent lamps (approximately 20 W m^{-2}). Except for experiments conducted in the greenhouse, all plants were placed in a lath house immediately following

Figure 3: Leaf and taproots of Chinese cabbage (left), hybrid (center), and turnip (right).



vernalization for 1 week prior to transplanting into the field to prevent devernalization.

A natural field vernalization experiment was conducted in order to compare bolting response under artificial vernalization at a constant temperature with vernalization with fluctuating temperatures. Yamasaki (1956) reported that the critical temperature for vernalization of Chinese cabbage was 13°C and proposed the following formula for predicting flower primorida induction: (13°C - X) Y = 87°C, where X is the temperature below 13°C and Y is the number of days with minimum temperatures below 13°C. When the sum of this equation equals or exceeds 87°C, then flower primordia formation is induced. This formula has proved to be adequate for predicting bolting in Chinese cabbage under artificial vernalization at a constant temperature of 5°C, followed by temperatures above 20°C. It has been observed that the Chinese cabbage cultivars Chee Hoo, Nozaki Early, Mandarin, and Matsushima did not bolt after 1 week, but bolted after 2 weeks at 5°C in the vernalization chamber. According to Yamasaki's formula, the sum after 1 week of vernalization at 5°C is 56°C, which is insufficient to induce bolting, while the sum after 2 weeks is 112°C which exceeded the level necessary to induce bolting.

Determination of Plant Age Effect on Vernalization Response

Chinese cabbage (Nakamura, 1976) and turnip (Sakr, 1944) have been reported to be sensitive to vernalization from seed germination to mature plants. Although no specific information on the effect of plant age on vernalization response was found for Siberian kale (_B_. _napus_), Cheng and Moore (1968) reported that the collard (_B_. _oleracea_ var. _acephala_) cultivar Vates requires stem diameters of at least 4 mm before it can be vernalized by low temperatures. Since _B_. _napus_ is an amphidiploid involving _B_. _oleracea_ and _B_. _campestris_ (Morinaga, 1929), a preliminary investigation was made to determine the effect of plant age (leaf number) on vernalization response for Chikale, Chinese cabbage, turnip, and Siberian kale in order to establish the proper plant age for screening populations for bolting. Plants of each of the parents were vernalized when they were 2 weeks (2 true leaves), 3 weeks (3-4 true leaves), and 4 weeks (5-7 true leaves) old. Chikale, Chinese cabbage, and turnip plants bolted regardless of plant age. None of the 2 week old Siberian plants bolted while only some of the 3 week old Siberian plants bolted, and all of the 4 week old plants bolted.

The effect of plant age or leaf number on vernalization of Siberian kale was further investigated. Plants with 2-3, 4-5, 6-7, and 7-8 true leaves were vernliazed for 5, 6, 7, or 8 weeks. The experimental design was a ramdomized complete block with 4 replicates. Each treatment combination had 16 plants and were transplanted into the field June 30, 1981. The number of bolted plants as determined by the visibility of flower buds at the apex of the plants, was recorded 55 days after removal from the cold room. Analysis of variance showed highly significant effect ($p = .01$) for plant size and number of weeks of vernalization. The interaction between plant size and length of vernalization and blocks effects were not significant ($p = .05$). The results are presented in Table 1. After 5 weeks of vernalization none of the plants bolted regardless of leaf number. The treatment combination, 2-3 true leaves and 6 weeks vernalization, only produced 66% bolters, while all the other treatment combinations produced 100% bolters. These results suggest that Siberian kale with a minimum of 4 true leaves can be induced to bolt after 6 weeks of vernalization. Since the parents Chinese cabbage, Chikale, turnip, and Siberian kale produce 5 to 7 true leaves in 4 weeks when grown in the greenhouse, all vernalization treatments were initiated when the plants were 4 weeks old.

Establishing Bolting Criteria

Nakamura (1976) reported that Chinese cabbage seedlings with 20-25 outer leaves developed prior to vernalization form heads before the flower stalk protrudes from the head and, therefore, are still marketable. Therefore, the length of time for the flower stalk to develop in relation to the heading becomes important. In the process of developing homozygous parent material, a plant was considered to have bolted when flower buds were visible at the apex. It was observed that the length of time between removal from the cold room to the visible appearance of flower buds was shorter for Chinese cabbage than for Siberian kale. This made it difficult to determine the proper time to record the data on bolting for the segregating populations from this interspecific cross.

Table 1. Effect of plant size (leaf number) and duration of exposure to 5°C on the percent bolting in Siberian kale.

Number of Weeks at 5°C	Number of True Leaves	Percent Bolting
5	2-3	0
6	2-3	66
7	2-3	100
8	2-3	100
5	4-5	0
6	4-5	100
7	4-5	100
8	4-5	100
5	6-7	0
6	6-7	100
7	6-7	100
8	6-7	100
5	7-8	0
6	7-8	100
7	7-8	100
8	7-8	100

A preliminary vernalization experiment was conducted in 1981 to observe bolting in response to 0, 1, 2, 3, 4, 5, 6, or 7 weeks of vernalization of Siberian, Chee Hoo, and an F_2 population (Siberian x Chee Hoo). The visibility of flower buds was the criterion used to classify plants as bolters. Data was recorded weekly for 8 weeks following vernalization. Chinese cabbage bolted in all treatments with 2 or more weeks of vernalization. The mean number of days after removal from the cold room to bolting for the cultivar Chee Hoo was significantly ($p = .05$) lower after 6 weeks of vernalization (10 ± .45 days) than after 2 weeks of vernalization (32 ± 1.37 days). Siberian kale bolted after 6 or more weeks of vernalization. The mean number of days after removal from the cold room to bolting of Siberian after 6 weeks of vernalization (26 ± 1.82 days) was significantly greater ($p = .05$) than for Chee Hoo vernalized for 6 weeks (10 ± .45). The lesser number of days to visible flower buds in Chee Hoo after 6 weeks of vernalization as compared to 2 weeks of vernalization suggest that when the threshold or minimum number of hours of thermo-induction is reached, vegetative growth ceases and reproductive growth begins and continues while the plants remain at the vernalizing temperatures. If this assumption is correct, the mean number of days to bolting in Chinese cabbage after 2 weeks of vernalization and the mean number of days to bolting in Siberian after 6 weeks of vernalization should be similar. The mean number of days to bolting in Chee Hoo vernalized for 2 weeks (32 ± 1.37) was not significantly different ($p = .05$) from the mean number of days to bolting in Siberian vernalized for 6 weeks (26 ± 1.82).

None of the F_2 plants bolted after 0, 1, 2, or 3 weeks of vernalization. The percentage of F_2 plants bolting after 4, 5, 6, and 7 weeks of vernalization was 15%, 41%, 48%, and 70% respectively. The absence of bolted F_2 plants in the 2 and 3 week treatments and the presence of some nonbolted plants after 6 and 7 weeks of vernalization suggested transgressive segregation or for a need of a more precise method for determining bolting. In an inheritance study on bolting in celery, Bouwkamp and Honma (1970) made longitudinal cuts through the apex of nonbolted plants to observe the growing point for elongation when plants reached a marketable size. Plants that showed pointed apices were classified as bolters. These authors suggested that this criterion for bolting eliminated the variability for rate of seed stalk development which is dependent on post-vernalization environmental factors.

An experiment was conducted in the greenhouse in 1981 to determine if a similar procedure for determination of bolting would be applicable for this study. Eighty plants of Siberian, Nozaki Early, and 160 F_2 plants (Siberian x Nozaki Early) were grown at 20°C for 4 weeks prior to vernalization. The F_2 and Siberian plants were vernalized for 6 weeks while Nozaki Early was vernalized for 2 weeks. The vernalized plants were transplanted into a greenhouse ground bed and the greenhouse temperature was maintained at 20°C. Thirty days after removal from the cold room, plants with visible flower buds were observed in all the populations. On the 30th, 37th, 44th, and 51st days after removal from the cold room, twenty parental plants and 40 F_2 plants were observed either for visible bolting or were cut longitudinally to observe apex elongation. Data were recorded on the percentage of plants visibly bolted and total percent bolted (visible and plants with pointed apices) (Figure 4). The results (Table 2) suggested that by cutting the plants, it was possible to classify bolted plants that would have been classified as nonbolters if only the visible bud technique was used. Since the number of bolted plants as determined by cutting through the apex did not change beyond 37 days in this study, it provided an accurate estimation of bolting.

Based on these findings, the following procedure was implemented for classifying bolting and nonbolting. Data was recorded at weekly intervals for number of visibly bolted plants until the bolt resistant parent visibly bolted or reached marketable size. All nonbolted plants were cut longitudinally through the apex to observe for elongation. Plants with pointed apices were classified as bolters while those with rounded apices were classified as nonbolters. The data on percent bolters as determined from the sum of visible bolters and those with pointed apices, for the various populations were used to develop genetic models for inheritance of bolting in the various crosses. Chi-square tests for goodness of fit and statistical tests were conducted as outlined in Little and Hills (1972).

Figure 4: Stages in seedstalk development. Left, not bolting; center and right, bolting; growing points outlined for ease of identification.

Table 2. Percentage of bolted plants at weekly intervals after vernalization

Generation	Percent Bolted Plants							
	30 days		37 days		44 days		51 days	
	A	B	A	B	A	B	A	B
Siberian kale	30	80	70	100	75	100	80	100
Nozaki Early	95	100	95	100	100	100	100	100
F_2	75	95	84	95	88	90	95	95

A = percent plants with visible flower buds at the apex.

B = total percent bolted plants, visible and from macroscopic observation of longitudinal sections of apices.

Vernalization Experiments

Experiment I: Natural Vernalization of Siberian Kale, Chinese Cabbage, an F_2 Population (Siberian x Chee Hoo) and Chikale

In the spring of 1982 an experiment was conducted to observe bolting under natural field conditions. This experiment included an F_2 population from the cross Siberian x Chee Hoo (derived from sib-mating 3 F_1 plants), Siberian kale, commercial Chinese cabbage cultivars Chee Hoo, Michihili, and Spring A-1, and 4 late-bolt Chikale lines (LB-129A, LB-129B, LB-217, LB-219). Seeds were sown in vermiculite on April 4, 1982. Seedlings with 1 true leaf were transplanted into No. 24 PCV growing trays filled with a mixture of 1 peat: 1 perlite: 1 vermiculite. The trays were placed under G.E. High Intensity Discharge lamps with 1000 watt multivapor bulbs (approximately 24 W m^{-2}) in a greenhouse maintained at 20°C. When the plants were 4 weeks old (5 to 7 true leaves), they were transplanted into the field in a randomized complete block design, with 4 replicates. Each replicate included 48 F_2 plants and 6 plants of each of the other cultivars. The spacing within rows was 44 cm and between rows was 120 cm. After transplanting all plants were watered with 120 ml of a starter solution and insecticide (Diazinon) mixture. The plot was irrigated, sprayed, and cultivated as necessary. A pre-plant application of 1135 kg per hectare of 12-12-12 was made and one sidedressing of ammonium nitrate at 113.5 kg per hectare was applied 2 weeks after transplanting. The air temperature was monitored with a Weather Hawk thermograph.

Experiment II: Artificial Vernalization Studies Siberian Kale x Chinese Cabbage

In 1982, an artificial vernalization experiment was conducted to determine the bolting response of parents, F_1, F_2, F_3 and backcross populations from the interspecific cross Siberian kale x Chinese cabbage after various amounts of vernalization. Asexual propagation of one Siberian x Chee Hoo F_1 hybrid plant was used to produce F_2 and backcross populations. This experiment also included F_2 and backcross populations from the cross Siberian x Nozaki Early and Mandarin x Siberian. Due to a limited amount of seeds, part of the populations were only grown in selected treatments. The vernalization treatments were 0, 2, 3, 4, 5, and 6 weeks. All treatments were removed from the cold room August 24, and transplanted into the field 1 week later. Planting in the field was a randomized complete block design with 3 replicates and cultural practices were similar to Experiment I. The experiment was terminated on October 18, 1982, when Siberian plants that were vernalized for 6 weeks visible bolted.

Experiment III: Artificial Vernalization Studies of Siberian x Nozaki Early F_3 Families

Observation of bolting response of Siberian, Nozaki Early, and F_3 families derived from selfing individual F_2 plants form the cross Siberian x Nozaki Early were made in the greenhouse during the Fall (1982). These F_2 plants all bolted after 6 weeks vernalization. Due to partial sterility, the number of plants used in each of the vernalization treatments (0, 2, 4, and 6 weeks) varied. After vernalization treatments, the plants were transplanted into a greenhouse ground bed at a temperature of 20°C. The experiment was terminated in December when Siberian plants after 6 weeks of vernalization visibly bolted.

Experiment IV: Artificial Vernalization Studies of Chinese Cabbage x Turnip

Inheritance studies of Chikale crosses with Wong Bok were made in 1982. Parents F_1, F_2 and backcross populations from reciprocal crosses: LB-7 x QB-2, LB-7 x Wong Bok, and QB-2 x Wong Bok were vernalized for 0, 1, 2, 3, 4, and 5 weeks. Each vernalization treatment included 12 plants of each parent and F_1, 24 plants of each backcross, and 66 plants of each F_2. The plants were randomized in 3 replicates, and transplanted into the field July 16, 1982.

The cultural practices were similar to Experiment I and the experiment was terminated September 10, 1982, when the late-bolt Chikale parent (LB-7) visibly bolted in the 5 week treatment.

Experiment V: Artificial Vernalization Studies of Chinese Cabbage x Turnip

This phase of the study on the inheritance of bolting involved the intra-specific cross between Chinese cabbage (Mandarin) and the turnip cultivar, Milan White. The various populations were vernalized for 0, 1, 2, 3, 4, and 5 weeks. Each treatment included 18 plants of each parent and F_1, 72 F_2 plants and 36 backcross plants which were divided into 3 replicates. This experiment was terminated on October 20, 1982, when Milan White after 5 weeks of vernalization visibly bolted. Cultural practices were similar to Experiment I.

RESULTS AND DISCUSSION

Experiment I: Natural Vernalization Studies of Siberian Kale, Chinese Cabbage, an F_2 Population (Siberian X Chee Hoo) and Chikale

The data on the percent bolters for the various populations grown under natural field conditions (May 4 to June 21, 1982) are presented in Table 3. The Chinese cabbage cultivars and the Chikale late-bolt (LB) lines had formed marketable heads by June 21, therefore, the experiment was terminated. Weekly observations were made for the visual appearance of flower buds at the apex of the plants, however, due to the formation of heads, it was not possible without damaging the plant. By June 21 none of the plants showed visible flower buds. All of the plants were cut longitudinally to observe for apex elongation. All of the Michihili and Chee Hoo cultivars had elongated apices with flower stalks of various lengths. Of the cultivar Spring A-1, 44 percent showed elongation of their apices, while the remaining 56% showed no signs of elongation. None of the plants from the late-bolt Chikale lines, Siberian kale, and the F_2 population (Siberian x Chee Hoo) showed signs of apical elongation.

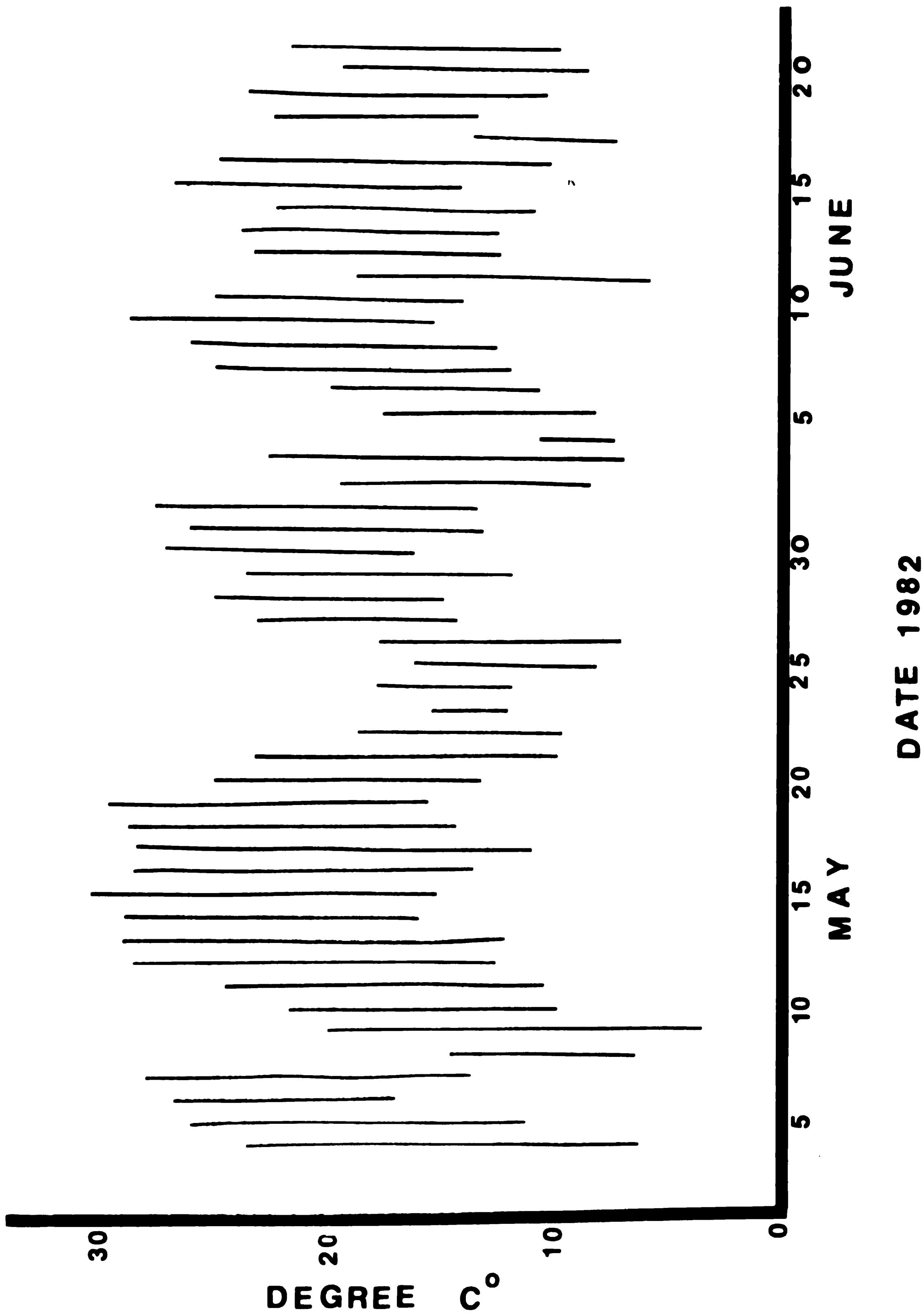
The minimum-maximum air temperatures 30 cm from ground level for each day from May 4 to June 20, 1982, are shown in Figure 5. Using Yamasaki's formula and the temperature data recorded for this natural vernalization experiment, the degree units obtained was

Table 3. Percentage of bolters under natural field conditions.

Population	Percent Bolters*
Chee Hoo	100
Michihili	100
Spring A-1	44
Siberian kale	0
LB-129A	0
LB-129B	0
LB-217	0
LB-219	0
(Siberain x Chee Hoo) F_2	0

*Percent bolters on June 21, 1982, 48 days from transplanting.

Figure 5. Minimum-Maximum air temperature chart for the period May 4 to June 21, 1982.



DEGREE C°
0
10
20
30
DATE 1982
MAY
JUNE
5
10
15
20
25
30
5
10
15
20

99.3°C which exceeded the amount (87°C) necessary to induce flower primordia in Chinese cabbage. Yamasaki's formula was adequate in predicting bolting in the cultivars Chee Hoo, Michihili, and Spring A-1 under natural vernalization even with fluctuating temperatures. Nakamura (1976) suggested that minimum night temperature was the important factor for induction of flower stalks in Chinese cabbage. He observed that 10 days with minimum temperatures of 5°C, 15 days with minimum temperatures of 8-9°C and 30 days with minimum temperatures of 13-14°C induced flower stalks in most Chinese cabbage cultivars. In this experiment the plants were exposed to 1, 11, and 26 days with minimum temperatures of 5°, 8-9°, and 13-14°C, respectively. The total cumulative cold units received by the Chinese cabbage cultivars used in this exerpiment were sufficient to induce flower stalks.

The fact that no visible bolting occurred in this experiment is explained as follows. The temperature after transplanting into the field was not conducive to initiate the development of the floral primorida, but continued to favor the vegetative phase which meant the development of 35 or more leaves which was sufficient to form a head and mask the visible flower stalks. It is also possible, as Nakamura (1976) suggested, that larger plants are more susceptible to low temperatures than smaller (younger) plants. Lorenz (1946) observed that the cultivar Pe-tsai bolted before forming a head if grown at temperatures between 10 and 15°C, while they bolted after forming a head if grown at temperatures between 15° and 20°C.

The absence of bolters in the late-bolt Chikale lines and the F_2 population suggest that bolt resistance from Siberian kale was transferred to Chinese cabbage. Although the F_2 population did not segregate for bolting response, the morphological variability observed suggested that hybridization of Siberian x Chee Hoo was successful.

Experiment II: Artificial Vernalization Studies of Siberian Kale x Chinese Cabbage

The percent of bolters in the parental, F_1, F_2, and backcross populations for the cross Siberian kale x Chee Hoo are presented in Table 4. Siberian kale required 6 weeks vernalization while Chee Hoo bolted after 2 weeks vernalization. Due to the limited number of F_1 seeds, samples of the F_1 population were vernalized for only 2, 4, and 6 weeks. Only F_1 plants which were vernalized for 6 weeks bolted, suggesting dominance for bolt resistance. Since no F_1 plants were included in the 5-week vernalization treatment, it was not possible to distinguish between partial and complete dominance. It was observed that after 6 weeks vernalization, the F_1 plants bolted 30 days after removal from the cold room, while the Siberian plants did not bolt until the 50th day. As discussed in the material and methods, the lower number of days to visible bolting in the F_1 population as compared to Siberian with the same degree of vernalization suggest that the F_1 plants required less cold than the Siberian plants.

The percent of bolters in the F_2 population increased with increased duration of vernalization. The percent bolters observed

Table 4. Bolting percentages after 5 durations of vernalization for the various populations from the interspecific cross Siberian kale x Chee Hoo

Generation	Weeks at 5°C				
	2	3	4	5	6
Siberian kale	0	0	0	0	100
Chee Hoo	100	100	100	100	100
F_1	0		0		100
F_2	10.9 (68)*	11 (64)	57 (108)	62.3 (61)	82.8 (88)
F_1 x Siberian	0 (38)	0 (36)	58 (66)	61.5 (30)	53.9 (28)
F_1 x Chee Hoo	13.6 (80)	53.6 (30)	55.4 (82)	76.9 (30)	89.9 (69)

*Number in parenthesis is the total number of plants observed for each respective population. The number of parental and F_1 plants (12) was equal for all treatments.

after 2 weeks (10.9%) and 3 weeks (11%) were similar. The percent bolters after 4 weeks (57%) and 5 weeks (62.3%) of vernalization were also similar and were both significantly greater (p = .05) than after 3 weeks. The percent bolters after 6 weeks vernalization (82.8%) was significantly greater (p = .05) than after 5 weeks, however, 27.2% of the F_2 plants were more bolt resistant than Siberian, the bolt resistant parent.

In the backcross to Siberian, no bolters were observed until after a minimum of 4 weeks vernalization (Table 4). The percent bolters observed after 4 weeks (58%), 5 weeks (61.5%), and 6 weeks (53.9%) of vernalization were not significantly different (p = .05). Similar to that observed in the F_2 population, plants requiring a longer period of vernalization than Siberian were observed. Of the plants in the backcross to Siberian population, 46% did not bolt after 6 weeks of vernalization.

In the backcross to Chee Hoo the percent bolters observed after 2 weeks vernalization was 13.6% (Table 4). There was a significant increase (p = .05) in percent of bolters after 3 and 4 weeks of vernalization (53.6% and 55.4%, respectively). The percent of bolters also increased between 4 weeks (55.4%) and 5 weeks (76.9%) vernalization and between 5 weeks and 6 weeks (89.9%) of vernalization. In this population. 10.1% of the plants were more bolt resistant than Siberian after 6 weeks vernalization.

This experiment also included F_2 and backcross progeny from the cross Siberian x Nozaki Early (Table 5) and Mandarin x Siberian

Table 5. Bolting percentages after five duration of vernalization for the various populations from the interspecific cross Siberian kale x Nozaki Early

Generation	Weeks at 5°C				
	2	3	4	5	6
Siberian kale	0	0	0	0	100
Nozaki Early	100	100	100	100	100
F_2	18.7 (65)*		59.6 (44)		83.3 (30)
F_1 x Siberian		18.2 (11)	41.6 (89)		82.6 (23)
F_1 x Nozaki Early	54.8 (31)	95.0 (19)	88.7 (53)	100 (29)	100 (26)

*Number in parenthesis is the total number of plants observed for each respective population; The number of parental plants (12) was equal for all treatments.

(Table 6). The observed segregation for percent bolters for the various durations of vernalization were similar for all 3 crosses in that (1) the percentage of bolters increased with increased duration, (2) the backcross to Siberian populations had fewer bolters in all treatments than the F_2 populations, (3) the backcross to Chinese cabbage populations had larger percentage of bolters in all treatments than the F_2 populations, and (4) plants were observed in some of the populations vernalized for 6 weeks which required longer durations of vernalization than Siberian. However, there were significant differences between these 3 crosses for percent bolters in certain treatments. For example, the percent bolters after 6 weeks vernalization in the Mandarin x Siberian F_2 population (100%) was significantly greater ($p = .05$) than in the Siberian x Chee Hoo F_2 population (82.8%) and the Siberian x Nozaki Early F_2 population (83.3%). The percent of bolters after 2 weeks vernalization in the Mandarin x Siberian F_2 population was also significantly greater ($p = .05$) than the percent of bolters observed in the Siberian x Chee Hoo F_2 population and the Siberian x Nozaki Early F_2 population. Significant difference ($p = .05$) for percent of bolters were observed in the backcross to Siberian populations for all 3 crosses after 3 weeks vernalization; the percent of bolters in the backcross population Siberian x (Siberian x Chee Hoo) after 4 weeks of vernalization was greater ($p = .05$) than the percent of bolters in the corresponding populations from the other 2 crosses, while after 5 and 6 weeks of vernalization, the percentage of bolters in the

Table 6. Bolting percentages after five durations of vernalization for the various populations from the interspecific cross Mandarin x Siberian kale

Generation	Weeks at 5°C				
	2	3	4	5	6
Siberian	0	0	0	0	100
Mandarin	100	100	100	100	100
F_2	42.6 (61)*				100 (30)
F_1 x Mandarin			78.8 (33)		100 (36)
F_1 x Siberian	13.0 (92)	40 (25)	34.5 (58)	92.5 (40)	82.0 (58)

*Number in parenthesis is the total number of plants observed for each respective population; the number of parental plants (12) was equal for all treatments.

backcross to Siberian populations from the Siberian x Chee Hoo cross were less ($p = .05$) than in the other 2 crosses. The percentage of bolters were also significantly different ($p = .05$) in the backcross to Chinese cabbage populations between at least 2 of the 3 crosses in all of the treatments. It appears that the Chinese cabbage cultivar used as a parent in crosses with Siberian affected the segregation for vernalization response. Although no differences were observed for percent bolters between these 3 cultivars after 1 and 2 weeks of vernalization, they may have shown differences if vernalized for 8, 9, 10, 11, 12, and 13 days, due to minor genes. Minor genes that effect bolting response may be responsible for the observed differences for percent bolting in the populations produced from crossing these 3 cultivars with Siberian.

The differences in the basic chromosome number between Siberian kale ($n = 19$) and Chinese cabbage ($n = 10$) may also explain the segregation ratios observed. Nwankiti (1970) and Mackay (1977) previously reported that the resulting hybrids from crossing _B. napus_ and _B. campestris_ had 29 chromosome pairs, 10 from _B. campestris_ (_a_ genome) and 19 from _B. napus_ (_a'c_ genome). The pairing at metaphase I in these hybrids included 10 bivalents (presumably the _campestris a_ genome and the _napus a'_genome) and 9 univalents (_c_ genome). Nwankiti (1970) observed some multivalent associations and therefore the number of univalents was variable. Pollen mother cells from the hybrids used in this study were observed to determine chromosome number and pairing at metaphase I. All the hybrids had

29 chromosomes and the most common association at metaphase I was 10 bivalents and 9 univalents (Figure 2). In general, the bivalents were centered along the equatorial plate and the 9 univalents were scattered randomly in the cell. Occasionally, only a portion of the 9 univalents were separated from the bivalents which suggest some multivalent associations. These observations agree with the reports by Nwankiti (1970) and Mackay (1977). Therefore, one would expect chromosome numbers in the gametes to range from 10 to 19 and they should occur in equal frequencies. The unequal chromosome numbers in the gametes probably explains the reduced fertility observed in the hybrids. Pollen viability as estimated by staining with acetic carmine ranged from 44 to 58%, which agrees with that reported by Mackay (1977). No attempt was made in this study to determine the chromosome numbers in the F_2 and backcross progenies. However, both Nwankiti (1970) and Mackay (1977) observed a greater than expected number of plants with chromosome numbers near to the euploid level (20 and 38). They attributed this to selection for gametes with extreme numbers of chromosomes, which Nwankiti (1970) suggested was the result of their competitive ability in achieving fertilization. They also observed sterility in the F_2 and backcross plants with aneuploid chromosome numbers which may help explain the sterility of many F_2 and backcross selections noted in this study. Since the 2 species used in this study, and observations on chromosome numbers, chromosome pairing, and fertility of the hybrids were similar to that reported by Nwankiti (1970) and Mackay (1977), it is

possible that selection for euploid gametes may also have occurred in the hybrids produced in this study.

A hypothesis for genetic control of bolting in this interspecific cross, Siberian kale x Chinese cabbage, is based on the following: (1) Chinese cabbage, a genome, bolts after 2 weeks vernalization; (2) common cabbage, c genome, bolts after 8 weeks vernalization; (3) Siberian kale, a'c genome, bolts after 6 weeks vernalization, and (4) the sesqui-diploid hybird, aa'c genome, bolts after 5 or 6 weeks of vernalization. According to the hypothesis of Purvis (1966) on vernalization and gene action, plants requiring thermo-induction are lacking genes or their products that are able to bring about flowering in related species without a cold requirement. That is, the cold period compensates physiologically for the absence of these genes' products. Such a hypothesis suggest that plants with a greater cold requirement have more negative or inactive alleles while those with a lesser cold requirement have more positive or active alleles. One can only speculate as to the nature of these genes or gene products. Melchers and Lang (Lang, 1965) and Purvis (1966) favor the hypothesis of a specific substance, "vernalin," which is produced through a series of biochemical intermediates and that the enzymes that catalyse these reactions leading to the formation of "vernalin" have lower temperature optimums or are activated at lower temperatures. Therefore, at low temperatures "vernalin" formation is favored. Assuming that the substrates for these hypothetical enzymes are not limiting, the production of more enzyme would hasten the reactions thereby shortening the length of cold period required to produce sufficient levels of "vernalin" to allow for bolting and flowering. Increased levels of enzyme could result from (1) changes in regulator genes which allows for increased production of these enzymes; (2) loss of suppressors that limit production of these enzymes; or (3) increased number of structural genes which code for these enzymes. It is difficult to determine the actual mechanism underlying the segregation of degree of vernalization requirement, however, it is possible that varying levels of catalytic enzymes effect the rate of "vernalin" formation. It is suggested that the rate of vernalin formation as effected by genotype is the basis for variable degrees of vernalization requirement in the progeny produced from crossing Siberian kale and Chinese cabbage.

Selfing the F_1 hybrid could produce progeny with 20, 29, or 38 chromosomes as well as some aneuploids, assuming selection for euploid gametes. The homology between chromomes from the a and a' genomes would make it possible for chiasmata formation and crossing over to occur, as well as allow for random assortment of the chromosomes from the a and a' genomes. Crossing over and random assortment of the chromosomes would allow for recombination of genes which condition the degree of vernalization requirement and could produce individuals with various levels of bolt resistance in each of the ploidy levels. This recombination of both major and minor genes may have resulted in the segregants that require greater periods of vernalization than Siberian kale. The majority of individuals selected for vernalization requirements greater than Siberian kale were sterile which could have resulted from aneuploidy as suggested by Nwankiti (1970) and Mackay (1977). Presumably, the individuals with greater vernlization requirements than Siberian kale are lacking genes present in Siberian kale and Chinese Cabbage which may have conditioned reduced vernalization requirement and that the absence of these genes may have resulted from either recombination, crossing over, or aneuploidy.

A similar relationship appears to exist in the backcross populations. In the backcross to Chinese cabbage (a genome), the majority of the individuals should have either 20 or 29 chromosomes, therefore the percent of bolters expected after 6 weeks of vernalization is 100%. In the backcross to Nozaki Early (Table 5) and Mandarin (Table 6) populations, 100% of the plants bolted after 6 weeks of vernalization. However, in the backcross to Chee Hoo population only 89.9% of the plants bolted after 6 weeks of vernalization. As mentioned, these nonbolters could be attributed to recombination or aneuploidy.

In the backcross to Siberian (a'c genome), the majority of the plants would have either 29 or 38 chromosomes, therefore would yield only a few bolters after vernalization of less than 4 weeks. In the Siberian x (Siberian x Chee Hoo) population no bolters were observed after 2 or 3 weeks of vernalization (Table 4). In the Siberian x (Siberian x Nozaki Early) population, 18.2 percent of the plants bolted after 3 weeks of vernalization (Table 5) and is probably due to individuals with 29 chromosomes which may have resulted from gametes which carried Chinese cabbage genes which conditioned shorter vernalization requirements. In the backcross population, Siberian x (Manarin x Siberian), 13% and 40% of the plants bolted after 2 and 3 weeks of vernalization, respectively (Table 6). These bolters are also probably due to aneuploidy. The frequency of these genotypes was higher than expected if chromosome distribution was at random. One possible explanation for this apparent nonrandom distribution of chromosomes is that the cytoplasm effected the ability of certain gametes to produce balanced pollen grains (Nwankiti, 1970). Another possible explanation for the higher than expected percentage of bolters in some of these populations may be due to cytoplasmic factors which influence the vernalization processes (Bouwkamp and Honma, 1970). The increased number of bolters observed when Manarin is used as the female parent (Table 6) as compared to when Siberian is used as the female parent (Table 4 and Table 5) may be attributed to one or both of these factors.

Observations made of the F_3 families tend to support the theory that the genomic constitution of the parents may have influenced the segregation for vernalization response. All of the F_2 plants selected that failed to bolt after 6 weeks of vernalization were nearly sterile which suggest that these plants had aneuploid chromosomes.

Only 2 F_3 families from the Siberian x Chee Hoo cross were observed for vernalization response. The percent bolters after varius durations of vernalization for these F_3 families are presented in Table 7. One of these F_3 families (SC-3) was produced

Table 7. Percent bolters after five durations of vernalization in two F_3 families from the cross Siberian kale x Chee Hoo

F_3 Family	Weeks at 50°C				
	2	3	4	5	6
SC-3	20		83		100
SC-6	0	0	0	16	36

from an F_2 plant that did not bolt after 2 weeks of vernalization while SC-6 was produced from an F_2 plant that did not bolt after 5 weeks of vernalization. The observed segregation for SC-3 suggest one major gene is segregating for vernalization requirement. The expected percent of bolters assuming one major additive gene is 25% after 2 weeks of vernalization. Using the gene symbols V for bolt susceptibility and v for bolt resistance, these 25% bolters after 2 weeks of vernalization have the genotype VV. The 75% bolters expected after 4 weeks vernalization are the sum of the genotypes VV and Vv, while the 100% bolters expected after 6 weeks vernalization are the sum of the genotypes VV, Vv, and vv. Although the chromosome number of this F_2 plant was not determined cytologically, its fertility in crosses with Chinese cabbage suggest it has 20 chromosomes. The observed segregation for bolting response can be explained if it is assumed that this F_2 plant has one _V_ allele from Chinese cabbage and one _v_ allele from Siberian kale.

The F_3 family SC-6 has 16% bolters after 5 weeks vernalization and 36% bolters after 6 weeks vernalization (Table 7). The F_2 parent of this population was not fertile in crosses with Chinese cabbage suggesting that it was polyploid. The absence of bolters after 2, 3, or 4 weeks of vernalization suggest that the F_2 parent did not have any major genes from Chinese cabbage that conditioned reduced vernalization requirement. The observed segregations suggest that the F_2 parent was heterozygous for major and minor genes from Siberian and that through recombination, individuals that require 5, 6, or more weeks of vernalization were produced. Possibly variation of ploidy level may also have effected the observed segregations.

Experiment III: Artificial Vernalization Studies of Siberian x Nozaki Early F_3 Families

The data for percent bolting after 2, 4, or 6 weeks of vernalization for 6 (Siberian x Nozaki Early) F_3 families are presented in Table 8. Although the population sizes were small, the observed segregations suggest 2 or 3 different F_2 genotypes, which produced these families. The F_3 families SN-2, SN-18, SN-20, and SN-25 segregated for 1 major additive gene, _V_ (less vernalization), and _v_ (greater vernalization). Individuals that bolted after 2 weeks vernalization have the genotype _VV_, those that bolt after 4 weeks vernalization have the genotypes _VV_ or _Vv_, and all three genotypes

Table 8. Percent bolters after three durations of vernalization of F_3 families from the cross Siberian x Nozaki Early

F_3 Family	Weeks at 5°C		
	2	4	6
SN-2	33 (12)	67 (12)	100 (12)
SN-7	0 (12)	37 (12)	100 (12)
SN-17	0 (12)	67 (12)	100 (12)
SN-18	28 (22)		100 (12)
SN-20	17 (12)		100 (12)
SN-25	23 (48)		96 (48)

*Number in parenthesis represents the total number of plants.

(VV, Vv, vv) bolt after 6 weeks vernalization. The few nonbolters observed after 6 weeks vernalization in SN-25 may be due to minor or modifier genes. Chi-square tests for goodness of fit to a single additive gene model for inheritance of bolting response in the F_3 families SN-2, SN-18, SN-20, and SN-25 all suggest a good fit (Table 9). The high degree of self-fertility of these F_2 plants and their observed fertility in crosses with Chinese cabbage suggest they had 10 chromosome pairs.

The 2 remaining F_3 families (SN-7 and SN-17) failed to yield bolters after 2 weeks of vernalization. The percent bolting of these 2 families differed after 4 weeks of vernalization (Table 8) suggesting that the individual F_2 plants were of different genotypes. SN-17 had 67% and 100% bolters after 4 and 6 weeks of vernalization,

Table 9. Chi-square test for goodness of fit to a single additive gene model for bolting after three durations of vernalization of F_3 families from the cross Siberian x Nozaki Early

Weeks of Vernalization	Population	Bolters		Nonbolters		X	p
		Observed	Expected	Observed	Expected		
2	SN-2	4	3	8	9	0.44	.7 - .5
2	SN-18	6	5.5	16	16.5	0.06	.9 - .8
2	SN-20	2	3	10	9	0.44	.7 - .5
2	SN-25	11	12	37	36	0.11	.8 - .7
4	SN-2	8	9	4	3	0.44	.7 - .5
6	SN-2	12	12	0	0	--	--
6	SN-18	12	12	0	0	--	--
6	SN-20	12	12	0	0	--	--
6	SN-25	46	48	2	0	--	--

respectively. The similarity in percent bolters after 4 and 6 weeks of vernalization between SN-17 and SN-2 and the observed fertility when SN-17 was crossed with Chinese cabbage suggest that this F_3 parent was an euploid with 10 chromosome pairs and that the segregation of modifiers genes produced all nonbolters in the 2-week treatment. Perhaps the absence of bolters in this population may also have been due to the small population size (12 plants).

The F_3 family, SN-7 had only 36% bolters after 4 weeks vernalization and was not grown in the 6-week treatment due to limited number of seeds. The F_2 parent was highly sterile and failed to produce any viable seeds in crosses with Chinese cabbage, suggesting polyploidy. Since there were no plants in the 6-week treatment, the determination of the parental genotype is not possible. The observed segregation in this F_3 family is attributed to both gene and chromosome number differences. It appears that the segregation for bolting response noted from Siberian kale x Nozaki Early F_3 families tends to support the theory that recombination of parental genes conditioning vernalization requirement and the variation in ploidy level were responsible for the segregation of this character.

Experiment IV: Artificial Vernalization of Chikale

This experiment included crosses between Chikale lines developed for quick-bolting habit (1 week of vernalization) and late-bolting habit (4 weeks of vernalization) and the Chinese cabbage cultivar Wong Bok.

Chikale LB-7 x Chikale QB-2

The data for percent bolters after 0, 1, 2, 3, and 4 weeks of vernalization for the parents, F_1, F_2, and backcross populations from reciprocal crosses between Chikale LB-7 and Chikale QB-2 are presented in Table 10. When Chikale QB-2, Chikale LB-7, and Wong Bok plants were placed in a greenhouse with a temperature of 20°C following artificial vernalization, bolting was observed after 1, 4, and 2 weeks of vernalization, respectively. However, since QB-2 plants in the control (0 weeks) treatment bolted and LB-7 bolted after 3 weeks of vernalization, it was evident that further thermo-induction occurred after the plants were removed from the cold room. While the plants were in the lath house awaiting transplanting into the field, the minimum night temperatures ranged from 9° to 11°C for 6 days, and this additional low temperature may have caused the bolting. Since all of the plants received similar post-vernalization temperatures, the data were interpreted as noted.

The quick-bolt parent, QB-2, bolted in all of the treatments while the late-bolt parent, LB-7, required a minimum of 3 weeks vernalization to bolt (Table 10). The F_1 population from reciprocal crosses differed in the duration of vernalization required to induce bolting, suggesting cytoplasmic influence in the vernalization responses; however, no significant reciprocal differences ($p = .05$) between the F_2 populations were observed. The intermediate vernalization requirement of the F_1 plants and the increase in percentage of bolters with increased duration of vernalization suggest additive

Table 10. Bolting percentages after five durations of vernalization for the various populations from the cross LB-7 x QB-2.

Generations	Weeks at 5°C				
	0	1	2	3	4
LB-7	0	0	0	100	100
QB-2	100	100	100	100	100
(LB-7 x QBp2) F_1	0	0	100	100	100
(QB-2 x LB-7) F_1	0	100	100	100	100
F_1 pooled	0	50	50	100	100
(LB-7 x QB-2) F_2	27	68	92	100	100
(AB-2 x LB-7) F_2	21	53	91	100	100
F_2 pooled	24	60	91.5	100	100
(LB-7 x QB-2) x LB-7	0	0	83	100	100
(QB-2 x LB-7) x LB-7	0	0	46	100	100
F_1 x LB-7 pooled	0	0	64.5	100	100
(LB-7 x QB-2) x QB-2	83	92	100	100	100
(QB-2 x LB-7) x QB-2	78	92	100	100	100
F_1 x QB-2 pooled	80.5	92	100	100	100

gene action, which supports the observations made in Experiment II and III. Based on the observed segregations for botling response, the following gene model is proposed. The model involves two coupling phase additive gene pairs. The gene V is used for reduced vernalization requirements, and v for greater vernalization requirement. Different gene pairs are designated by use of subscripts.

The quick-bolt parent having the least cold requirement has the genotype $V_1V_1V_2V_2$ and bolts after 0 weeks of vernalization. The late-bolt parent has the genotype $v_1v_1v_2v_2$ and bolts after 3 weeks of vernalization. The F_1 is heterozygous for both loci, $V_1v_1V_2v_2$ and is phenotypically intermediate of the parents bolting after 1 or 2 weeks of vernalization. Based on the hypothesis on vernalization (Purvis, 1966, and Lang, 1965) that the cold period leads to the formation of a specific flower-inducing substance, vernalin, which brings about changes in the cells of the meristematic region leading to flowering, the cytoplasmic differences noted may be attributed to differential sensitivity of the cytoplasm to intermediate levels of the gene products, if it is assumed that the cytoplasm is the receptor of the vernalizing stimulus and that the chemical and biochemical changes which lead to bolting and flowering take place in the cytoplasm (Bouwkamp and Honma, 1970). The absence of reciprocal differences in the F_2 populations probably resulted from recombination of nuclear genes which effected the cytoplasm, thereby reducing cytoplasmic differences in the reciprocal F_2 populations. The backcross populations were produced by using the recurrent parent as the female in crosses with the F_1 hybrids, therefore, the cytoplasm of the recurrent parent makes up the majority of the cytoplasm in the backcross progeny, and may also have effected the vernalization response of this population. The Chinese cabbage parent from which these Chikale lines resulted was the cultivar Mandarin which was the parent used in crosses with Siberian kale (Experiment II). In this cross cytoplasmic differences were also noted.

Based on the proposed 2 additive gene model, the F_2 population would have 1/16 (4 "V" alleles): 4/16 (3 "V" alleles): 6/16 (2"V" alleles): 4/16 (1 "V" allele) and 1/16 (no "V" alleles). The observed frequency of individuals with the Quick-bolt parental phenotype is approximately 4/16 which suggest that individuals with 3 and 4 "V" allele bolt after similar durations of vernalization. Therefore, it is proposed that individuals with 3 or 4 "V" alleles bolted after 0 weeks, plants with 2 or more "V" alleles bolt after 1 week, plants with 1 "V" allele bolt after 2 weeks and plants with no "V" alleles at these 2 loci bolt after 3 weeks of vernalization. Chi-square tests were conducted to determine if the observed ratios in the segregating populations for each duration of vernalization fit the 2 additive gene model. The observed percent bolters suggested a good fit to the proposed model in all populations except the backcross to LB-7 populations (Table 11). The poor fit to the expected ratios in the backcross to LB-7 population after 1 week of vernalization may be due to cytoplasmic factors.

Table 11. Chi-square tests for goodness of fit to a two additive gene model for bolting after four durations of vernalization for the cross LB-7 x QB-2.

Weeks at 5°C	Generation	Bolted		Nonbolted		X^2	P
		Observed	Expected	Observed	Expected		
0	F_2	32	41.25	100	90.75	3.02	.1 - .05
o	F_1 x LB-7	0	0.00	48	48	--	--
0	F_1 x QB-2	106	99.00	26	33.00	1.98	.2 - .1
1	F_2	80	90.75	52	41.25	3.50	.1 - .05
1	F_1 x LB-7	0	12.00	48	36.00	16.00	$p < .01$
1	F_1 x QB-2	45	48.00	4	0.00	--	--
2	F_2	121	123.75	11	8.25	0.98	.5 - .3
2	F_1 x LB-7	31	36	17	12.00	2.78	.1 - .05
2	F_1 x QB-2	48	48	0	0.00	--	--
3	F_2	132	132	0	0.00	--	--
3	F_1 x LB-7	48	48	0	0.00	--	--
3	F_1 x QB-2	48	48	0	0.00	--	--

Chikale LB-7 x Wong Bok

The proposed 2 additive gene model is further supported by the segregation for bolting response in the cross Chikale LB-7 x Wong Bok (Table 12). The Chinese cabbage parent, Wong Bok, bolted

Table 12. Percentage of bolters* after five durations of vernalization in the various populations from the cross LB-7 x Wong Bok.

Generations	Weeks at 5°C				
	0	1	2	3	4
LB-7	0	0	0	100	100
Wong Bok	0	100	100	100	100
(LB-7 x Wong Bok) F_1	0	0	100	100	100
(LB-7 x Wong Bok) F_2	0	26.5	80.5	100	100
F_1 x LB-7	0	0	33	96	100
F_1 x Wong Bok	0	73	100	100	100

*Data from reciprocal populations pooled.

in all treatments with 1 or more weeks of vernalization while the late-bolt parent, LB-7, bolted in all treatments with 3 or more weeks of vernalization. The data were pooled since no significant differences (p = .05) were observed between reciprocal populations. The F_1 was intermediate for bolting response and bolted after 2 weeks of vernalization (Table 12). The F_2 and backcross populations segregated for bolting response. The percent bolters in the F_2 (26.5%) and the backcross to Wong Bok (73%) populations after 1 week of vernalization suggested that only one gene was segregating in this cross. The following gene model is proposed based on the model developed for the previous cross (LB-7 x QB-2). Wong Bok has the genotype $\underline{V_1V_1v_2v_2}$ and bolts after 1 week of vernalization. LB-7 has the genotype $\underline{v_1v_1v_2v_2}$ and bolts after 3 weeks of vernalization. The F_1 has the genotype $\underline{V_1v_1v_2v_2}$ and bolts after 2 weeks vernalization. The expected F_2 segregation is 25% bolters after 1 week, 75% bolters after 2 weeks, and 100% bolters after 3 weeks of vernaliztion. Chi-square tests (Table 13) for goodness of fit to these expected ratios suggest a good fit. The deficiency of bolters in the backcross to Chikale LB-7 population after 2 weeks of vernalization and the presence of 2 nonbolted plants after 3 weeks of vernalization may have resulted from recombination of modifier genes or crossing over.

Chi-square analysis of goodness of fit to the expected ratio of 75% bolters to 50% nonbolters in the backcross to Wong Bok population after 1 week of vernalization showed a good fit ($p > .50$) and all of the plants bolting after 2 or 3 weeks of vernalization supports the single additive gene model proposed (Table 13).

<u>Wong Bok x Chikale QB-2</u>

The segregation for bolting for the parents, F_1, F_2, and backcross populations from the cross Wong Bok x QB-2 also supports the proposed additive gene model (Table 14). Chikale QB-2 has the genotype $\underline{V_1V_1V_2V_2}$ and bolts after 0 weeks of vernalization. Wong Bok has the genotype $\underline{V_1V_1v_2v_2}$ and bolts after 1 week of vernalization.

Table 13. Chi-square tests for goodness of fit to a one additive gene model for bolting after 3 durations of vernalization for the cross LV-7 x Wong Bok.

Weeks at 5°C	Generation	Bolted		Nonbolted		X^2	P
		Observed	Expected	Observed	Expected		
1	F_2	35	33	97	99	0.16	.7 - .5
1	F_1 x LB-7	0	0	48	48	--	--
1	F_1 x Wong Bok	35	36	13	12	0.11	.8 - .7
2	F_2	106	99	26	33	1.98	.2 - .1
2	F_1 x LB-7	16	24	32	24	5.33	.05 - .02
2	F_1 x Wong Bok	48	48	0	0	--	--
3	F_2	132	132	0	0	--	--
3	F_1 x LB-7	46	48	2	0	--	--
3	F_1 x Wong Bok	48	48	0	0	--	--

Table 14. Percentage of bolters* after five durations of vernalization in the various populations from the cross Wong Bok x QB-2

Generations	Weeks at 5°C				
	0	1	2	3	4
Wong Bok	0	100	100	100	100
QB-2	100	100	100	100	100
(Wong Bok x QB-2) F_1	100	100	100	100	100
(Wong Bok x QB-2) F_2	63	93	97	100	100
F_1 x Wong Bok	71	100	100	100	100
F_1 x QB-2	100	100	100	100	100

*Data from reciprocal populations were pooled.

The data were pooled since no significant reciprocal differences were observed ($p = .05$). The F_1 has the genotype $\underline{V}_1\underline{V}_1\underline{V}_2\underline{v}_2$ and bolts after 0 weeks of vernalization. The vernalization treatments plus the additional post vernalization in this series of experiments did not make it possible to differentiate plants with the genotypes $\underline{V}_1\underline{V}_1\underline{V}_2\underline{V}_2$ and $\underline{V}_1\underline{V}_1\underline{V}_2\underline{v}_2$. The percent bolters expected in the F_2 population after 0 weeks of vernalization is 75%. Chi-square tests (Table 15) suggest a poor fit ($p < .01$). This poor fit and the presence of 9 nonbolting plants after 1 week and 4 nonbolting plants after 2 weeks of vernalization in the F_2 population are attributed to recombination or crossing over.

The segregation for bolting in the backcross populations supports the proposed additive gene model. After 0 weeks of

Table 15. Chi-square tests for goodness of fit to a one additive gene model for bolting after three durations of vernalization for the cross Wong Bok x QB-2.

Weeks at 5°C	Generation	Bolted		Nonbolted		X^2	p
		Observed	Expected	Observed	Expected		
0	F_2	83	99	49	33	7.81	p < .01
0	F_1 x Wong Bok	34	36	14	12	0.44	.7 - .5
0	F_1 x QB-2	48	48	0	0	--	--
1	F_2	123	132	0	0	--	--
1	F_1 x Wong Bok	48	48	0	0	--	--
1	F_1 x QB-2	48	48	0	0	--	--
2	F_2	128	132	4	0	--	--
2	F_1 x Wong Bok	48	48	0	0	--	--
2	F_1 x QB-2	48	48	0	0	--	--

vernalization the expected percent bolters in the backcross to Wong Bok population is 75% and Chi-square tests suggested a good fit (Table 15). The absence of nonbolters after 1 and 2 weeks of vernalization in the backcross to Wong Bok populations and in all of the backcross to Chikale QB-2 populations was expected based on the proposed single additive gene model.

Based on the observed bolting segregations from these 3 crosses following various durations of vernalization and the origin of the Chiklae lines, it appears that genes for lesser vernalization requirement were contributed by both Siberian kale and Chinese cabbage. The segregations observed in the Siberian kale x Chinese cabbage crosses (Experiment II and III) and in the Chikale x Chinese cabbage crosses are explainable if 4 major genes control vernalization requirement. As mentioned previously, the gene symbol "V" conditions a reduced vernalization requirement while "v" conditions a greater vernalization requirement. Alleles of these 4 genes have an equal and additive effect on vernalization requirement. Chinese cabbage has the genotype $V_1V_1V_2V_2V_3V_3v_4v_4$ and bolts after 2 weeks of vernalization. Siberian kale has the genotype $v_1v_1v_2v_2v_3v_3V_4V_4$ and bolts after 6 weeks of vernalization. It is suggested that these genes for reduced vernalization requirement are on chromosomes of the a and a' genomes since cabbage (c genome) requires 8 weeks of vernalization. The F_1 hybrid from the cross Siberian kale (female) x Chee Hoo has the genotype $V_1v_1V_2v_2V_3v_3V_4v_4$ and bolts after 5 weeks of vernalization. Based on additive gene action, the hybrid with 4 "V" alleles should bolt after 4 weeks vernalization. Since reciprocal differences have been observed in this study, the increased vernalization requirement of this F_1 may be attributed to cytoplasmic factors. Due to pairing of the $\underline{a}$ and $\underline{a}'$ genomes in the hybrid and assuming random assortment, individuals with 10 chromosome pairs in the offspring of this hybird have between 8 "V" alleles and no "V" alleles. As suggested previously, polyploidy could also produce individuals with more or less "V" alleles than the parents. That is, individuals which resulted from the pairing of gametes which both were lacking chromosomes from the parents carrying the "V" alleles would have a vernalization requirement greater than Siberian kale. If these gametes had chromosomes from the $\underline{c}$ genome substituted for chromosomes from the $\underline{a}$ or $\underline{a}'$ genomes they would theoretically be functional and able to complete fertilization, however, the resultant zygote would be aneuploid for specific chromosomes. This aneuploidy could explain the observed association between greater vernalization requirement and sterility.

Both Chikale lines used in this study appear to have 10 chromosome pairs based on fertility in crosses with Chinese cabbage (n = 10). Cytological observation of the pollen mother cells from Chikale LB-7 revealed 10 chromosome pairs (Figure 2). The following genotype is proposed for Chikale LB-7, $\underline{V_1V_1v_2v_2v_3v_3V_4V_4}$, however, other genotypes with 4 "V" alleles are possible. The vernalization requirement of Chikale LB-7 is 4 weeks. Chikale QB-2 has the genotype $\underline{V_1V_1V_2V_2V_3V_3V_4V_4}$ and it bolts after 1 week of vernalization.

The proposed genotypes and phenotypes for diploid individuals with 10 chromosome pairs are presented in Table 16.

Table 16. Proposed genotypes and phenotypes for bolting response produced from the cross Siberian kale x Chinese cabbage.

Genotype	Phenotype
$V_1V_1V_2V_2V_3V_3V_4V_4$	Bolts after 1 week of vernalization
$V_1V_2V_2V_2V_3V_3v_4v_4$	Bolts after 2 weeks of vernization
$V_1V_1V_2V_2v_3v_3v_4v_4$	Bolts after 4 weeks of vernalization
$V_1V_1v_2v_2v_3v_3v_4v_4$	Bolts after 6 weeks of vernalization
$v_1v_1v_2v_2v_3v_3v_4v_4$	Bolts after 8 weeks of vernalization

Experiment V: Artificial Vernalization of Chinese Cabbage x Turnip Populations

The results of the vernalization response from the cross between Chinese cabbage (Mandarin) and turnip (Milan White) are shown in Table 17.

Mandarin bolted after 2 weeks of vernalization while Milan White bolted after 5 weeks vernalization. The F_1 hybrids from the unilateral cross Mandarin x Milan White bolted after 3 weeks of vernalization. The intermediate vernalization requirement of the F_1 and the observed increase in percent of bolters with increased vernalization in the F_2 and backcross populations suggest additive gene action with a few number of genes. The segregating populations

Table 17. Percentage of bolters after four durations of vernalization in the various populations from the cross Mandarin x Milan White.

Generations	Weeks at 5°C			
	2	3	4	5
Mandarin	100	100	100	100
Milan White	0	0	0	100
F_1	0	96	100	100
F_2	25	67	95	100
Mandarin x F_1	88	100	98	100

showed variation for leaf type, heading tendencies, and root shape. Although most plants were intermediate for these characters, both parental types were observed. No apparent association was noted between the morphological characteristics and bolting response; however, the 4 nonbolting plants in the F_2 population after 4 weeks vernalization had turnip-like roots which were phenotypically similar to Milan White, while their leaf morphology was intermediate to the parents. Wester and Magruder (1938) reported that most turnip cultivars, including Milan White, did not bolt until reaching marketable size even after prolonged exposure to low field temperatures. This type of phasic development has also been reported in radish (Yun and Pyo, 1977). The development of an enlarged root prior to bolting even under thermo-inductive conditions suggest a complex phasic development. This complex phasic development may explain the absence of recombinants in the segregating populations from this cross having Chinese cabbage leaf morphology and head formation with bolt resistance equal to turnip (nonbolting after 4 weeks of vernalization). Since the desired recombinant was not found, it would appear that hybridization between Chinese cabbage and turnip to develop bolt resistance in Chinese cabbage may be difficult.

Crane (1943) suggested that kale cultivars with 38 chromosomes were produced by natural hybridization between turnip (2n = 20) and kale (2n = 18). Thus the similarity between Siberian kale (a'a'cc) and turnip (aa) for vernalization requirement suggest that inheritance for vernalization response in the cross Mandarin x Milan White is similar to Chinese cabbage x Siberian kale. Based on the observed segregations for bolting response, the following additive gene model is proposed. Mandarin has the genotype $V_1V_1V_2V_2V_3V_3v_4v_4$ and bolts after 2 weeks of vernalization. Milan White has the genotype $V_1V_1v_2v_2v_3v_3v_4v_4$ and bolts after 5 weeks of vernalization. The lesser vernalization requirement of Milan White than Siberian kale with the same number of "V" alleles could result from the differences in genomic constitution.

The F_1 hybrid is heterozygous at the V_2 and V_3 loci and has 4 "V" alleles, which condition bolting after 3 weeks of vernalization. The F_1 hybrid from crossing Mandarin and Milan White was also intermediate for leaf morphology and degree of fleshy root (Figure 3). Since no reciprocal cross was made, determination of cytoplasmic effects are not possible in this population.

The expected percentage of bolters in the F_2 population after 2, 3, 4, and 5 weeks of vernalization are 31%, 69%, 94%, and 100%, respectively. Chi-square tests (Table 18) show a good fit to the proposed additive gene model. The backcross to Mandarin is expected to have 75% bolters after 2 weeks of vernalization and 100% bolters after 3 or more weeks of vernalization. The Chi-square value for the backcross to Mandarin after 2 weeks of vernalization (6.35) suggest a poor fit to this additive gene model. The percent of bolters in this population may be attributable to recombination of modifier genes and also to the complex phasic development in turnips.

Table 18. Chi-square tests for goodness of fit to a two additive gene model for bolting after four durations of vernalization for the cross Mandarin x Milan White

Weeks at 5°C	Generation	Bolted		Unbolted		X	p
		Observed	Expected	Observed	Expected		
2	F_2	15	18.75	45	41.25	1.09	.3 - .2
2	Mandarin x F_1	60	51.00	8	17.00	6.35	.02 - .01
3	F_2	28	29	14	13.00	0.11	.8 - .7
3	Mandarin x F_1	36	36	0	0.00	--	--
4	F_2	83	81.5	4	5.50	0.44	.7 - .5
4	Mandarin x F_1	44	45	0	0.00	--	--
5	F_2	120	120	0	0.00	--	--
5	Mandarin x F_1	45	45	0	0.00	--	--

SUMMARY AND CONCLUSIONS

The inheritance of bolting in Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) was investigated by hybridizing Chinese cabbage with Siberian kale (*B. napus*), Chikale (*B. campestris* L. ssp. *pekinensis* x *B. napus*), and turnip (*B. campestris* L. ssp. *rapifera*). The inheritance model was developed from segregation ratios observed in segregating populations from these crosses after various durations of vernalization at 5°C and 16 hour daylength. Percent of bolters was determined by the sum of visible bolters and longitudinally cut plants with pointed apices, observed when the bolt-resistant parent either visibly bolted or reached a marketable size.

Segregation for bolting under natural field vernalization was observed in Chinese cabbage, Chikale late-bolt lines, Siberian kale, and an F_2 population (Siberian kale x Chee Hoo). The observed bolting in the Chinese cabbage cultivars while Chikale, Siberian kale and the F_2 population failed to bolt suggested (1) differences in bolting response observed after artificial vernalization at a constant temperature (5°C) are also expressed after natural vernalization with fluctuating temperatures and (2) bolt resistance from Siberian kale was transferred to Chikale and the F_2 population.

The genetic model was based on observations from a series of hybridizations and included four major genes, modifier genes, and cytoplasmic factors. Segreation for bolting response in the progeny from the crosses Siberian kale x Chee Hoo, Siberian kale x Nozaki Early, and Mandarin x Siberian kale suggested bolting response was conditioned by a few major additive genes and that percent of bolters was dependent on the Chinese cabbage cultivar. An increase in the percent bolters in crosses where Chinese cabbage was used as the female suggested cytoplasmic factors. The difference in chromosome number between the two parents (Chinese cabbage, n = 10 and Siberian kale, n = 19) produced varying degrees of fertility in the progeny. Variable ploidy level, random assortment, and probable crossing over of chromosomes may have produced the observed segregations.

Results from selfing F_2 plants assumed to have euploid chromosome numbers based on their fertility suggested one or more additive genes were conditioning bolting response.

Results from crossing quick-bolt and late-bolt Chikale lines with Chinese cabbage, cultivar Wong Bok, suggested 2 major additive genes were conditioning bolting response. Cytoplasmic influences were noted in certain crosses.

Results from crossing Chinese cabbage, cultivar Mandarin, with turnip, cultivar Milan White, also suggested 2 major additive genes and modifiers were conditioning bolting response.

The gene model was expanded from the 2 major additive gene theory to 4 major additive genes to hypothesize the probable genotypes of the parents based on the above crosses. The following genotypes and phenotypes are proposed for the parents: Chinese cabbage has the genotype $V_1V_1V_2V_2V_3V_3v_4v_4$ and bolts after 2 weeks of vernalization. Siberian kale has the genotype $v_1v_1v_2v_2v_3v_3V_4V_4$ and bolts after 6 weeks of vernalization. Chikale LB-7 has the genotype $V_1V_1v_2v_2v_3v_3v_4v_4$ and bolts after 4 weeks of vernalization, while Chikale QB-2 has the genotype $V_1V_1V_2V_2V_3V_3V_4V_4$ and bolts after 1 week of vernalization. The turnip cultivar Milan White has the genotype $V_1V_1v_2v_2v_3v_3v_4v_4$ and bolts after 5 weeks of vernalization. Recombination of these 4 major additive genes and modifier genes accounts for most of the observed segregations.

The complex nature of the vernalization processes and the interplay between environmental and genetic factors would suggest quantitative inheritance. Apparently in *Brassica*, variation in the vernalization requirement was evolved through selection for specific genes which had major effects on the vernalization processes. As mentioned in the literature review, the primary genomes of *Brassica* have developed through duplication of specific chromosomes of the basic set of 6 chromosomes. If these major genes are situated on a specific chromosome which is found in higher dosage in one species as compared to others, then variation for vernalization requirement between these species could result from a dosage effect of particular chromosomes. For example, Chinese cabbage (*a* genome) has three *F* chromosomes while cabbage (*c* genome) has only one *F* chromosome (Robbelen, 1960). A similar dosage effect was observed by Nwankiti (1971) for leaf serration in progeny from a cross between *B. campestris* ssp. *chinensis* and *B. napus*. Therefore, if such a dosage effect does exist, breeding for bolt resistant Chinese cabbage through interspecific crosses with _B_. _napus_ should be possible as noted in this study of the late-bolt Chiklae line LB-7. Based on the additive gene action theory on vernalization requirement, a system to concentrate the alleles into the inbreds for use in hybrid production is suggested. Interspecific hybridization drastically reduces the horticultural quality required in Chinese cabbage and therefore recombining desirable quantitative traits such as heading, flavor, and texture with genes for bolt resistance will require time and patience.

BIBLIOGRAPHY

BIBLIOGRAPHY

Asian Vegetable Research and Development Center. 1980. Annual Report for 1979. Shanhua, Taiwan, Republic of China.

Bailey, L. H. 1922. The cultivated Brassicas. Gentes Herbarium. 1: 53-108.

Balicka, M. 1981. Some features of the F_2 generation obtained in the result of interspecific crossing between Brassica campestris and Brassica oleracea. Cruciferae Newsletter No. 6: 30.

________, B. Barcikowska, W. Mlyniec, and E. Zwierzykowska. 1980. Synthesis of new genotypes within the genus Brassica. Cruciferea Newsletter No. 5: 28.

Borchers, E.A. and R.T. Taylor. 1978. Intercrossing in the genus Brassica. The Vegetable Growers News 33 (6): 3-4.

Boswell, V.R. 1929. Studies of premature flower stalk formation in wintered-over cabbage. Bull. Agr. Exp. Sta. Univ. Maryland 313: 69-145.

Bowkamp, J.C. and S. Honma. 1970. Vernalization response, pinnae number, and leaf shape in celery. J. Hered. 6(3): 115-118.

Calder, R.A. 1929. Interpollination of Brassicas: Its significance in relation to seed production. New Zeal. J. Agr. 55: 299-308.

Catcheside, D.G. 1934. The chromosomal relationships in the swede and turnip groups of Brassica. Ann. Bot. 48: 601-633.

Chailakhyan, M. 1968. Flowering hormones of plants. In Biochemistry and Physiology of Plant Growth Substances. Eds F. Wightman and G. Settlefield. pp. 1317-1340.

Cheng, K.H. and E.L. Moore. 1968. Relation of seedling size and length of cold exposure to the incidence of flowering in Brassica oleracea L. var. acephala D.C. Proc. Amer. Soc. Hort. Sci. 93: 363-367.

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

Chroboczek, E. 1931. Premature seedstalk formation in table beets. Proc. Amer. Soc. Hort. Sci. 28: 323-327.

________. 1934. A study of some ecological factors influencing seed-stalk development in beets (Beta vulgaris L.). Cornell Agr. Exp. Sta. Mem. 154: 83 pp.

Cooper, H.P. 1923. The inheritance of spring and winter growing habit in crosses between typical spring and winter wheats and the response of wheat plants to artificial light. J. Amer. Soc. Agron. 15: 15-24.

Crane, M. B. 1943. The origin and relationship of the Brassica crops. Nature 150: 431.

Curtis, D.F. and H.T. Chang. 1930. The relative effectiveness of the temperature of the crown as contrasted with that of the rest of the plant upon the flowering of celery plants. Amer. J. Bot. 17: 1047-1048.

Curtis, D.F. and D.G. Clark. 1950. An Introduction to Plant Physiology. McGraw Hill Book Co., New York.

Detjen, L.R. and C.A. McCue. 1933. Cabbage characters and their heredity. Del. Agr. Exp. Sta. Bull. 180 Tech. Bull No. 13.

Dickson, M.H., B. Rieger, and C.E. Peterson. 1961. A cold unit system to evaluate bolting resistance in carrots. Proc. Amer. Soc. Hort. Sci. 77: 401-405.

Elmsweller, S.L. 1934. Premature seeding in inbred celery lines. Proc. Amer. Soc. Hort. Sci. 31: 155-159.

Fantes, J.A. and G.R. Mackay. 1979. The production of disomic addition lines of Brassica campestris. Cruciferae Newsletter No. 4.

Gaines, E.F. 1917. Inheritance in wheat, barley, and oat hybrids. Wash. Agr. Exp. Sta. Bull. 135.

Gotoh, T. 1979. Genetic studies on growth habit of some important spring wheat cultivars in Japan, with special reference to the identification of the spring genes involved. Japan. J. Breed. 29: 133-145.

________. 1980. Gene analysis of the degree of vernalization requirement in winter wheat. Japan. J. Breed. 30(1): 1-10.

Hall, A.D. 1928. Bateson's experiments on bolting in sugar beets and mangolds. J. Genet. 10:219-231.

Harberd, D.J. and E.D. McArthur. 1980. Meiotic analysis of some species and genus hybrids in the Brassiceae. Chapter 4. In Brassica Crops and Their Wild Allies. S. Tsunoda, K. Hinata, and C. Gomez-Campo, eds. Scientific Soc. Press, Tokoyo, Japan.

Heide, O.M. 1970. Seedstalk formation and flowering in cabbage I. Daylength, temperature and time relationships. Scientific Rep. Agr. Coll. Norway 49(27): 1-21.

Honma, S. 1959. A method for evaluating resistance to bolting in celery. Proc. Amer. Soc. Hort. Sci. 74: 506-513.

________. 1981. Challenges in breeding for bolt resistance in Chinese cabbage. In Chinese Cabbage Proc. 1st Intl. Symp. Eds. N.S. Talekar and T.D. Griggs. A.V.R.D.C., Shanhua, Tainan, Taiwan, China.

________, and O. Heeckt. 1960. Results of crossing Brassica pekinensis (lour.) Rupr. with B. oleracea L. var. acephala Dc. Euphytica 9: 243-246.

Hosoda, T. 1950. On new types of napus crops obtained from artificial amphidiploids I. A new type as a forage crop. Ikushukenkyu 4: 91-95. (Japanese).

Inomata, N. 1978. Production of interspecific hybirds in B. campestris x B. oleracea by culture in vitro of excised ovaries I. Development of excised ovaries in the cross of various cultivars. Japan. J. Genet. 53(1): 1-11.

________. 1980. Hybrid progenies of the cross, Brassica campestris X B. oleracea I. Cytogenetical studies on F_1 hybrids. Japan. J. Genet. 55(3): 189-202.

Ito, H. and T. Saito. 1961. Time and temperature factors for flower formation. Tohoku J. Agr. Res. 12: 297-316.

Jahr, W. 1962. Befruchtungsbiologie und allopolyploidie bei der artkreuzung sommerraps x Chinakohl. Zuchter 32: 216-225. (English Summary.).

Jelinkova, E. 1971. Some information on cross pollination in Chinese cabbage. Hort. Abst. 6464.

Kagawa, A. 1966. Studies on the effect of thermo-induction in floral initiation of Chinese cabbage. Res. Bull. Fac. Agr. Gifu Univ. 22: 29-39. (English Summary).

Kagawa, A. 1971. Studies on the inheritance of flower inductive habits in Brassica crops. Res. Bull. Fac. Agr. Gifu Univ. 31: 41-62.

Kamala, T. 1978. Basic chromosome number and probable origin of the genomes in Brassica. Curr. Sci. 47(4): 128-129.

Karpechenko, G.D. 1922. The number of chromosomes and the genetic correlation of cultivated cruciferae. Bull. Appl. Bot. Genet. Pl. Breed. 13: 3-14.

Lammerink, J. 1970. Interspecific transfer of clubroot resitance from B. campestris L. to B. napus L. N. Z. J. Agr. Res. 13: 105-110.

Lang, A. 1965. Physiology of flower initiation. Encyl. Plant Physiol. 15: 1422-1536.

Little, T.M. and F.J. Hills. 1978. Agricultural Experimentation. John Wiley and Sons, Inc. 350 pp.

Lorenz, O. 1946. Response of Chinese cabbage to temperature and photoperiod. Proc. Amer. Soc. Hort. Sci. 47: 309-319.

Mackay, G.R. 1977. The introgression of S-alleles into forage rape Brassica napus L. from turnip, Brassica campestris L. spp. rapifera. Euphytica 26: 511-519.

Marrewijk, N.P.A. 1976. Artificial cold treatment, gibberellin application and flowering response of kohlrabi (Brassica oleracea L. var. gongylodes) Scientia Hort. 4(4): 367-375.

Matsui, T., H. Eguchi and K. Mori. 1978. Mathematical model of flower stalk development in Chinese cabbage in response to low temperature. J. Fac. Agr. Kyushu Univ. 22: 233-241.

McNaughton, I.H. 1973. Brassica napocampestris L. (2n = 58): Synthesis, cytology, fertility and general considerations. Euphytica 22: 301-309.

Miller, J.C. 1929. A study of some factors affecting seed-stalk development in cabbage. Cornell Univ. Agr. Exp. Sta. Bull. 488: 1-46.

Mizushima, U. 1950. Karyogenetic studies of species and genus hybrids in the tribe Brassiceae of Cruciferae. Tohoku J. Agr. Res. 1: 1-14.

Moore, E.L. 1958. Eleven varieties in collards tests. Miss. Agr. Exp. Sta. Farm Res. 21: 10.

Mori, K., H. Eguchi and T. Matsui. 1979. Mathermatical model of flower stalk development in Chinese cabbage affected by low temperature and photoperiod. Environmental Control in Biol. 17(1): 17-26.

Morinaga, T. 1929. The cytology of F_1 hybrids of B. napella and various other species with 10 chromosomes. Cytologia 1: 16-27.

Moue, T. 1979. Interspecific hybridization between an A-genome species (Chinese cabbage) and a C-genome species (cabbage). Bull. Fac. Agr. Niigata Univ. 31: 29-36.

Nakagawa, H. and S. Henmi. 1955. Studies on the effect of low temperature treatment on the production of seeds of Brassica pekinensis in the autumn. Jap. J. Breed. 4: 161-163. Abstr.

Nakamura, E. 1976. The culture of Chinese cabbage in Japan. Shiga Agr. Coll. Kusatsu, Shiga, Japan. Mimeo. Rep.: 1-18.

Namai, H. 1971. Studies on the breeding of oil rape (Brassica napus var. oleifera) by means of interspecific crosses between B. campestris ssp. oleifera and B. oleracea. Japan. J. Breed. 21(1): 40-48.

Nieuwhof, M. Cole Crops, Botany, Cultivation, and Utilization. London L. Hill, 1969.

Nishi, S. 1981. Hakuran, an interspecific hybrid between Chinese cabbage and common cabbage. In Chinese cabbage Proc. 1st Intl. Symp. Eds. N.S. Talekar and T.D. Griggs. A.V.R.D.C., Shanhua, Tainan, Taiwan, China.

________, J. Kawata and M. Toda. 1962. Studies on the embryo culture in vegetable crops. II. Breeding of interspecific hybrids between cabbage varieties and Chinese cabbage varieties through the application of embryo culture techniques. Bull. Hort. Res. Sta. Japan Ser. A. 1: 111-156.

________, M. Toda and T. Toyoda. 1970. Studies on the embryo culture of vegetable crops. III. On the conditions affect to embryo culture of interspecific hybrids between cabbage and Chinese cabbage. Bull. Hort. Res. Sta., Japan Ser. A. 9: 75-100.

Nwankiti, O. 1970. Cytogenetic and breeding studies with Brassica I. Cytogenetic experiments with Brassica napocampestria. Hereditas 66: 109-126.

Nwankiti, O. 1971. Cytogenetic and breeding studies with Brassica II. Progenies from backcrosses involving primary hybrids between B. napus and B. campestris. Hereditas 68: 35-46.

Olsson, G. 1960. Species crosses within the genus Brassica II. Artificial Brassica napus L. Hereditas 46: 351-386.

Parham, P.H. and E.L. Moore. 1959. The effect of low temperature exposure on the vegetative and reproductive growth of collards. Proc. Amer. Soc. Hort. Sci. 73: 367-373.

Patil, J.A. 1981. Studies on the effect of temperature on the physiological response of vegetable crops. Res. J. Mahatma Phule Agr. Univ. 3(2): 82-88.

Pearson, O.H. 1928. A suggested classification of the genus Brassica. Proc. Amer. Soc. Hort. Sci. 25: 105-110.

Permadi, A.H. 1974. A method to induce the flowering of Chinese cabbage, variety Granat, under tropical conditions. In Res. Rep. Agr. Coop. Project Sec. II: Tech. Contribution: 345-356.

Peto, F.H. 1934. The cause of bolting in swede turnips (Brassica napus var. napobrassica L. peterm.). Canadian J. Res. 11(6): 733-749.

Prakash, S. and K. Hinata. 1980. Taxonomy, cytogenetics and origin of Brassicas, a review. Opera Bot. 55: 1-57.

Purvis, O.N. 1939. Studies in vernalization of cereals. V. The inheritance of the spring and winter habit in hybrids of Petkus rye. Ann. Bot., N.S. 3: 719-729.

________. 1940. Vernalization of fragments of embryo tissue. Nature 145: 462-467.

________. 1966. The physiological analysis of vernalization. Encyl. Plant Physiol. 16: 76-117.

________, and F.G. Gregory. 1937. Studies in vernalization of cereals. I. A comparative study of vernalization of winter rye by low temperature and by short days. Ann. Bot., N.S. 1: 569-592.

Ragionieri, A. 1920. Brassica crosses. Gard. Chron. 68: 60.

Richharia, R.H. 1937. Cytological investigations of 20-chromosome species of Brassica and their F_1 hybrids. J. Genet. 34: 45-55.

Robbelen, G. 1960. Beitrage zue analyse des Brassica genoms. Chromosoma 11: 205-226. (English Summary).

Sakr, E.S. 1944. Effect of temperature and photoperiod on seed-stalk development in turnips. Proc. Amer. Soc. Hort. Sci. 49: 473-478.

Sarashima, M. 1964. Studies on the breeding of artificially synthesized rape (Brassica napus). I. F_1 hybrids between B. campestris group and B. oleracea group and the derived F_2 plants. Japan. J. Breed. 14: 226-236.

Shinohara, S. 1959. Genecological studies on the phasic development of flowering centering on the cruciferous crops, especially on the role of the vernalization of ripening seeds. Shizuoka Perfectur Agr. Exp. Sta. Tech. Bull. No. 6: 2-120.

Sikka, S.M. 1940. Cytogenetics of Brassica hybrids and species. J. Genet. 40: 441-509.

Sinskaia, E.N. 1927. Geno-systematical investigations of cultivated Brassica. Bull. Appl. Bot. Genet. Pl. Breed. 17: 3-166.

Snell, C.L. 1977. Brassica oleracea x B. campestris hybrids in forage rape and swede (B. napus) breeding. In Interspecific Hybridization in Plant Breeding. Proc. 8th Eucarpia Congress. Madrid, Spain.

Stout, M. 1946. Relation of temperature to reproduction in sugar beet. J. Agr. Res. 72: 49-68.

Street, H.E. and H. Opik. 1975. The Physiology of Flowering Plants: Their Growth and Development. Amer. Elsevier Publishing Co., New York. 280 pp.

Sutton, E.P.F. 1924. Inheritance of bolting in cabbage. J. Hered. 15: 257-260.

Thomas, T.H. 1980. Flowering of brussels sprouts in response to low temperature treatment of different stages of growth. Scientia Hort. 12(198): 221-229.

Thomas, P.T. and M.B. Crane. 1942. Genetic classification of Brassica crops. Nature 150: 431.

Thompson, H.C. 1939. Temperature in relation to vegetative and reproductive development in plants. Amer. Soc. Hort. Sci. Proc. 37: 672-679.

Tsunoda, S. and S. Nishi. 1968. Origin, differentiation and breeding cultivated Brassica. Genetics in Asian Countries XII International Cong. Genet.

U., N. 1935. Genome analysis in Brassica with special reference to the experimental formation of B. napus and peculiar mode of fertilization. Jap. J. Bot. 7: 389-452.

Van Heel, J.P.D. 1927. Inheritance or bolting in sugar beets. Genetica 9: 217-236.

Verkerk, K. 1954. The influence of low temperature on flower formation and stem elongation in brussels sprouts. Proc. Kon. Akad. Wet. Ser. C. 57: 339-345.

Wang, P.J. 1969. Studies on flower differentiation and fruiting in Brassica chinensis To-pe-tsai. J. Jap. Soc. Hort. Sci. 38: 144-149. (English Summary).

Weibe, H.J. 1972. Effect of temperature and light on growth and development of cauliflower. I. Duration of juvenile phase for vernalization. Gartenbauwissenschaft 37: 165-178.

Wellensiek, S.J. 1965. Recent developments in vernalization. Acta Bot. Neer. 14: 308-314.

Wester, R.E. and R. Magruder. 1938. Varietal and strain differences in bolting of turnips. Proc. Amer. Soc. Hort. Sci. 35: 594-598.

Yamasaki, K. 1956. Thermo-stage for the green plant of Chinese cabbage grown in spring. Tokai-Kinki Agr. Exp. Sta. Hort. Bull. 3: 31-47. (English Summary).

Yarnell, S.H. 1956. Cytogenetics of the vegetable crops. II. Crucifers. The Botanical Rev. 22(2): 81-166.

Yun, H.M. and H.K. Pyo. 1977. Studies on the genetics of bolting, flowering time and other characters in radish. Korean J. Breed. 9(1): 45-57.

Zwierzykowska, E. 1981. Interspecific hybridization within the genus Brassica by in vitro culture. Cruciferae Newsletter No. 6: 36-37.