Vernalization response in spring oilseed rape (*Brassica napus* L)

A thesis
submitted to
Faculty of Graduate Studies
University of Manitoba

by

Lee Anne Murphy, P.Ag.

In Partial Fulfilment of the
Requirements for the Degree
of
Doctor of Philosophy

Department of Plant Science
April, 1997

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-23642-0

Canada

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

VERNALIZATION RESPONSE IN SPRING OILSEED RAPE (*Brassica napus* L.)

BY

LEE ANNE MURPHY

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of DOCTOR OF PHILOSOPHY

Lee Ann Murphy 1997 (c)

Permission has been granted to the Library of The University of Manitoba to lend or sell copies of this thesis/practicum, to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film, and to Dissertations Abstracts International to publish an abstract of this thesis/practicum.

The author reserves other publication rights, and neither this thesis/practicum nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

TABLE OF CONTENTS

LIST OF TABLES

List of Tables, continued

LIST OF FIGURES

ABSTRACT

Due to the relatively short growing season, early maturity is an important characteristic for spring oilseed rape (Brassica napus L.) cultivars adapted to western Canada. Factors that could delay maturity must be assessed because of their impact on crop production.

Vernalization involves the exposure of imbibed seed or young plants to low temperatures. Response to vernalization influences plant growth prior to flowering by promoting the initiation of reproductive structures. The presence of a significant response to vernalization may delay flowering until the response has been satisfied.

A range of spring B. napus cultivars were evaluated for vernalization and photoperiod response. A series of vernalization treatments and indices of plant response were used.

The treatment of germinated seeds to a 2 week exposure at 4°C under 20 hr photoperiod was sufficient to identify a significant response to vernalization in spring B. napus. A rapid screen for vernalization response was developed which permitted the evaluation at 20 days after planting when the plants were at the 4 leaf stage. F1 hybrids, S4 inbred lines, F3 families and doubled haploid lines derived from F1 donors were evaluated for vernalization response.

Genetic analysis revealed that one major gene appears to condition the response to vernalization. The use of doubled haploid lines permitted the recovery of winter type progeny from the spring B. napus parents.

ACKNOWLEDGEMENTS

The completion of this research program and production of the doctoral thesis was possible due to the support and guidance of a number of individuals.

This thesis would not have been possible without the support of my husband, Keith. Through the planning, the coursework, the examinations, the writing and defense of this thesis, he was by my side- encouraging, assisting and providing the balance that is required for life. When our family grew to include Ryan and Brent, he was there- to help, to play and to support. My success is our success.

To my parents- Peter and Jane Armstrong; to Irene and the late William Murphy and the rest of my extended family- I thank you for your support, patience and for helping me keep my feet planted firmly on the ground.

I am grateful for the support, patience and understanding of my main advisor, Dr Rachael Scarth. The topic developed from our initial discussions, and her suggestions were appreciated. Her professionalism has been a model for me as I continue my career in agriculture.

Thank you to my committee members, Dr Peter McVetty, Dr Don Palmer and Dr Sam Badour, who provided insight and suggestions on the variety of topics examined in this thesis. My appreciation to Dr Isabel Waters, Department of

Botany, and Dr Peter Pauls, University of Guelph, for their review of the final thesis.

I would also like to thank Judith Nugent-Rigby for her technical assistance, especially in the production of the F3 plant material, and for her solid guidance on the doubled haploid technique.

I would like to recognize my colleagues at the Faculty of Agricultural and Food Sciences, for their fellowship, conversation and opinions. The University experience includes the development of a network of peers and I look forward to the continuation of our relationships, both professional and personal.

I was fortunate to receive several scholarships during the course of my doctoral studies. I would like to acknowledge the support of the Natural Sciences and Engineering Research Council and the University of Manitoba for fellowships that I received.

FOREWORD

This thesis was written using manuscript format. The first manuscript, "Vernalization response in spring oilseed rape (Brassica napus L.) cultivars" has been published in the Canadian Journal of Plant Science. The second manuscript "The effects of vernalization on apical development in spring oilseed rape (Brassica napus L.)" was presented at the Eighth Crucifer Genetics Workshop, held in Saskatoon, Saskatchewan in 1994. The third manuscript "The inheritance of vernalization response in segregating generations of spring oilseed rape (Brassica napus L.)" has not been submitted for publication, but has been written in the style of the Canadian Journal of Plant Science. The fourth manuscript "The inheritance of vernalization response in spring oilseed rape (Brassica napus L.) using doubled haploid lines" has been submitted to Crop Science.

GENERAL INTRODUCTION

The growth and development of field crops is affected by a wide range of environmental factors. Due to the relatively short growing season in western Canada, early maturity is a goal in most breeding programs. Factors that could delay maturity and hence reduce yield potential, must be studied in order to assess their impact on crop production.

Temperature and photoperiod have the greatest influence on plant development. In particular, the response to vernalization influences plant growth prior to flowering. As flowering and maturity are well correlated in Brassica napus, any factor that affects time of flowering will have an effect on maturity. Thus, the presence of a significant response to vernalization that could delay flowering until the response has been satisfied must be identified.

In addition to understanding the physiology of the plant response to an environmental cue, plant breeders are also interested in determining the effect of a response on a breeding population. Thus, the inheritance of the response, as well as an indication of the number of genes involved, must be determined.

Given the importance of environmental cues, and the limited information available, the following study was initiated. The general objective of this study was to determine the significance of the vernalization response in spring B. napus cultivars.

In the first manuscript, a range of B. napus cultivars were assessed for their response to vernalization. In the second, the effects of vernalization on the

developing apex were identified. This was be done by examining the time of transition from a vegetative to a reproductive apex and relating this to the visual appearance of the plant. The last two manuscripts examined the inheritance of the vernalization response for a select group of S4 lines. Both conventional and doubled haploid approaches were used.

The research conducted in this study provided information to breeders on the significance of a response to vernalization on the parental material prior to introduction into a spring B. napus breeding program.

2.0 LITERATURE REVIEW

Development of Canola

There are three primary Brassica species in the family Cruciferae: B. nigra, B. oleracea and B. rapa and three amphidiploid species, B. carinata, B. juncea and B. napus, developed from paired crossing between the primary diploid species (U, 1935).

History suggests that oilseed rape (B. napus L.) and oilseed turnip rape (B. rapa) were cultivated as early as the 20th century B.C. in India, and was introduced into China and Japan about 5 B.C. (Appelqvist, 1972). Oilseed rape and oilseed turnip rape are two of the few oilseeds that can be grown in temperate areas.

B. rapa and B. napus are the two species grown in the major rapeseed/canola growing areas of Canada. According to Thomas (1984), the original seed stocks of B. napus grown in Canada came from Argentina. The seed stock contained a mixture of plant types that provided the genetic material for the development of varieties in Canada.

Early breeding programs in Canada concentrated on improvements in agronomic characteristics and in oil content and quality (Downey and Rakow, 1987). In particular, the nutritional status of the oil was of concern, due to high levels of the long chain fatty acids, eicosenoic (approx. 5%) and erucic (approx. 40%) acids. In 1959, researchers identified a line, Liho, in oilseed rape that contained low levels of erucic acid. A program of backcrossing and selection

was initiated to transfer the low erucic trait into agronomically suitable cultivars for western Canada. Further selection for low erucic acid levels led to the development of zero erucic acid varieties and allowed Canada to set a maximum level of 5% erucic acid in the edible oil. Rapeseed meal was considered an excellent source of protein, with a favourable balance of amino acids, but high glucosinolate levels lead to palatability and nutritional problems. The variety Bronowski from Poland was found to be low in glucosinolates and the trait was incorporated into new varieties (Downey and Rakow, 1987).

In order to distinguish the new "double-low" varieties, with their altered oil and meal properties, from common oilseed rape, the term "canola" was trademarked in 1978 by the Canola Council of Canada, then known as the Rapeseed Association of Canada. Canola cultivars are those modern oilseed rape varieties known as double-low and characterized by containing 2% or less erucic acid content in the seed oil and less than 30 micromoles of glucosinolates per gram of oil-free meal (Canola Oil and Meal Standards and Regulations, 1987).

Rapeseed Growth and Development

Upon emergence, which occurs 4 to 10 days after seeding, the B. napus seedling develops a short stem with the growing point located above ground, between the open cotyledons. Successive leaves develop in a rosette, with up to 30 leaves present in some varieties (Daniels et al., 1986). The production of leaf primordia occurs in a spiral pattern around the apex (Smith and Scarisbrick,

1990). Bud formation is triggered by suitable conditions of temperature and daylength. A cluster of buds becomes visible at the center of the rosette of leaves and is carried up as the stem bolts. The cluster of buds enlarges as the main stem lengthens. The main stem reaches 30 to 60% of its maximum length just prior to flowering (Thomas, 1984). Flowering in B. napus begins with the opening of the lowest bud on the main stem and continues with 3 to 5 or more flowers opening per day. The first flower opened is destined to become the first pod (Daniels et al., 1986). Oilseed rape is an indeterminate plant, because it retains the capacity to initiate new reproductive racemes throughout most of its lifecycle. Thus, the full range of reproductive structures may be present on the plant at any one time.

Growth Stage Keys for Oilseed Rape

Many agronomic decisions are made based on operations performed at specific stages of growth. However, the division of oilseed rape growth and development into distinct phases is difficult due to its indeterminate pattern of growth. By restricting observations to the main stem, distinctions concerning stage of development can be readily determined.

Harper and Berkenkamp (1975) developed a growth stage key which has been widely used for B. napus and B. rapa. They divided the life cycle into six distinct stages, which can be further subdivided (Table 1).

Table 1. Harper and Berkenkamp (1975) key to growth stages in rape (Brassica rapa and B. napus).

Stage	Description	Sub Groups
0	Pre-emergence	
1	Seedling	
2	Rosette	
2.1		First true leaf expanded
2.2		Second true leaf expanded add 0.1 for each additional leaf
3	Bud	
3.1		Inflorescence visible at center of rosette
3.2		Inflorescence raised above level of rosette
3.3		Lower buds yellowing
4	Flower	
4.1		First open flower
4.2		Many flowers open; lower pods elongating
4.3		Lower pods starting to fill
4.4		Flowering complete; seeds enlarging in lower pods
5	Ripening	
5.1		Seeds in lower pods full size, translucent
5.2		Seeds in lower pods green
5.3		Seeds in lower pods green-brown and mottled
5.4		Seeds in lower pods brown
5.5		Seeds in all pods brown, plant senescent

Sylvester-Bradley et al. (1984) used the Harper and Berkenkamp key as the basis for a new system that could be used for all the oilseed Brassica species and production systems in the world. The new scale allowed for a greater degree of precision, as well as an improved data entry system that was more amenable to winter oilseed rape development. A revision of the original

Sylvester-Bradley scale was required in order to reflect the conflicting requirements of researchers for detail, and farmers for ease of use (Sylvester-Bradley, 1985).

A concern expressed about the use of growth stage keys is that they tend to mask a wide range of physiological stages (Smith and Scarisbrick, 1990). For example, the Harper and Berkenkamp stage of 3.1 covers the internal period of transition from a vegetative to a reproductive apex to sepal formation on flower buds. Tommey and Evans (1989) constructed a key that defined the stages of flower initiation and floral development for winter oilseed rape. Based on samples of the terminal growing point, their key provided a score from 1 (flat, vegetative index) to 10 (bud visible to the naked eye). In order to use the Tommey and Evans (1989) key, large populations are required to permit the frequent, destructive sampling required for assignment of a score.

Environmental Effects on Development

Smith (1990) used the term 'phenotypic plasticity' to describe the ability of individual plants to adapt or acclimate to changing circumstances. Phenotypic plasticity has three features: flexible expression of a single genotype, an intimate connection with environmental variation, and an adaptive value of the capacity of an individual to adapt to environmental conditions. The sequence of response includes: perception of the environmental signal, transduction of the

environmental signal to a biological signal, and evocation of a physiological response to the environmental condition.

Development in crops is a function of both genetic and environmental parameters. Thermal and light conditions appear to have the greatest influence of the environmental parameters on plant development (Daughtry et al., 1984).

A major objective in Canadian oilseed rape breeding programs has been early maturity (Campbell and Kondra, 1978). Breeding for early maturity usually involves selection for earliness to first flower, which is used as an indicator of maturity. Campbell and Kondra (1978) found that the correlation between time to first flower (in days) and time to maturity (in days) was significant and large to justify the use of appearance of first flower as a selection criteria. Collins and Wilson (1974) indicated that time of floral initiation and flowering were closely correlated with each other and with maturity. They concluded that time of initiation and flowering were the primary determinants of maturity. Thus, any factor, such as vernalization or photoperiod requirement, which affected the period from seeding to first flower would have a significant influence on time to reach maturity.

Tommey and Evans (1992) found that temperatures during early vegetative growth greatly influence the time of floral initiation and mainstem leaf number. Exposure to low temperature from the time of cotyledon expansion onward reduced the length of the pre-floral growth stages. Tommey and Evans

(1992) reported that the subsequent developmental pattern of oilseed rape was largely dependent on the date of floral initiation.

Thurling and Kaveeta (1992) divided pre-anthesis development in oilseed rape into three distinct segments: vegetative development (the period from sowing to floral initiation); post-initiation (initiation to commencement of stem elongation) and stem elongation (stem elongation to appearance of the first open flower). The summation of the three phases equaled the time to flower.

Both soil and air temperature affect oilseed rape growth and development (Thomas, 1984). There are few reports available on the direct effects of temperature on rapeseed development (Morrison et al., 1989). Studies that have identified the indirect effects of increasing temperature by manipulation of seeding date can be confounded by interactions with changing photoperiod and moisture conditions available for growth. To mitigate these confounding factors, Morrison et al. (1989) conducted both field and controlled environment studies of the phenological development of the canola cultivar 'Westar'. Morrison et al. (1989) found that the phenological development of 'Westar' grown under controlled conditions was not significantly different from 'Westar' grown in the field. Morrison et al. (1989) developed a regression equation to describe the phenological development of Westar. The model developed by Morrison et al. (1989) was less accurate in describing the development of 'Westar' during the early and mid phases of growth, and more accurate when the crop was maturing.

Early seeding of oilseed rape is desirable, in order to use as much of the frost-free period as possible (Kondra et al., 1983). However, cold soil conditions could result in delayed emergence. Morrison et al. (1989) determined the baseline temperature, defined as that temperature at which phenological development ceases, for the oilseed rape cultivar 'Westar' to be 4.8 +/- 1.5 °C.

Thomas (1984) reported an optimum temperature for oilseed rape growth and development of just over 20 °C. An optimum temperature is one at which the rate of growth is within a range of the maximum rate (Fitter and Hay, 1981).

Photoperiod.

Photoperiodism is a growth response to the relative lengths of day and night. Oilseed rape has been classified as a facultative long day plant, in that flowering will occur over a wide range of photoperiods, but is significantly accelerated by an increase in daylength (Gauss and Taylor, 1969 and King and Kondra, 1986). Major (1980) studied the photoperiodic responses of nine representative cultivars of crop species in Canada, including B. napus and B. rapa. He indicated that there were differences between the crop species grown in Canada with respect to optimal photoperiods and photoperiodic sensitivities. The optimal photoperiod was found to exceed 18 hours for both Brassica species.

Natural daylength in the majority of the oilseed rape growing area in Canada is between 15 and 17 hours during the period of spring growth prior to flowering. Therefore the optimal photoperiod may not be reached prior to floral

initiation (King and Kondra, 1986). The influence of photoperiod on development of oilseed rape requires further investigation in order to determine the practical significance of its influence on development.

Photoperiod and Temperature Interaction.

Research on the effects of the photoperiod and temperature interaction on the development of oilseed rape is very limited. However, work done in other crop species may have application to oilseed rape.

Weibel (1958) found that the photoperiod during the vernalization treatment of wheat embryos did not influence either the length of the cold treatment required, or the response to photoperiod after the vernalization treatment. Weigand et al. (1981) indicated that winter wheat grown under 11 hour days in a 24 hour cycle could lose the earliness to flower effect produced by vernalization. Extending the vernalization period was found to partially overcome the limiting effects of short days. Mosaad et al. (1995) examined a series of selected and unselected spring wheat lines and found that vernalization resulted in a decrease of 3.5 leaves on the main stem at flowering for the unselected group. They found no response in the selected group, which was based on selection under a heat stress environment. Mosaad et al. (1995) concluded that the selection pressure resulted in the elimination of lines with strong vernalization response.

The interaction between photoperiod and vernalization appears to be of value in adapting a cultivar to a particular growing area (Berry and Aitken, 1979 and Davidson et al., 1985). Potential cultivars are assessed under conditions that are indicative of the growing area. Myers et al. (1982) screened 48 lines of oilseed rape for possible use in Australia, using vernalization and photoperiod conditions that would be comparable to the winter conditions in New South Wales. Myers et al. (1982) cautioned that their treatment of 2 week old plants exposed to 8/6 °C day/night temperatures under an 8 hour photoperiod was meant to allow differentiation among lines, not saturation of responses. They felt that relative comparisons, not absolute values, were of the most significance in their study. Mosaad et al. (1995) felt that, while both photoperiod and vernalization response affected development, selection of spring wheat cultivars for growth in the tropics would be more effective and consistent if based on the photoperiod response (Mosaad et al. 1995). Mosaad et al. (1995) cite the cultivar 'Cham 6' as an example of a cultivar that is insensitive to both photoperiod and vernalization, which they felt contributed to its wide adaptability.

Vernalization.

The term vernalization is the anglicized version of the Russian word "jaroxization", which refers to the process that made a winter cereal behave like a summer cereal (Lysenko, 1928, cited by Chouard, 1960). Further investigation

revealed that the determining factor was exposure to cold temperatures for a few weeks.

There is some confusion in the literature regarding the usage of the term vernalization. McKinney (1940) indicated that the term was used to refer to almost all environmental factors or other methods applied at any time in the plant's development which were capable of accelerating sexual reproduction. Chouard (1960) recommended that researchers follow the original definition, which specifically involved the substitution of chilling of a plant for the natural exposure to winter, in order to make possible the initiation of floral primordia. Chouard (1960) indicated that there were four conditions required for effective vernalization: minimum moisture, so as to allow sufficient seed imbibition; a period of activation prior to application of the vernalization treatment, approximately 24 hours; presence of oxygen and an appropriate temperature and duration of chilling.

Theories on the mechanism of control of flowering range from the florigen-antiflorigen model (Chouard, 1960) to the multifactorial model of control advanced by Bernier (1988). Bernier (1988) felt that the possibility of a complex, integrated response such as flowering being controlled by a simple promoter:inhibitor model was not satisfactory. Instead, Bernier (1988) felt that a multifactorial model of control, involving the diversion of nutrients and involving Plant Growth Regulators, best explained the complexity of the floral process.

Bernier (1988) recommended comprehensive studies, using flowering and PGR mutants, in order to determine the molecular biology and genetics of flowering.

Vernalization is an unusual biological process in that it appears to have a negative Q10, with the lower the temperature, the faster the reaction proceeds (Napp-Zinn, 1987).

Effects on plant development As with most experiments that examine environmental effects on plant development, researchers working with the vernalization response have attempted to relate effects to the phenological development of the plant. Presence of a vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied (Flood and Halloran, 1984). In fall-planted oilseed rape cultivars grown in Europe, the transition to a floral apex occurs during the first two weeks of November (Scarisbrick et al., 1989). However, the date of transition is influenced by cultivar, sowing date, climate and geographic location.

Measurements of the days required for floral initiation would therefore appear to be an important method for assessing vernalization response. Investigators have used this approach in Triticum aestivum (Wort, 1941; Halse and Weir, 1970, Syme, 1973 and Manupeerapan et al., 1992), Trifolium subterraneum (Evans, 1959 and Salisbury et al., 1987) and Pisum sativum (Berry and Aitken, 1979). One major disadvantage of using days to initiation is

that it is determined by destructive sampling and thus requires a large number of plants.

Non-destructive measurements usually focus on the visually distinct flowering phase, including heading in cereals and appearance of first open flower in dicots. Levy and Peterson (1972) considered days to head to be a good indicator of vernalization in spring wheat. Flood and Halloran (1984) indicated that there was a strong linear relationship (r=0.86) between days to initiation and days to ear emergence in wheat. Given that days to ear emergence was easier to determine than days to initiation on large numbers of plants, they concluded that days to ear emergence could provide an accurate index of relative vernalization response.

Trione and Metzger (1970) focused on the apical meristem when they studied vernalization in wheat and Hordeum vulgare. They used the distance between the apical meristem and the crown as a measure of degree of vernalization. The longer the cold treatment, the greater the distance between apex and crown. Manupeerapan et al. (1992) recorded apex length and width midway up the spike in order to determine apex volume. They discovered that apex size in wheat at the time of initiation was relatively uniform (0.13 mm^3), despite a wide range in time (26 to 224 days) and growing degree days (220 to 1900 GDD) required for floral initiation.

In cereals, leaves are initiated on the meristem until a floral bud is initiated (Quinby and Liang, 1969). If initiation is delayed, more leaves are

formed. In wheat, floral organs appear soon after differentiation of the last leaf primordium (McKinney and Sando, 1935). Early differentiation of floral organs results in fewer leaves being formed.

Halloran (1975) used regression of final leaf numbers between vernalized and nonvernalized wheat lines to indicate the significance of the vernalization response. Differences in slopes of the lines indicated a significant vernalization response. In a later study, however, Salisbury et al. (1979) cautioned that it was not valid to use leaf number to evaluate the effect of vernalization unless uniform post-vernalization temperature conditions are maintained. Berry et al. (1980) indicated that final leaf number was not as sensitive a parameter as days to anthesis for measuring vernalization response in wheat. Jedel et al. (1986) found final leaf number and days to anthesis to be positively correlated in wheat. They suggested that days to flag leaf emergence, which included the environmental effects on final leaf number, was a useful parameter that was easy to measure. Karlsson et al. (1993) used both final leaf number and number of days to flowering for determination of the effects of vernalization and photoperiod on 32 Arabidopsis thaliana (Brassicaceae) ecotypes. The study by Karlsson et al. (1993) found a high correlation between leaf numbers and days to flower ($r=0.94$), but indicated that they preferred to use leaf numbers, although they did not provide any additional reasons for this preference.

Friend (1985) reported that it is often difficult to determine the final leaf number in the Brassica species, as the size of the vegetative leaves becomes

reduced at the base of the inflorescence. Upper leaves may be missing or reduced to bracts. Thurling and Kaveeta (1992) felt that leaf number in oilseed rape, as an ontogenic measure of development, reflected more closely the morphogenetic mechanisms determining floral initiation. Their study found variability in leaf number on the main stem of the cultivars they examined.

Vernalization response patterns. The expression of vernalization response can be characterized by its pattern of development. The majority of the research on characterization of the vernalization response has been done in cereals, particularly wheat. Winter wheat, in general, has an absolute requirement for cold treatment in order to respond to light and temperature conditions that result in flowering under conditions conducive to optimum growth in the spring. Spring wheats, however, have been considered to be unresponsive to cold treatment, although this distinction is not absolute.

Three vernalization response patterns have been identified. They include: cumulative, with quantitative decreases in length of vegetative period with increased duration of cold treatment (Levy and Peterson, 1972); irregular, characterized by plateaus and increases in length of the vegetative period with extended cold (Halloran, 1977) and a threshold response, an all-or-nothing response that only occurs with extended periods of cold (Berry et al., 1980).

Spring sown crops can vary in the amount of vernalization that will produce a response. Rahman and Gladstones (1973) found a quantitative

response in Lupinus, with different species varying in the period of vernalization required to saturate the response. A quantitative response in spring wheat has been identified (Purvis and Gregory, 1937; Levy and Peterson, 1972; Wall and Cartwright, 1974 and Halloran, 1975).

Physiology of vernalization. Vernalization has been found to be effective in germinating or actively growing seeds, apical meristems, leaf cuttings and root cuttings (Wellensiek, 1964). Wellensiek (1962) identified the locus of vernalization as consisting of cells which are in mitosis, with dividing cells appearing to be a prerequisite to effective vernalization.

Weibel (1958) described the standard method of vernalization as a cold treatment of germinating seeds. Variations in methodology involve differences in temperature and duration of temperature treatment. Weibel (1958) discussed competence of seedlings as being a characteristic of those seedlings with a growing apex that can respond to stimuli that induce floral differentiation. Vernalization response acts to confer competence early in vegetative development.

Cold treatments are most frequently given to imbibed or germinating seeds (Gauss and Taylor, 1969; Weinberger, 1975; Halloran, 1977 and Weigand et al., 1981). However, vernalization effects have been observed when the cold treatment is applied to other stages of development. Purvis (1939) successfully treated excised embryos of Petkus winter rye, while Gauss and

Taylor (1969) found that vernalization in the early seedling stage of spring wheat reduced the time to head emergence. Evans (1959) found that vernalizing developing embryos, germinating seeds or young seedlings of clover hastened floral initiation. Tommey and Evans (1992) found that exposure to vernalizing temperatures from cotyledon expansion onward in the winter rape cultivar Mikado markedly reduced the duration of the pre-floral growth stage. Whelan and Schaalje (1992) successfully vernalized the embryogenic callus of immature embryos of Norstar winter wheat.

Different temperature regimes have been employed with varying degrees of success in the study of vernalization. Trione and Metzger (1970) identified temperatures capable of vernalizing different crop species, ranging from -5 to +15 °C in winter wheat, to +1 to -1.5 °C in Beta. In general, temperatures in the range from +1 to +7 °C were the most effective. As vernalization involves a biochemical process, temperatures below freezing are ineffective, as metabolic activity ceases below a minimum temperature (Ahrens and Loomis, 1963).

There are other environmental factors both during and after cold treatment which may influence the vernalization response. Peterson and Bendixen (1963) indicated that photoperiod conditions before and after cold treatment had a confounding effect. They eliminated the photoperiod interaction by keeping Lolium temulentum seeds in the dark during imbibition and chilling, after which continuous light was used. Barber (1959) had indicated a similar interaction in peas, as well as in the Cruciferae family. As seed vernalization

occurs in the dark before emergence under field conditions, it is not apparent why light during vernalization would be necessary.

Temperature and moisture conditions can also affect the vernalization process. Work with clover by Evans (1959) indicated that fully vernalized plants had a consistent hastening of flowering with increased mean temperature in the post-vernalization period.

Salisbury et al. (1979) cautioned that, at least for some genotypes, the way in which the vernalization treatment is applied could influence the response. They indicated that the rate of reproductive development of cultivars vernalized as imbibed seeds would not necessarily reflect their development rate when vernalized naturally in the field.

Weinberger (1975) stressed the importance of maintaining moisture conditions above a critical level during vernalization. There is disagreement concerning the ideal moisture conditions, with some researchers preferring to limit moisture in order to restrict growth, while others prefer to imbibe seeds for a standard length of time, without controlling moisture (Syme, 1968, and Klaimi and Qualset, 1974).

The phenomena of devernalization, or loss of the vernalized condition, has been suggested (Gregory and Purvis, 1938; Weibel, 1958; Gowers and Gemmell, 1968 and Trione and Metzger, 1970). The most common devernalizing agent is high temperature, although devernalization by short day conditions (Wellensiek, 1964) and dry storage (Weibel, 1958) has also been

reported. Although exposure to high temperature can lead to devernalization, the vernalized condition is considered to be stable and appears to be perpetuated in the cell division products of vernalized cells (Thomas and Vince-Prue, 1984).

Vernalization response in the Brassica species. The majority of research on the process of vernalization has been conducted in the cereals. As the importance of the Brassica species to producers has increased, so too has the amount of research.

Most members of the Cruciferae possessing a cold requirement are biennial. Research work involving B. oleracea, including Chinese cabbage, cauliflower and Brussels sprout, indicates that there is a quantitative requirement for low temperature induction of early flowering (Gauss and Taylor, 1969). The temperature range required for vernalization response in Brassica species varied from 0 to 8 °C, with duration of effective treatment ranging from 10 days to 20 weeks (Friend, 1985).

Thurling and Vijendra Das (1977) examined the effects of different temperatures and photoperiods on the pre-anthesis development of spring oilseed rape. They studied a range of cultivars from Canada, Europe and Japan in order to determine the relative importance of different climatic variables on the duration of pre-anthesis development stages. Research on the Canadian cultivars Target and Oro revealed that these cultivars had no response to

vernalization under a 24 hour photoperiod. The flowering of the European cultivars Bronowski and Masoweicki was accelerated by vernalization at 3 °C under all combinations of temperature and photoperiod. Thurling and Vijendra Das (1977) indicated that the differences between spring oilseed rape cultivars in vernalization response appeared to be much greater than those observed between cultivars of spring wheat.

Hodgson (1978), examined the phenology of the B. napus cultivar Midas and found that it exhibited a significant facultative response to vernalization. Hodgson concluded that vernalization significantly affected plant development prior to the bud stages, but had no direct effects on subsequent phases.

Myers et al. (1982) working with the Canadian cultivars Torch, Span and Midas, found that there was a reduction in time to flowering in response to vernalization treatment or increasing photoperiod. The response to both vernalization and photoperiod was similar and highly correlated (r=0.93). Of interest was the observation by Myers et al. (1982) that variation in response existed between two samples of the Span that came from different sources. The variation was attributed to contamination or to strong differential selection pressures on the original seed stock.

Environmental Influence on Apical Development

The visual appearance of a flower is but one step in the flowering process. There are six sequential phases of flowering: induction, evocation, primordia differentiation, flower opening, anthesis and pollination and senescence (Orr, 1984). McDaniel (1996) reviewed the developmental physiology of Nicotiana tabacum L as a conceptual model for understanding floral initiation in angiosperms. McDaniel (1996) described morphogenesis as the 'fating' of specific areas of the cell for unique patterns of growth and differentiation. Cells are competent if they respond to a particular developmental signal in a predictable way. Induction occurs when a particular signal acts on competent cells to determine them for a specific developmental fate. Expression of their fate results in the determination of the cells, whether in their normal location in the organism or in isolation.

Upon satisfaction of the vernalization requirement, and exposure of plants to the appropriate photoperiod, a floral stimulus is formed in the leaves (Bernier, 1971). The stimulus is translocated to the apical meristem, where it evokes the production of flower buds at the meristem. McDaniel (1996) felt that the term 'evocation' more precisely describes the action of the floral stimulus, since induction has already occurred at the responsive part of the plant. Bernier (1971) described the presence of target tissues, which were those meristematic tissues which, in response to the floral stimulus, specifically initiated floral

morphogenesis. In dicots, the target tissues include the central and peripheral zones of the apical meristem.

Several studies on the changes to the meristem of Brassica species have been reported. An understanding of the time of inflorescence development is important in commercial B. napus production in that, once the growing point is committed to flowering, the number of potential leaves from that meristem is also fixed and thus the size of the leaf canopy is determined within the limits of variation in individual leaf size (Mendham and Scott, 1975). The time of floral initiation can also indirectly limit yield through its effect on time of flowering. Mendham and Scott (1975) indicated that early initiation could result in floral development before plants had reached maximum potential size. Tommey and Evans (1992) reported that there was potential in oilseed rape for a profitable redistribution of metabolites to developing seeds by manipulation of the plant hierarchical structure. The goal would be to restrict assimilate availability to an excessive number of branches and flowers by restricting the period of pre-floral growth. The results of the Tommey and Evans (1992) experiment indicated that plants given a pre-floral cold treatment produced significantly higher total seed weights in spite of having fewer leaves and a shorter pre-floral growth stage. Plants that were intermediate in terms of duration of pre-floral growth and meristem leaf number produced the highest seed yields.

Friend (1985) reported that, in general, Brassica vegetative apices are dome-shaped, and become very convex at the time of transition to reproductive

development. Once floral differentiation begins, no new leaf primordia are formed and flower bud primordia initials begin to differentiate.

Orr (1978) studied the inflorescence development in B. rapa in considerable detail. He used a highly photo-sensitive Ceres strain, which had been found to respond to one inductive cycle given 4 to 5 days after sowing. Orr (1978) described the phases of inflorescence development as a series of discrete processes, with the transition from a vegetative meristem to a reproductive meristem requiring approximately 2 to 5 days. A series of papers (Orr, 1981, 1984, 1985, 1987) examined changes in cellular events occurring in the meristem during the transition to flowering. The prefloral stage is defined by a constant volume and number of cells in the meristem. During transition to reproductive apex, there are two 'waves' or series of increases in the mitotic index of the peripheral and central zones of the apex (Orr 1981). The second increase occurs simultaneously with the initiation of floral primoridia in the peripheral zone. Along with the increase in cell division, Orr (1985) measured an increase in production of enzymes (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase), increased C02 activity and increased respiration.

Polowick and Sawhney (1986) undertook a scanning electron microscope study of the initiation and development of the B. napus cultivar 'Westar'. They were able to determine that the vegetative apex, under their growing conditions of 23/18 °C day/night temperature and 16 hour photoperiod, was slightly

convex, while the floral apex was domed. An earlier study (Daniels et al., 1984) using field-grown material of winter oilseed rape, determined that the appearance of a small protuberance at the base of the apical dome was the first visible sign of initiation.

A study of B. rapa cultivar 'Wisconsin Fast' indicated that the developing floral apex follows the same pattern of development indicated by other researchers, but in a much condensed time frame (Hobbs et al., 1996). Floral induction occurred Day 7, with buds opening by Day 18.

Mendham and Scott (1975) studied field-grown winter oilseed rape in order to determine when the change to the reproductive phase occurred. The procedure used by Mendham and Scott (1975) involved the scoring of apices for stage of development. Leaf numbers at the time of initiation ranged from 18 to 24, depending on the sowing date.

Crisp and Walkey (1974) described the effects of vernalization on B. oleracea by examining meristem cultures. They found that, in the vegetative state, there is a single meristematic shoot apex with no apparent axillary apices. When vernalized, the shoot apex entered a generative phase and underwent many divisions to give the edible curd, which is a mass of apical meristems. Each of the meristems could then give rise to floral primordia.

Cauliflower has an obligate cold requirement for flowering that can only be fulfilled once the plant has passed the juvenile phase (Sadik and Ozbun, 1967). Sadik and Ozbun (1967) examined the nuclei of the vegetative shoot

apices and found them to be smaller than those that had received cold treatment.

The availability of improved laboratory techniques for isolation and identification of genes has lead to an improved understanding of the complex events occurring during the transition to flowering. Mutant strains of Arabidopsis thaliana have been identified that constitutively express the floral-meristem-identity gene *LEAFY* (Lfy), which encodes a developmental switch capable of conversion of lateral shoots to flowers from a range of different species (Weigel and Nilsson, 1995). This approach offers several advantages for the researcher over induced responses, including the fact that transcription of the Lfy gene is independent of other genes or factors affecting floral induction; and the ability to assay for activity in tissues where the genes would not normally be active, such as in shoot meristems.

Hobbs et al. (1996) studied the changes in lipid composition during floral development and found that the floral apex represents an active system with rapidly changing lipid composition. They speculated that the lipid changes could be involved in the regulation of the transition to a floral apex through permeability changes in membranes that could permit transport of metabolites during evocation.

In an attempt to understand the process involved in the change from a vegetative to a reproductive apex, Kitashiba et al. (1996) screened for genes specifically expressed in the shoot apical meristem of B. rapa during transition to

flowering. They found two cDNA clones being preferentially expressed in the floral apex that did not show significant similarity to known genes Further studies are being conducted to characterize the clones and their possible involvement in flowering.

Inheritance of the Vernalization Response

Numerous studies on the inheritance and genetics of vernalization response have been reported for cereals, with limited research done in other crops. There is some discrepancy with regards to the nature of the inheritance of the vernalization response, or more specifically, the inheritance of the spring growth habit. Complete dominance of the spring habit, indicative of no response to vernalization, was identified in the F1 progeny of wheat by several researchers (Purvis, 1939; Pinthus, 1967; Pugsley, 1971 and Pugsley, 1972). Klaimi and Qualset (1974) reported that analysis of the F1 generation indicated partial dominance of the spring growth habit. All researchers reported that the winter habit was recessive to the spring habit.

Dominance of the spring habit appears to be incomplete in the segregating generations (Purvis, 1939; Pugsley, 1963; Pinthus, 1967 and Roberts, 1990). This has been explained as being due to multiple factors affecting time of heading. Purvis (1939) suggested that the factors which controlled earliness were segregating independently of those controlling growth habit. Jedel (1994) reported that transgressively late segregants arose

whenever crosses were made between cultivars having different alleles at the 'Vrn' locus. She indicated that the different combinations of alleles could be used to create widely adapted material. Early planting of cultivars with responsive alleles in short season areas could allow induction of the vernalization response. The same cultivars, planted later in areas with longer growing seasons, would use the longer growing season due to the fact that the vernalization response may not be fully satisfied.

There have been several attempts to determine the number of genes involved in the vernalization response. In wheat, Powers (1934) and Pugsley (1971) indicated the presence of three genes. In addition to major gene action, minor genes and modifiers have been identified. Halloran and Boydell (1967) considered the expression of the vernalization response to be genetically complex, with closely linked genes producing minor effects. Klaimi and Qualset (1974) considered a lack of distinct classes in the F2 and subsequent generations to indicate quantitative inheritance, with the involvement of both major and minor genes. Flood and Halloran (1986) reported continuous variation in time of leaf emergence in wheat from spring to strong winter habit and suggested the action of many genes, rather than a small number of genes.

Studies on the inheritance of vernalization response in other crops have been done, but not as extensively as the studies in cereals. Flowering time in clover has been shown to be influenced by vernalization. Davern et al. (1957) and Salisbury et al. (1987) reported that polygenic inheritance of time to flower

was involved. Use of controlled environment conditions by Salisbury et al. (1987) allowed them to study the segregation of the vernalization response. The results from the F1 generation suggested complete dominance of early flowering, which was interpreted as indicating no vernalization requirement. F2 distributions were continuous and unimodal.

In peas, the Sn gene confers the ability to respond to photoperiod as well as the potential to respond to vernalization (Murfet, 1977). Arabidopsis thaliana has numerous genes controlling flowering that vary in their response to vernalization.

Rahman and Gladstones (1973), working with Lupinus, found partial dominance for lack of a vernalization requirement. They considered that, for practical purposes, the presence of the dominant genes would mean no vernalization was required, since some natural vernalization could be expected in the field.

Inheritance of the Vernalization Response in Brassica.

Few studies have been conducted in the Brassica species. From crosses between annual and biennial B. oleracea, Wellensiek (1960) reported that the annual habit was monogenic and dominant. The inheritance of growth habit for the biennial Brussels sprout was found to be complex, (polygenic), with additive gene action. Wellensiek (1960) speculated that since the Brussels sprout originated in the Mediterranean region from a wild cabbage, the originator was

probably annual, as phylogenetically older characters are usually dominant. He felt that the biennial form probably arose as a point mutation. Mero and Honma (1985) examined bolting resistance in an intraspecific cross between chinese cabbage and turnip and found that there was a progressive increase in bolting with increased vernalization. Their study identified two major additive genes that conditioned bolting resistance, and by inference, vernalization requirement.

While these reports do not deal directly with the response to vernalization, vernalization requirements are often used to differentiate between annual and biennial habits of growth (Murfet, 1977).

Thurling and Vijendra Das (1977) speculated that the vernalization requirement of some B. napus cultivars arose from the B. rapa genome. They based their conclusion on the fact that the Japanese cultivars used in their study were developed from crosses between B. napus and B. rapa, which resulted in a transfer of genes affecting vernalization response from B. rapa. European cultivars, in contrast, were presumed to be derived from the ancestral form of B. rapa, which is one genome donor in the allopolyploid origin of B. napus.

In a subsequent study, Thurling and Vijendra Das (1979a) determined that the vernalization requirement of B. napus was determined by recessive alleles. For example, the cultivar 'Isuzu', which had a marked response to vernalization under controlled environment conditions, possessed several recessive alleles for vernalization response.

Analysis indicated two types of genes were controlling flowering- genes for regulating vernalization response, and genes for early flowering, with no influence on vernalization. Thurling and Vijendra Das (1979b) assigned the following alleles to describe the vernalization response:

- v1- causes delay in flowering of unvernalized material, but is not expressed after 4 weeks of vernalization
- v2- slight (5 to 7 day) delay in flowering of unvernalized material
- v3- moderate (15 to 20 day) delay in flowering of unvernalized material, but is not expressed after 4 weeks of vernalization
- v4- slight (5 to 7 day) delay in unvernalized material, but is not expressed after 4 weeks of vernalization. Presence will delay flowering after 4 weeks of vernalization if v1 and v2 are present.

Based on the description of effects of the alleles, genotypes were assigned to the cultivars that were used as the source of the parental lines (Thurling and Vijendra Das, 1979b):

Target: V1V1V2V2V3V3V4V4

Isuzu: v1v1v2v2V3V3V4V4

Bronowski: V1V1V2V2v3v3v4v4

The authors concluded that the genetic mechanism in B. napus was similar to that of wheat, in that winter habit was conferred by the presence of two recessive alleles. Spring habit was determined by the presence of dominant alleles.

A third study by Thurling and Vijendra Das (1979c) proposed a breeding scheme whereby flowering time could be modified in order to maximize yield in areas where the growing season was limited. Considerable variation in the pre-

anthesis growth stages, specifically the stem elongation phase, was observed. The variation was attributed mainly to additive effects, but dominance effects were also substantial. The authors felt that it should be possible to combine a short vegetative period, a longer period for stem elongation and optimal flowering time into one cultivar. They proposed using three parents, from different geographic areas, in order to generate a segregating population that would then be screened under non-vernalizing conditions of 25 °C and continuous light. Selection for early flowering and longer stem elongation phase would be carried out prior to preliminary yield assessment.

While variation in response is essential in order to have response to selection, concerns have been raised by several researchers as to the stability of expression of vernalization response. Thurling and Vijendra Das (1979a) indicated that, while they had used single plants from the S3 generation as parents, the assumption of virtual homozygosity was probably not completely accurate.

Brandle and McVetty (1989) reported that there were few segregating loci contributing to the expression of earliness in the canola lines they studied. They suggested that the cultivars used in their study consisted of a mixture of generally homozygous lines that mainly differed by additive genes.

The development and refinement of tools of biotechnology has added new methodology to the study of inheritance of the vernalization response.

Van Deynze and Pauls (1994) examined DH lines from the F1 of a cross between spring and winter B. napus. They detected two gene differences that were vernalization dependent between the winter and spring parent and classified them as major gene "A" and minor gene "B". The presence of major gene "A" was sufficient to allow flowering in less than 62 days without vernalization. Minor gene "B" allowed plants to flower in between 62 and 77 days. Plants with the double recessive genotype required more than 77 days to flower in the absence of vernalization.

Ferreira et al. (1995) used DH lines derived from the F1 of a cross between B. napus cultivars Major (biennial) and Stellar (annual) and mapped the loci controlling vernalization requirement and flowering time. They confirmed that vernalization response was conditioned by recessive gene action. The results indicated that a major locus of linkage group 9 (LG9), or closely linked loci, affected flowering time through vernalization requirement. The early lines had alleles predominately from the annual parent, Stellar, while the non-flowering lines had alleles primarily from the biennial parent, Major. In a related study in B. rapa, Teutonico and Osborn (1995) reported that more than one major gene was involved in the response of B. rapa to vernalization. They reported that vernalization requirement was dominant, as the F1 did not flower without vernalization. They also concluded that vernalization requirement should be treated as a quantitative, rather than qualitative trait. The assignment of annual vs. biennial or spring vs. winter is a qualitative one. Physiologists also

need to consider the findings that individual lines or cultivars within each growth habit classification vary in flowering time, suggesting the involvement of genes regulating both a qualitative (growth habit) as well as a quantitative (flowering time) component.

In an attempt to inter-relate inheritance studies of vernalization in the Brassica species, Teutonico and Osborn (1995) speculated that some of the different loci for vernalization requirement identified in various studies could encode the same genes that are conserved across the Brassica species. Thurling and Kaveeta (1992) had earlier speculated as to the possibility that the absence of very early plants in an interspecific cross between B. napus and B. rapa was a manifestation of the napus "C" genome chromosomes imposing a limit on expression of earliness genes from the "A" genome. The progress in analytical techniques made it possible for Teutonico and Osborn (1995) to compare the positions of trait loci in different populations using RFLP and linkage maps constructed with a common set of DNA probes. Their results indicated some common regions, but additional common markers between Brassica species would have to be developed to permit a comprehensive examination of their hypothesis.

Use of Haploidy in Breeding and Genetic Studies of Brassica species

Interest in the use of microspore culture in B. napus arose out of the observation by Thomas and Wenzel (1975) that a haploid embryo was produced in culture that was completed isolated from the anther.

The haploid condition refers to the chromosome number of the normal gametes of a species (Sharp et al., 1984) and are derived from the haplo-phase of the life cycle, following production of the male and female gametes (Jensen, 1986). According to Keller and Armstrong (1977), Brassica breeding objectives, including alteration of fatty acid composition and glucosinolate content, may be more efficiently achieved by the creation of doubled haploid (DH) from the parental crosses.

Several attributes of DH have lead to their use in breeding programs. DH can offer a considerable time savings during the development of homozygous lines (Keller and Stringam, 1978). In addition, the hemizygous conditions for all loci in haploids reduces some of the difficulties encountered in identifying and manipulating desired genetic traits (Collins, 1977). Henderson and Pauls (1992) and Thiagarajah and Stringam (1993) indicated that doubled haploid techniques were valuable, especially when the desired trait was determined by the presence of multiple recessive alleles in the homozygous state.

Ferrie et al. (1994) summarized the main advantages to plant breeders as: reduction in time to develop new varieties; rapid fixing of traits in homozygous condition; improved efficiency of selection because phenotype is

not masked by dominance effects; smaller numbers of plants are needed to screen for desirable recombinants than in conventional populations.

Microspore culture is of value to a breeding program, however, only if high numbers of androgenetic plants can be obtained on a consistent basis and with no in-vitro selection pressure (Hoffman et al., 1982, Snape et al., 1986 and Mandal, 1987). Factors influencing the success of microspore culture include the environmental conditions of the donor plant (Keller and Armstrong, 1978); genotype of the cultured species (Collins, 1977); stage of pollen development (Keller and Stringam, 1978); media composition (Keller et al., 1982) and physical culture conditions (Keller and Armstrong, 1978). Chen et al. (1994) indicated that, with direct application of colchicine to microspores, the process of generating a gametic array, from planting the F1 to producing sufficient amounts of true-breeding seed to field test could take as little as eight months.

The developers of new techniques in genetic analysis have used DH lines as the foundation material for experiments. Ferreira et al. (1994) described the potential of combining DH lines and molecular markers. They felt that DH lines were desirable because the fixed genotype can be propagated indefinitely. Restriction fragment length polymorphisms (RFLP) are used to construct genetic linkage maps that provide insight into genome organization and assist location of loci controlling important agronomic traits. For mapping purposes, the DH population was treated as a backcross population, with an expected Mendelian segregation of 1:1 for 2 alleles present in the F1.

In order for the DH method to provide useful information to the breeder, the resulting haploid plants must represent a random set of gametes (Park et al., 1976; Choo et al., 1985 and Kubba et al., 1989). In general, it appears that, barring culture-induced abnormalities or linkage disequilibrium, and based on population mean, range of expression and frequency distributions, the DH method performs as well as conventional methods such as pedigree or single seed descent methods (Park et al., 1976).

Haploidy and Brassica

The potential of the DH techniques in a B. napus breeding program can be illustrated by the development of the European oilseed rape variety 'Maris Haplona', the first commercially grown agriculture crop variety in the world to be derived directly from a haploid (Thompson, 1979). More recently, the development and release of the Canadian oilseed rape cultivar, Quantum (Stringam et al., 1995) marked the first registration of a DH- derived cultivar for production in western Canada.

Of greater potential use to B. napus breeders is the increased ability to identify desirable combinations of characteristics from smaller populations. Henderson and Pauls (1992) indicated that for the introgression of recessive traits such as yellow seed coat into canola quality cultivars, extremely large populations would have to be assessed in order to obtain one desirable plant. For example, in order to obtain a yellow-seeded, canola-quality line from a cross

between a yellow-seeded oilseed rape and a black-seeded canola, the breeder would theoretically have to screen more than 4096 plants in an F2 population, versus 64 plants from a DH population. For their program, Henderson and Pauls (1992) did not attempt to isolate a yellow-seeded canola-quality line from a F2 population, but they did identify three in the 99 DH lines they screened. A similar scenario was outlined by Thiagarajah and Stringam (1993) in order to obtain yellow-seeded, canola quality Brassica juncea.

DH lines are also of benefit in the genetic analysis of quantitative characteristics, since they immediately form the F-infinity generation (Snape et al., 1984). As the goal of many breeding programs is to obtain the most desired genotype, Snape et al. (1984) felt that the use of DH offered a 2n advantage over diploid selfing, where n represents the number of segregating factors. In addition, simpler ratios of DH result in smaller populations needed to be produced in order to obtain extreme genotypes (Siebel and Pauls, 1989). Siebel and Pauls (1989) reported that class separation for erucic acid content was greater in androgenetic populations, making it easier to distinguish classes than in the F2 population.

Chen and Beversdorf (1990) examined the main fatty acids in oilseed rape (B. napus) in order to determine whether populations derived through microspore culture (MD) and SSD had the same distributions. Their results indicated that the MD populations formed a random, homozygous F1 plant-derived gametic array for erucic, oleic, linoleic and linolenic acid. There were,

however, differences between the MD and SSD populations. For example, three classes of erucic acid content were identified for the MD population, while four classes were identified for SSD. Chen and Beversdorf (1990) assigned the differences between the populations to residual heterozygosity in the SSD lines, which were evaluated at the F3 generation.

Charne and Beversdorf (1991), Scarth et al. (1991) and Stringam and Thiagarajah (1991) compared SSD and DH in B. napus populations and concluded that, while some traits showed significant differences, in most cases SSD and DH were equally effective in obtaining desirable lines. Of interest are the traits in which differences occurred between SSD and DH populations. Means and variances for lodging and frequency distributions for days to maturity, seed oil and seed protein content showed differences between SSD and DH in Charne and Beversdorf (1991). One factor of importance is that the DH populations used by Charne and Beversdorf (1991) were from F1 donor plants, while Stringam and Thiagarajah (1991) used F2 donor plants.

Kubba et al. (1989), Chen and Beversdorf (1990), Charne and Bevesdorf (1991), Scarth et al. (1991), Stringam and Thiagarajah (1991) and Thiagarajah and Stringam (1993) felt that the use of DH in Brassica breeding programs would result in breeders obtaining desirable lines with considerable savings in time and resources.

Haploids and Vernalization

Reports of vernalization requirement in DH lines of spring B. napus have been reported. Wenzel et al. (1977) reported that several stem embryo progeny from spring type plants required cold treatment prior to flowering. The appearance of the vernalization requirement was considered to reveal the occurrence of new allelic combinations in the microspore-derived plant progenies.

Hoffman et al. (1982) reported the appearance of a winter-type B. napus line that required cold for flowering in an otherwise homogeneous population of spring B. napus cultivar Tower. Hoffman et al. (1982) used this line as evidence supporting their hypothesis that there was a higher degree of homozygosity in DH lines, compared to inbred lines of B. napus.

Orr et al. (1990) found that microspore-derived embryos from the winter B. napus cultivar Jet Neuf that had been subjected to hardening treatments did not require vernalization to flower. Further investigation revealed that the ability of the embryo-derived plants to flower in the absence of vernalization did not result from the culture procedure alone, as non-hardened embryos still required vernalization in order to flower. Spring B. napus from the cultivar Topas did not respond to the hardening regime.

Flowering is an integrated process that encompasses several phases and is affected by external environmental and internal metabolic variables. Species-specific information, such as that for Brassica, and organ-specific information,

including leaf and apical and floral meristem development, should be considered as part of the continuum of whole plant development, rather than isolated events.

Vernalization Response in Spring Oilseed Rape (*Brassica napus* L.) Cultivars

L.A. MURPHY and R. SCARTH

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada R3T 2N2

Contribution No. 927. Received 25 May 1993 , accepted 17 December 1993.

Short Title: Vernalization Response in Spring Canola

ABSTRACT

Early maturity is a major objective of oilseed rape (Brassica napus) breeding programs in western Canada. Maturity of crops is influenced by time of initiation and flowering. The presence of a vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied. A range of spring oilseed rape cultivars were screened for their response to vernalization. Vernalization treatments consisted of exposure of germinated seeds to 0 to 42 days at 4° C. Plants were assessed under a 20 hr photoperiod. In general, there was a cumulative response to vernalization, with a decrease in days to each developmental stage as exposure to 4° C was increased. Vernalization treatment of 6 d at 4° C was sufficient to decrease both the days to first flower and the final leaf number. The characterization of vernalization response is of interest because variation in flowering time in response to year to year variations in the environment could result.

Key words: Brassica napus, canola, oilseed rape, vernalization

INTRODUCTION

Early maturity is a major objective of oilseed rape (Brassica napus) breeding programs in western Canada. Breeding for early maturity usually involves selection for earliness to first flower, as appearance of the first flower and maturity are sufficiently correlated to justify its use as a selection criterion (Campbell and Kondra 1978). Collins and Wilson (1974) indicated that time of floral initiation and flowering were closely correlated with each other and with maturity. Collins and Wilson (1974) concluded that time of initiation and flowering were the primary determinants of maturity. Thus, any factor which affected the period from seeding to first flower would have important consequences with regards to maturity.

The presence of a vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied (Flood and Halloran 1984). This delay in floral initiation results in an increase in final leaf number (Quinby and Liang 1969). Three vernalization response patterns have been characterized. The cumulative response elicits a quantitative decrease in the length of the vegetative period with an increased duration of cold treatment. The irregular response is characterized by increases in the length of the vegetative period with extended cold, while the threshold response is a response that only occurs with extended periods of cold (Salisbury et al. 1979; Berry et al. 1980; Flood and Halloran 1984; Jedel et al. 1986).

Most members of the Brassicaceae possessing a cold requirement are biennial. Research work involving Brassica oleracea, including Chinese cabbage, cauliflower and Brussels sprout, indicated that there was a cumulative requirement for low temperature induction of early flowering (Gauss and Taylor 1969). Hodgson (1978) reported that the B. napus cultivar Midas exhibited a cumulative response to vernalization and that vernalization significantly affected plant development prior to the bud stages, but had no direct effects on subsequent stages. Myers et al. (1982) found that there was a reduction in time to flowering in response to vernalization treatment.

Thurling and Vijendra Das (1977) examined the effects of different temperatures and photoperiods on the duration of pre-anthesis development of a range of spring oilseed rape cultivars from Canada, Europe and Japan. The Canadian cultivars Target and Oro had no response to vernalization under a 24 hour photoperiod, while the flowering of the European cultivars, Bronowski and Masoweicki, was accelerated by vernalization at 3° C. Thurling and Vijendra Das (1977) indicated that the range in response of spring oilseed rape cultivars to vernalization appeared to be much greater than those observed between contrasting cultivars of spring wheat.

This study was initiated in order to characterize the vernalization response of a range of spring oilseed rape cultivars grown in western Canada.

MATERIALS AND METHODS

The selection of cultivars used in this study was based on several criteria: commercially produced in western Canada; used in the University of Manitoba hybrid canola breeding program; were of canola quality and represented a range of geographic origins (Table 2).

Table 2. B. napus cultivar descriptions and country of origin.

Cultivar	Country of Origin	Year Released
Global	Sweden	1985
Karat	Sweden	1980
Marnoo	Australia	1980
Regent	Canada	1977
Westar	Canada	1982

Seeds were placed in petri dishes containing acid washed sand and moistened with distilled water. In Experiment 1, the vernalization treatment consisted of 14, 28 and 42 days at 4° C. In Experiment 2, seeds of Global and Marnoo were exposed to 2, 4, 6, 8, 10, 12 and 14 days at 4° C. Seeds of the nonvernalized control in both experiments were germinated at 22° C for 48 hr prior to the end of the vernalization treatment in order to reach a growth stage

similar to that of the vernalized seeds. All vernalization treatments were done in the dark.

After vernalization, the germinated seeds were planted with 2 seeds per pot and placed in a growth room under 22/16° C day/night temperature, with a 20/4 hr day/night photoperiod. The growth room was lit with VHO Grolux wide spectrum fluorescent lamps which provided approximately 300 $\mu E\ m^{-2}s^{-1}$ of photosynthetically active radiation, measured at the top of the plant canopy.

In Experiment 1, the number of days required to the first appearance of buds within the rosette (DB) and the first open flower (FF) were determined. In Experiment 2, FF was determined. The days to each stage were counted from the date of planting the seeds in the growth rooms. To facilitate leaf counts, each leaf was punched once with a single hole punch near the leaf margin at full emergence. The resulting 5 mm hole was visible throughout the experiment and was used to determine final leaf number (FLN) for both experiments.

The mean of the two plants per pot was determined for each measurement in each treatment. A total of nine replicates of each treatment were conducted for Experiment 1, while six replicates were conducted for Experiment 2.

LSD (P=0.05) was calculated for the treatments within each cultivar. Treatment effects were considered fixed effects.

RESULTS

Experiment 1

In general, there was a cumulative response to vernalization, with DB, FF and FLN decreasing with larger exposure to 4° C (Table 3). There were differences between cultivars and development stages. Westar appeared to have reached its maximum response to vernalization after 28 d, whereas Karat did not begin to respond until after 14 d at 4° C. The remaining cultivars Global, Marnoo and Regent continued to respond over the range of vernalization treatments examined.

Experiment 2

The cultivars Global and Marnoo were selected for further study because of their dramatic response to 14 d of vernalization in Experiment 2. Both cultivars responded to vernalization treatments of less than 14 d (Table 4). The results appeared to indicate that at least 6 d exposure to 4° C was required to elicit a response.

Table 3. Effect of 0 to 42 d vernalization treatment at 4° C on days to bud (DB) and to first open flower (FF) and final leaf number (FLN) of 5 spring B. napus cultivars.

Cultivar	Treatment (d)	DB (days)	FF (days)	FLN
Global	0	35.8	42.7	18.8
	14	27.1	33.3	9.3
	28	21.3	27.9	7.9
	42	19.6	26.2	6.8
	LSD (P=0.05)	2.7	1.8	1.9
Karat	0	27.0	33.7	11.1
	14	27.4	33.8	9.2
	28	23.3	30.0	7.4
	42	21.7	28.1	7.2
	LSD (P=0.05)	2.1	2.2	1.7
Marnoo	0	30.4	36.6	14.2
	14	22.8	29.3	10.0
	28	18.8	25.4	8.0
	42	17.3	23.7	6.9
	LSD (P=0.05)	2.5	2.4	2.8
Regent	0	28.1	33.8	9.9
	14	24.1	30.0	8.0
	28	21.0	28.1	6.8
	42	19.2	25.6	5.4
	LSD (P=0.05)	1.6	1.5	1.2
Westar	0	26.8	32.8	9.9
	14	22.1	28.3	9.1
	28	19.0	25.3	6.7
	42	18.7	25.0	6.0
	LSD (P=0.05)	1.6	1.6	1.0

Table 4. Effect of 0 to 14 d vernalization treatment at 4° C on days to first open flower (FF) and final leaf number (FLN) of cultivars Global and Marnoo.

Treatment (d)	Cultivar			
	Global		Marnoo	
	FF	FLN	FF	FLN
0	44.5	19.2	36.6	12.8
2	44.0	18.6	37.0	13.0
4	42.3	15.2	36.8	13.2
6	41.5	16.3	34.5	11.8
8	38.8	11.7	31.6	10.8
10	35.8	11.0	31.7	10.3
12	36.8	10.8	31.8	9.3
14	35.7	10.8	30.7	9.5
LSD(P=0.05)	4.8	3.1	4.5	3.6

DISCUSSION

The spring B. napus cultivars in this study showed significant response to vernalization. Vernalizing the imbibed seed at 4° C for at least 6 d decreased the length of each of the phenological growth stages and reduced FLN on the main stem. Further investigation revealed that the response to vernalization was

cumulative, and that there were differences in response among the five cultivars studied.

The cumulative response to vernalization is similar to the results obtained by Hodgson (1978) for the B. napus cultivar Midas and by Gauss and Taylor (1969) for B. oleracea. The results differ from those of Thurling and Vijendra Das (1977) who found that the Canadian B. napus cultivar, Target, had no response to vernalization under their experimental conditions. The response of the Canadian cultivar, Westar, is similar to the results reported for vernalization response in Canadian spring wheat cultivars (Jedel et al. 1986). The existence of a cumulative response to vernalization that is saturated by a relatively short exposure to cool temperatures may permit Canadian cultivars to respond favourably should vernalizing conditions occur during early growth, yet allow development to proceed to maturity even if conditions are not conducive to vernalization.

The characterization of the vernalization response is of particular interest to hybrid canola breeding programs. Variation in flowering time of responsive cultivars due to year-to-year variations in the environment could result in the failure to produce seed in field crossing blocks. Cultivars from different geographic origins are used to achieve maximum combining ability in canola hybrids (Brandle and McVetty, 1990). The results of this study indicate the importance of characterizing responsive cultivars such as Global and Marnoo before they are introduced into a breeding program.

The effects of vernalization on apical development in spring oilseed rape (Brassica napus L.)

L.A. MURPHY AND R. SCARTH

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada R3T 2N2

Submitted to the Eighth Crucifer Genetics Workshop

Saskatoon, Saskatchewan

1994

ABSTRACT

An understanding of the time of inflorescence development is important in crop production. Once the apical meristem is committed to reproductive development, the number of potential leaves from that meristem is also fixed. The presence of vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied.

The objective of this study was to identify the effects of vernalization on the developing apex and relate this to the Harper and Berkenkamp (1975) phenological development scale. Vernalized and nonvernalized populations of the doubled haploid (DH) line GK16 were used. Vernalization treatment consisted of exposure of germinated seeds to 2 weeks at 4° C. Apices were collected at two day intervals and examined under a dissecting microscope in order to determine stage of development.

The transition from a vegetative to a reproductive apex for the DH line GK16 occurred less than 20 days after planting and at approximately the Harper and Berkenkamp (1975) stage 2.35 to 2.4. Plants in both the vernalized and nonvernalized treatments underwent the same sequence of development. The fact that time of initiation occurs within 20 days after planting is of value for the development of a rapid method of determining relative vernalization response. As the transition to a reproductive apex is occurring relatively early in the life cycle of spring B. napus, there are important implications with regard to time of planting and the potential for vernalization in the field.

INTRODUCTION

An understanding of the time of inflorescence development is important in crop production. Once the apical meristem is committed to reproductive development, the number of potential leaves from that meristem is also fixed and thus the size of the leaf canopy is determined (Mendham and Scott, 1975). The time of floral initiation can also indirectly limit yield through its effect on time of flowering. Mendham and Scott indicated that early initiation could result in floral development before plants had reached maximum vegetative size.

Several studies on the changes to the development of the apical meristem in B. napus have been reported (Mendham and Scott, 1975; Daniels et al., 1984; Polowick and Sawhney, 1986 and Smith and Scarisbrick, 1990). In general, the changes described in the transition from the vegetative to the reproductive meristem are slight and involve a reorientation of the apical dome from dome-shaped to slightly convex. Of interest are the reports that reproductive meristems show small protuberances or axillary apices (Crisp and Walkey, 1974 and Daniels et al., 1984). Many researchers have determined that the appearance of protuberances was the first visible sign of floral initiation.

The presence of a vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied (Flood and Halloran, 1984). Measurement of the number of days required to floral initiation is an important method of assessing vernalization response. Investigators have used this approach in B. napus (Mendham and Scott, 1975; Smith and

Scarisbrick, 1990), Triticum aestivum (Wort, 1941; Halse and Weir, 1970 and Syme, 1973), Trifolium subterraneum (Evans, 1959 and Salisbury et al., 1987), Pisum sativum (Berry and Aitken, 1979). One major disadvantage of using days to initiation is that the assessment requires destructive sampling of a large number of plants.

Non-destructive measurements usually focus on the visually distinct flowering phase, including heading in cereals and appearance of the first open flower in dicots. Flood and Halloran (1984) found a strong linear relationship (r=0.86) between days to initiation and days to ear emergence in wheat. Days to ear emergence has the advantage of being easier to determine on large numbers of plants without destructive sampling.

The division of growth and development in B. napus into distinct phases is difficult due to its indeterminate pattern of growth. Harper and Berkenkamp (1975) developed a growth stage key which has been widely used for B. napus . It divides the life cycle into six distinct stages, which can then be further divided. Stage 0 and 1 refer describe the pre-emergence and emergence of the seedling. Stage 2 refers to the development of the plant in the rosette stage, and involves assignment of 0.1 units to each leaf that is fully expanded. Thus, the stage 2.2 refers to the stage at which the second true leaf has expanded. Stage 3 involves the bud stages, beginning at the time that the inflorescence or bud structure becomes visible within the rosette. Stage 4 refers to the flowering phase, which begins when the first flower opens. Stage 5 refers to the ripening stages, and describes seed development

within the pod until the plant senesces. Harper and Berkenkamp (1975) consider stages 0 to 3 to represent vegetative stages, while stages 4 and 5 refer to reproductive development.

It is important to note that when using a key such as Harper and Berkenkamp (1975) that the stages are not mutually exclusive (Mendham and Scott, 1975). Thus, stages 3 to 5, the period from buds being visible within the leaf rosette, to the presence of seeds in the developing pods, may be present on the same plant at a given time. By restricting observations to the main stem, distinctions concerning stage of development can be more readily determined. Another concern expressed about the use of growth stage keys is that they tend to mask a wide range of internal physiological stages (Smith and Scarisbrick, 1990). For example, the Harper and Berkenkamp (1975) stage of 3.1 covers the internal period of transition from a vegetative to a reproductive apex, to sepal formation on flower buds.

The objective of this study was to identify the effects of vernalization on the developing apex and to relate this to the Harper and Berkenkamp (1975) phenological development scale.

MATERIALS AND METHODS

Vernalized and nonvernalized populations of the DH line GK16 were examined. GK16 was selected from a DH population of a cross between S4 lines from Global and Karat and characterized as responsive to vernalization, for both FF and FLN (Murphy and Scarth, 1997). The vernalization response for Global-16,

Karat-17 and the DH line GK16 determined by Murphy and Scarth (1997) are summarized in Table 5.

Table 5. Effect of 0 vs 2 wk at 4°C on the days to first open flower (FF) and final leaf number (FLN) of B. napus S4 lines Global-16 and Karat-17 and DH line GK16 (Murphy and Scarth, 1997).

S4/DH Line	Vernalization Treatment (Weeks)	FF	FLN
Global-16	0	44.5*	16.8*
	2	35.0	10.2
Karat-17	0	38.2 ns	11.7 ns
	2	36.3	9.8
GK16	0	46.0 *	11.8 ns
	2	35.5	11.8

*: significant at P=0.05; ns: nonsignificant at P=0.05

Vernalization treatment consisted of petri plates filled with 30 ml silica sand and mixed with 9 ml distilled water. Seeds of GK16 were placed on the moist sand, covered and held at 22° C for 24 hr. The vernalization treatment (V) consisted of 2 weeks at 4 C in the dark. Seeds of the nonvernalized treatment (N) were germinated at 22° C for 48 hr prior to the end of the vernalization treatment in order to reach a growth stage similar to that of the vernalized seeds.

Germinated seeds were planted in 2 cm x 2 cm peat pots containing MetroMix potting medium. The pots were transferred to a growth room and grown under 22 C / 16 C day/night temperature, with a 20 hr day/4 hr night photoperiod.

Plants received approximately 300 $\mu E\ m^{-2}\ s^{-1}$ of photosynthetically active radiation at the canopy level.

Once plants had reached the 2.1 stage of Harper and Berkenkamp (1975), apices were collected from the V and N every day. Five representative samples were examined under a dissecting microscope at 40X magnification in order to determine the stage of development of the apex. Harper and Berkenkamp (1975) growth stage (H-B), state of apical development, final leaf number (FLN) and calendar days were recorded for each observation. Image processing equipment was used to record the developing apex at each stage of development.

RESULTS

The transition from a vegetative to a reproductive apex for the DH line GK16 occurred less than 20 days after planting for both V and N (Table 6). For both treatments, the transition to a reproductive apex under the experimental conditions used in this study occurred at H-B 2.35 to 2.4. This is the stage at which the third true leaf is fully expanded and the fourth true leaf is visible, but not yet fully expanded.

The plants in V and N differed in the amount of time required to reach H-B 2.35 to 2.4. On average, the plants in V reached initiation 14.5 days after planting, while the plants in N reached initiation 18 days after planting. The number of leaves emerged and initiated was determined for each Harper and Berkenkamp stage (Table 6). Determination of leaf number at the early growth stages proved difficult, as the small size of the apex and the newly formed leaves made counting

difficult. However, from H-B 2.3 and 2.45, FLN was easier to determine. There were differences in FLN between plants in V and in N. The average FLN for plants in V was 10.8, while the average FLN for N was 11.9.

Table 6. Harper and Berkenkamp (1975) growth stage (H-B), number of days after planting, description of the developing apex and final leaf number (FLN) and standard deviation for vernalized (V) and nonvernalized (N) plants of the B. napus DH line GK16.

H-B Stage	Treatment	Days after Planting	Description of Developing Apex	FLN
2.0	V	10	no apex visible	na*
	N	10		
2.1	V	11	no apex visible	na
	N	11		
2.2	V	12	no apex visible	na
	N	12		
2.25	V	13	domed apex visible	10.0 (0.8)
	N	13	no apex visible	
2.3	V	13	apex is domed	10.8 (0.6)
	N	15	domed apex visible	11.5 (0.8)
2.35	V	14	domed apex	11.0 (0.5)
	N	17	domed apex	12.0 (0.8)
2.4	V	15	floral structures visible	11.3 (0.5)
	N	19	floral structures visible	12.8 (0.8)
2.45	V	17	floral buds visible to naked eye	11.0 (0.6)
	N	20	floral structures visible; axillary branching	13.0 (0.8)

*na indicates that it was not possible to determine FLN at this stage

Plants in both V and N underwent the same sequence of development. From H-B 2.0 and 2.2, no apex was visible within the rosette (Figure 1). At H-B 2.25, the apex became visible within the rosette and was dome-shaped (Figure 2). The apex became more prominent, yet remained dome-shaped, from H-B 2.3 and 2.35 (Figure 3 and 4). By H-B 2.4, the reproductive structures, first seen as protuberances around the previously smooth apex, became visible (Figure 5). By H-B 2.45, floral buds were visible for the plants in V, while axillary branches and buds were apparent in the plants from the N treatment (Figure 6).

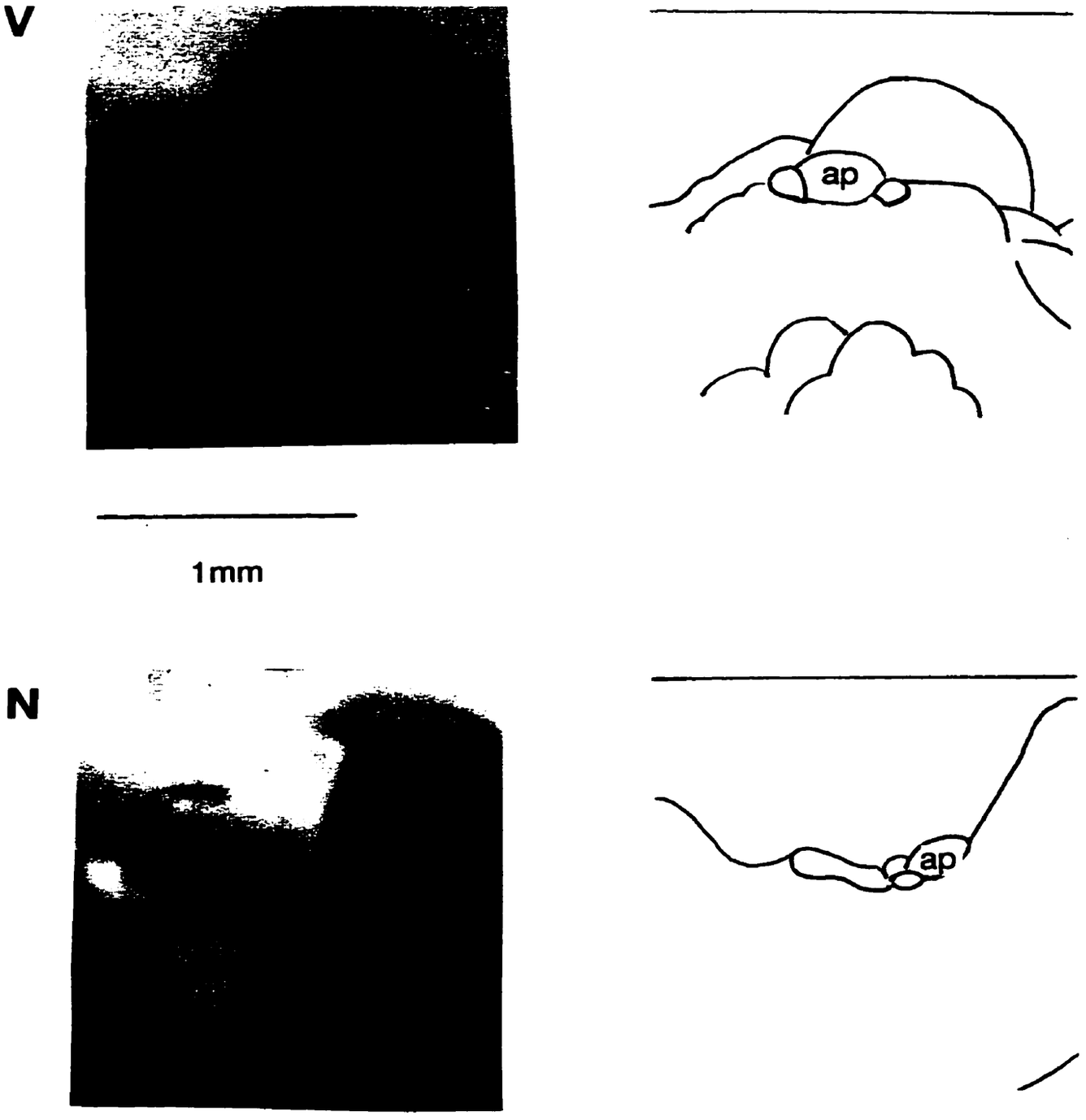
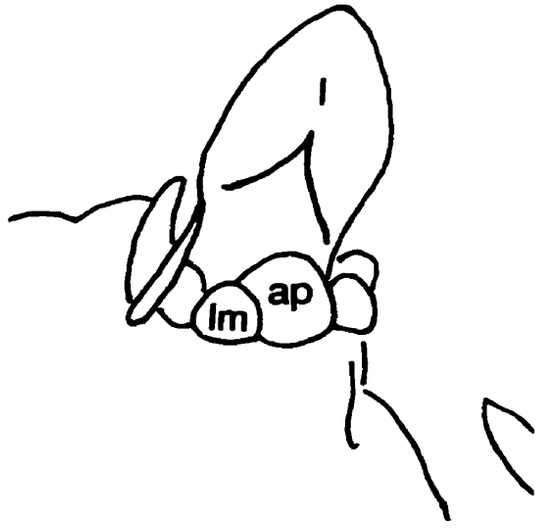


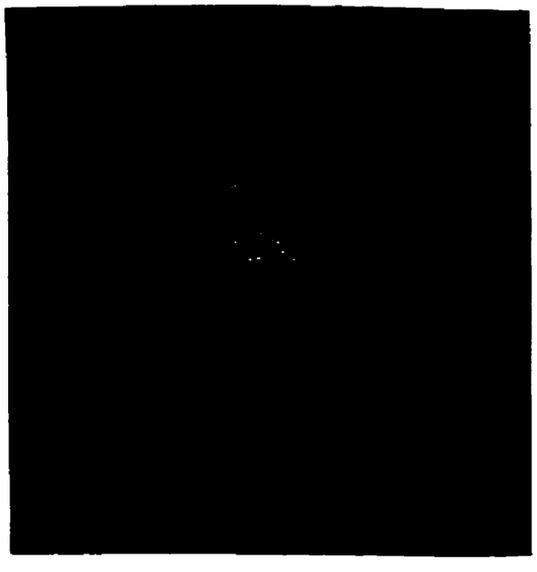
Figure 1. H-B stage 2.2 for vernalized (V) and nonvernalized (N) treatments of B. napus line GK16 (ap=apex).

V



1mm

N



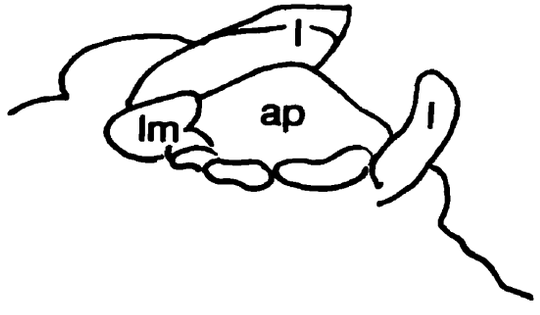
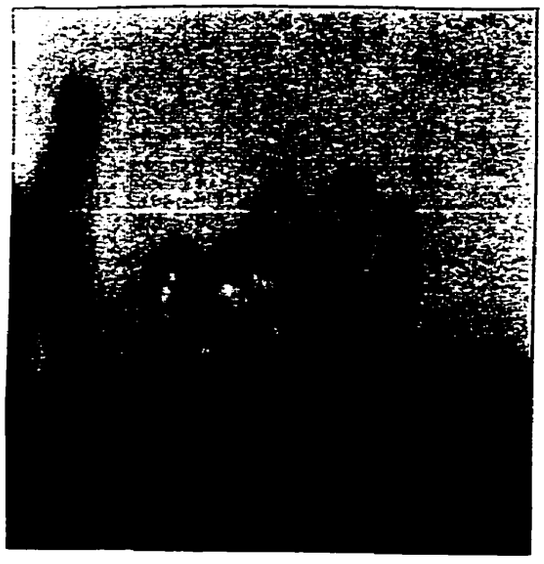
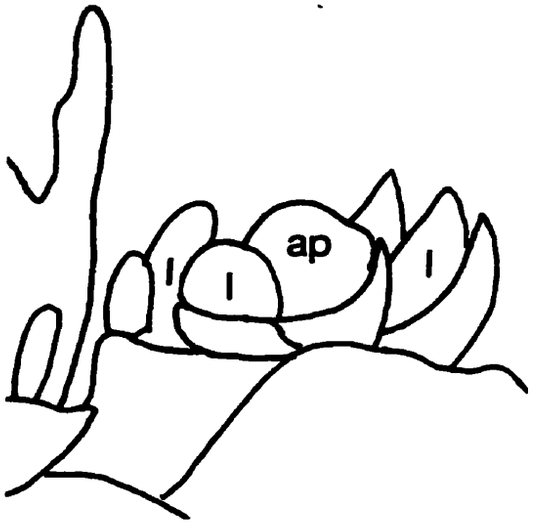


Figure 2. H-B stage 2.25 for vernalized (V) and nonvernalized (N) treatments of B. napus DH line GK16 (ap=apex; lm=leaf meristem; l=leaf).

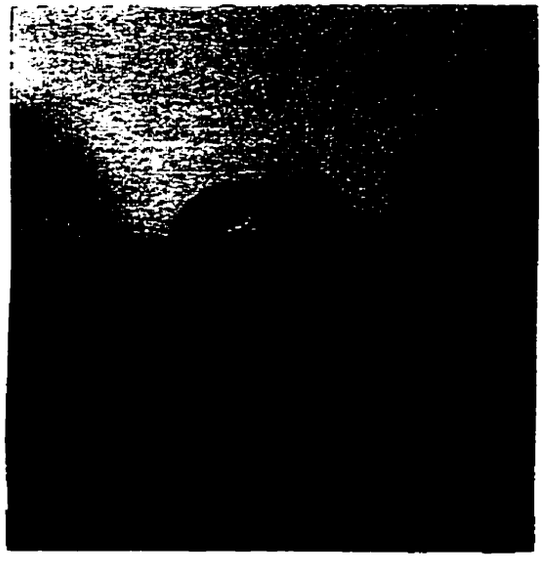
V





1mm

N



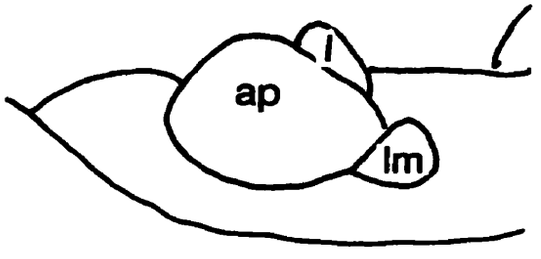
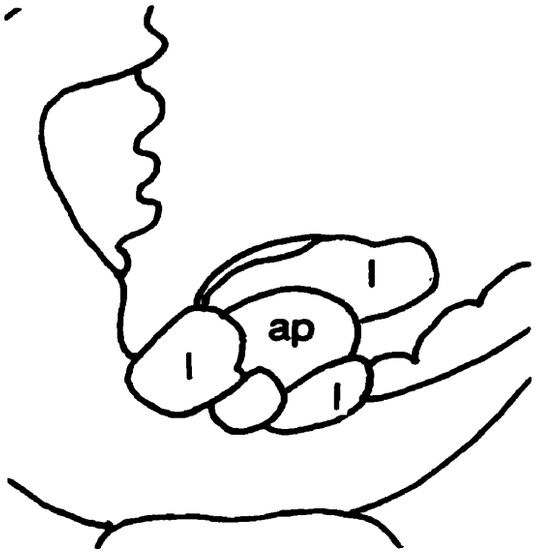


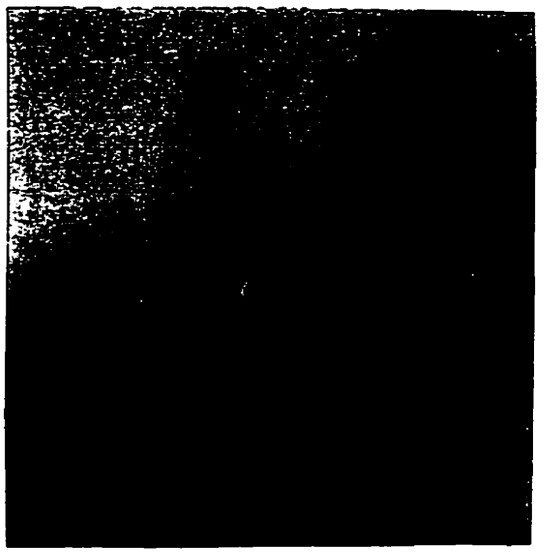
Figure 3. H-B stage 2.3 for vernalized (V) and nonvernalized (N) treatments of B. napus DH line GK16 (ap=apex; lm=leaf meristem; l=leaf).

V



1mm

N



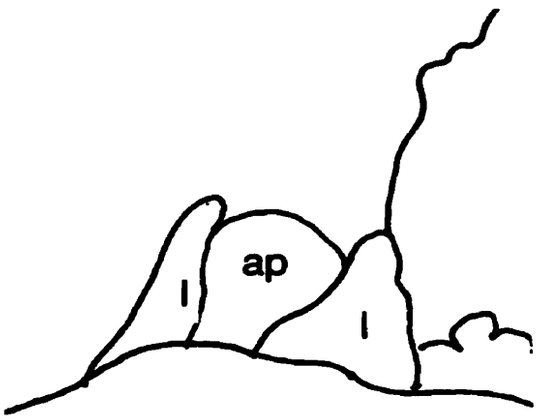
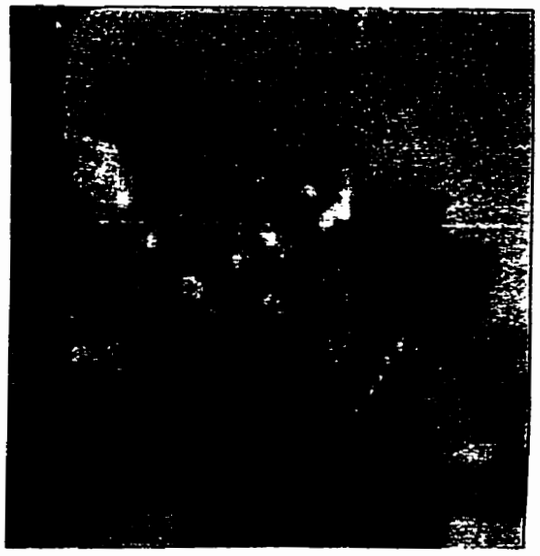
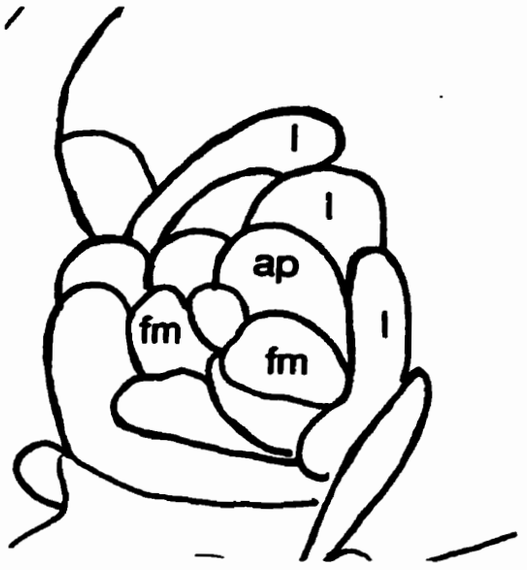


Figure 4. H-B stage 2.35 for vernalized (V) and nonvernalized (N) treatments of B. napus DH line GK16 (ap=apex; lm=leaf meristem; l=leaf).

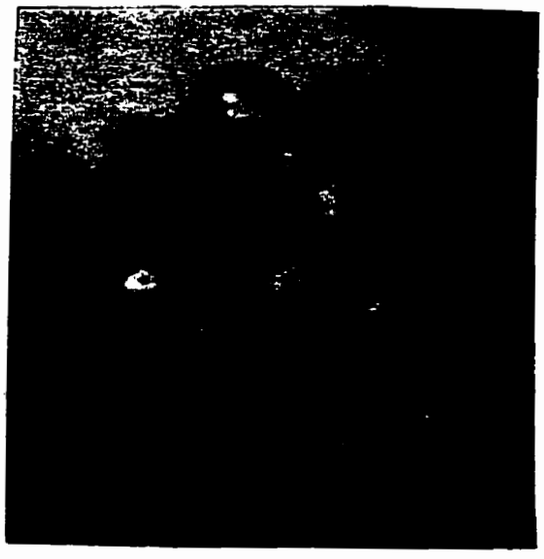
V





1mm

N



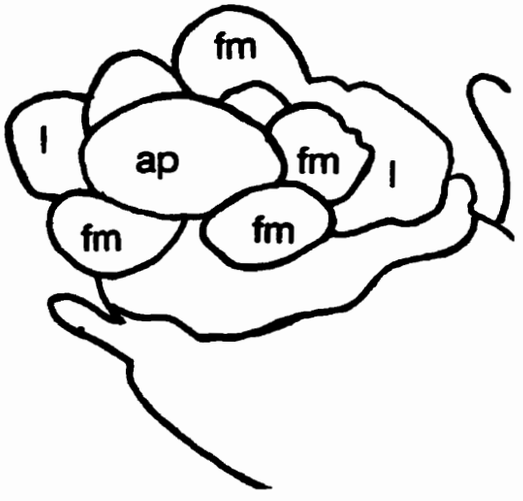


Figure 5. H-B stage 2.4 for vernalized (V) and nonvernalized (N) treatments of B. napus DH line GK16 (ap=apex; fm=floral meristem; l=leaf).

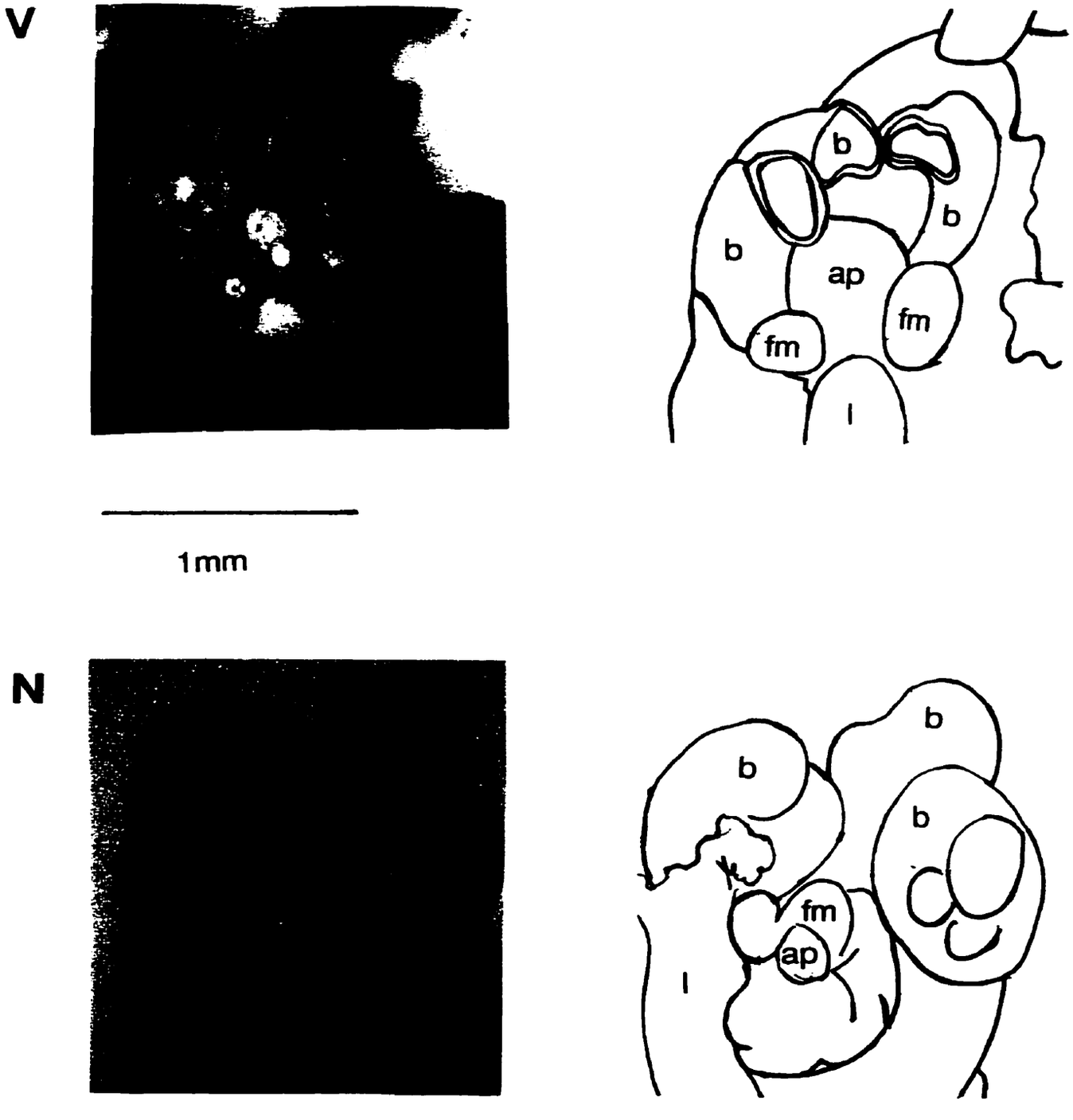


Figure 6. H-B stage 2.45 for vernalized (V) and nonvernalized (N) treatments of B. napus DH line GK16 (ap=apex; fm=floral meristem; l=leaf; b=floral bud).

DISCUSSION

The results of this study indicate that the transition of the apex from a vegetative to a reproductive meristem occurs before 20 days of development and at approximately the 4 visible leaf stage. The transition to a reproductive apex means that no new leaves will be initiated by that apex. This has important implications for final yield. Mendham and Scott (1975) indicated that early initiation may indirectly limit yield by permitting floral development before plants have reached maximum vegetative size.

The stages of development described in this study are in agreement with the sequences and stages reported by other researchers working with B. napus (Mendham and Scott, 1975; Daniels et al., 1984; Polowick and Sawhney, 1986 and Smith and Scarisbrick, 1990). The fact that transition from a vegetative to a floral apex occurs prior to H-B 2.4, or the 4 visible leaf stage, and before 20 days after planting, is of value for the development of a rapid method of determining relative vernalization response.

The potential for an easy and early determination of vernalization response has important time and resource implications. The potential for determination of vernalization response in a shorter time frame would mean that more plants, and hence more potential parental material, could be evaluated. Further research on different genotypes would be required in order to confirm the time and growth stage relationship established in this study.

The ability to relate internal physiological development to external plant development has been a goal of many researchers (Mendham and Scott, 1975; Flood and Halloran, 1984 and Smith and Scarisbrick, 1990). As the transition from a vegetative to a reproductive apex is occurring relatively early in the life cycle of spring B. napus, there are important implications with regard to time of planting, potential for vernalization in the field and the influence of photoperiod on time of transition. The scope of this study was limited to the response to vernalization under long day conditions. It is not unreasonable to expect that a similar rapid screening technique could be developed for assessment of response to photoperiod.

The ability to determine final leaf numbers early in development is important, both as a measure of relative response to vernalization, and as a preliminary indicator of potential yield. The relationship between final leaf number, canopy size and yielding ability was not determined in this study, yet work by Mendham and Scott (1975) indicates that reduced leaf numbers will result in a smaller canopy, which may impact negatively on yield potential.

Determining the Vernalization Response in Segregating Generations in Spring Oilseed Rape (Brassica napus L.)

L.A. MURPHY AND R. SCARTH

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada R3T 2N2

ABSTRACT

Plant breeders are interested in the inheritance of physiological responses of crop plants because of their significance in a cultivar development program. For example, the presence of a vernalization requirement that delays floral initiation until the cold requirement of the plant has been satisfied may result in unacceptably late maturity in an otherwise agronomically acceptable cultivar. The objective of this study was to determine the mode of inheritance of the vernalization response in spring oilseed rape B. napus. In Experiment I, a range of S4 inbred lines and F1 hybrids were evaluated for their response to vernalization. In Experiment II, 150 F3 families from two F1 populations were characterized for vernalization response. The vernalization treatment consisted of exposure of germinated seeds to 2 weeks at 4 °C. Two measurements were used to assess the vernalization response: days to first open flower (FF) and final leaf number (FLN) Measurement of FLN indicated that the majority of S4 lines and F1 hybrids did not respond to vernalization. When FF was used as an indicator of vernalization response, the F1 hybrids were generally classified as responsive to vernalization. Inheritance of the response to vernalization is complex, and involves at least three genes. Reciprocal effects were detected, as the response of the segregating population depended on the genotype of the female parent. The use of FLN as a measurement of response to vernalization offers physiological advantages, as the end of vegetative development and leaf initiation is more directly related to response to vernalization than FF.

INTRODUCTION

The term vernalization is the anglicized version of the Russian word "jaroxization", which refers to the process that made a winter cereal behave like a summer cereal (Lysenko, 1928, cited by Chouard, 1960). Further investigation revealed that the determining treatment was exposure of actively growing seeds or young plants to cold temperatures for a period of time. While most members of the Cruciferae possessing a cold requirement are biennial, it has been established that some spring B. napus cultivars exhibit a cumulative response to vernalization (Thurling and Vijendra Das, 1977; Hodgson, 1978 , Myers et al., 1982 and Murphy and Scarth, 1994). The presence of a vernalization requirement can delay floral initiation until the cold requirement of the plant has been satisfied (Flood and Halloran, 1984). Breeders attempting to develop early maturing cultivars must understand the inheritance of the vernalization response in order to assess the potential impact on their programs.

Studies on the genetic control of the vernalization response have been reported for cereals, with limited research done in other crops. There are differences between crop plant species with regards to the nature of the inheritance of the vernalization response, or more specifically, the inheritance of the spring growth habit. Studies of F1 progeny in Triticum aestivum indicated complete dominance of the spring habit (Purvis, 1939; Pinthus, 1967 and Pugsley, 1972) or partial dominance (Klaimi and Qualset, 1974). Analysis conducted using segregating generations indicated that the dominance of the

spring habit was incomplete (Purvis 1939; Pugsley, 1963 and Pinthus, 1967), and was attributed to the interaction of multiple genes. In Trifolium subterraneum, the F1 showed complete dominance for early flowering, while the F2 distributions were continuous and unimodal indicating polygenic inheritance (Davern et al., 1957 and Salisbury et al., 1987). In Lupinus , partial dominance for nonresponse to vernalization was found (Rahman and Gladstones, 1973).

From crosses between annual and biennial Brassica oleracea var gemmifera, Wellensiek (1960) reported that the annual habit was dominant. Thurling and Vijendra Das (1979b) concluded that the genetic mechanism in B. napus was similar to that of wheat, in that winter habit was conferred by the presence of recessive alleles.

There have been several attempts to describe the number of genes involved in the vernalization process. In wheat, Powers (1934) and Pugsley (1971) indicated the presence of three major genes, as well as minor genes and modifiers. Halloran and Boydell (1967) considered the expression of the vernalization response to be genetically complex, with closely linked genes producing minor effects. Klaimi and Qualset (1974) and Flood and Halloran (1986) reported that the continuous variation seen in the F2 and subsequent generations indicated quantitative inheritance, with the involvement of both major and minor genes.

The presence of many genes conditioning vernalization response has been reported in Trifolium subterraneum (Davern et al., 1957 and Salisbury et al.

1987), Arabidopsis thaliana (Murfet, 1977) and B. oleracea var gemmifera (Wellensiek 1960). In B. napus , Thurling and Vijendra Das (1979a) were able to identify 4 alleles of the 'V' gene, which accounted for the quantitative response to vernalization.

The objective of this study was to determine the mode of inheritance of the vernalization response in spring B. napus.

MATERIALS AND METHODS

The criteria for selection of cultivars and S4 lines used in this study included vernalization response of parent cultivars, canola quality in both seed oil and seed meal and a range of geographic origin (Table 7). Murphy and Scarth (1994) characterized the B. napus cultivars Global, Marnoo, Regent and Westar as responsive to a vernalization treatment of 2 weeks at 4C., while Karat was determined to show no response to 2 weeks at 4C. Brandle and McVetty (1989) developed S4 lines from Global, Karat, Marnoo, Regent and Westar by using single seed descent. The lines were inbred to S3, then increased in the S4 generation by selfing the S3 plants. F1 hybrids were produced by crossing the S4 parent lines in the greenhouse. The S4 lines selected in this study were characterized by Brandle (1989) as intermediate in days to flower within the sample of S4 lines from each parent cultivar.

Table 7. B. napus cultivar descriptions, country of origin and pedigree.

Cultivar	Country of Origin	Year Released	Pedigree
Global	Sweden	1985	Sv 701034 x Baldo
Karat	Sweden	1980	Hermes/Bronowski x Gulle
Marnoo	Australia	1980	/ Chikuzen x Zephyr/Bronowski
Regent	Canada	1977	/Turret/Turret x Liho/ x /Turret/Turret x
Westar	Canada	1982	/SD x s68-2895 x /Midas/Tower/F4

Experimental Material. Two experiments were conducted. In Experiment I, the S4 lines and resulting F1 hybrids were evaluated for vernalization response. In Experiment II, 150 F3 families from a reciprocal cross between the lines Marnoo-14 and Karat-17 were produced. F1 plants were selfed in the greenhouse to provide the F2 generation. 150 seeds from the F2 of each cross were planted and selfed. The resulting 150 F3 families were used to characterize the response to vernalization of the F2 seed. F2 plants could not be characterized directly, because of the requirement for the two environments, V and N.

Seeds from each cross were sown in MetroMix potting medium. The plants were grown in a controlled environment growth room under a 16 hr photoperiod and a 22° C / 16 °C day/night temperature regime. VHO Grolux wide

spectrum and cool white fluorescent lamps supplied a photosynthetic photon flux density of approximately 300 μE $m^{-2}sec^{-1}$ at the canopy.

Vernalization screening Seeds were placed on moist coarse acid silica sand, covered and held at 22 °C for 24 hours. The vernalization (V) treatment consisted of 2 weeks at 4° C in the dark. Seeds of the nonvernalized (N) treatment were germinated at 22° C for 48 hours prior to the end of the vernalization treatment in order to reach a growth stage similar to that of the vernalized seeds at the end of the V treatment.

Germinated seeds were planted in 2 cm x 2 cm peat pots containing MetroMix potting medium. The pots were grown under a regime of 22° C / 16° C day/night temperature, with a 20 hr day/ 4 hr night photoperiod. Plants received approximately 300 μE $m^{-2}s^{-1}$ of photosynthetically active radiation at the canopy level.

In Experiment I, plants were transferred to 10 cm peat pots, with two plants per pot, when they had reached the 2.3 growth stage (Harper and Berkenkamp, 1975). Plants in Experiment II were grown in the original 2 cm x 2 cm peat pots, with 25 plants for V and 25 plants for N. In Experiment I, pots were randomized weekly within the growth room. In Experiment II, the flats of 50 plants (25 V, 25N) were randomized in the growth room weekly.

The number of days required to reach first open flower (FF) and the final leaf number (FLN) for each experiment was determined. In Experiment I, the mean of the two plants in each pot was determined for each measurement. The

mean and standard error of the mean were used to determine response to vernalization. Standard errors were calculated from the standard deviations and number of pots screened per family. The difference between V and N for both FF and FLN, as well as the significance of the response, was determined by comparison of the means and standard errors at P=0.05. Response was defined as a significant difference (P=0.05) between the means and standard errors of V and N.

In Experiment II, the F2 plants could not be characterized directly, because of the requirement for the two environments, V and N. Thus, the mean of the V and N treatments was determined for each F3 family, which was then used to characterize the F2 individual that produced the F3 family. In order to determine the genotype of the F2 plants, the characterization of each F3 family was assigned NR if the mean difference between V and N was less than or equal to the NR parent, Karat-17; IR if the mean difference between V and N was intermediate to the NR parent, Karat-17 and the R parent, Marnoo-14; and R if the mean difference between V and N was equal to or greater than the R parent, Marnoo.

Tests for normal distribution was conducted and the chi-square test was used to test ratio of R, IR and NR, in an attempt to determine the number of genes involved in the response to vernalization. The level of significance used to test the fit of the data was P=0.05.

RESULTS

Experiment I- FF

The response of S4 inbred lines to vernalization was defined as a significant (R) or nonsignificant (NR) difference between V and N treatments. The response to vernalization was different between parent cultivars and within S4 lines from the same parent cultivars (Table 8) The three S4 lines developed from Karat were classified as varied from NR for Karat-17 and Karat-02 and R for Karat-03. The two S4 lines developed from Regent were both R, as were the two S4 lines developed from Global. Marnoo-14 was R, while Westar-07 was NR.

The F1 hybrids between selected S4 inbred lines had a range of response to vernalization (Table 9). The majority of F1 hybrids were R, with only Karat-03 x Karat-02, Karat-17 x Marnoo-14, Marnoo-14 x Westar-07 and Westar-07 x Marnoo-14 showing NR. There was evidence of reciprocal differences in vernalization response for the crosses involving Karat-02 and Karat-03, and Karat-17 and Marnoo-14. Karat-02 and Karat-17 were characterized as NR, while Karat-03 and Marnoo-14 were R. The F1 hybrids Karat-02 x Karat-03 and Marnoo-14 x Karat-17 were R, while the reciprocals were NR.

FLN.

S4 line response to vernalization using FLN also differed within and between parent cultivars . For example, the inbred lines developed from Karat

were classified as NR for Karat-03 and Karat-17 and R for Karat-02 (Table 8). Regent-06 showed R while Regent-17 was NR. The two S4 lines developed from Global were R. Marnoo-14 was R, while Westar-07 was NR.

In addition to differences in vernalization response within S4 lines from the same cultivar, there were also significant differences in mean leaf number in the same treatment. Karat-02 had the lowest FLN under both V and N of the three S4 lines derived from Karat. There were also differences in mean FLN for the two Regent lines, Regent-06 and Regent-17 under both V and N. Only the V treatment of Global-13 and Global-16 had similar FLN.

When FLN was used as the determining measurement for vernalization response, the majority of F1 hybrids were NR (Table 9). Only Regent-17 x Regent-06, Global-13 x Karat-17 and Karat-17 x Global-13 were R. As was the case with FF, one cross involving Regent-06 and Regent-17 showed reciprocal differences. However, when Regent-06, a responsive S4 line was the female parent, the resulting F1 hybrid was NR. Likewise, Regent-17 was characterized as NR, while the resulting F1 hybrid was R.

The two methods used to characterize response to vernalization, FF and FLN, provided some differences in characterization of vernalization response. The two methods provided the same characterization of response for the S4 inbreds Karat-17 and Westar-07 (NR) and Marnoo-14, Regent-06, Global-13 and Global-16 (R). There were differences in characterization for Karat-02, Karat-03 and Regent-17.

The characterization of response for F1 hybrids using FF and FLN resulted in the same characterization (NR) for Karat-03 x Karat-02, Karat-17 x Marnoo-14, Marnoo-14 x Westar-07, Westar-07 x Marnoo14, Regent-17 x Regent-06, Global-13 x Karat-17 and Karat-17 x Global-13. F1 hybrids involving the NR line Westar-07, were also NR.

Table 8. Response (R) and nonresponse (NR) of a range of S4 lines of spring B. napus to 0 (N) and 2 weeks at 4° C (V) on days to first open flower (FF) and final leaf number (FLN)

S4 lines	Treatment	FF mean	Standard Error	FF response	FLN mean	Standard Error	FLN response
Karat-02	N	32.9	2.1	NR*	7.6	0.4	R
	V	29.3	2.1		6.2	0.5	
Karat-03	N	37.3	1.2	R	13.3	1.1	NR
	V	33.7	1.2		11.7	1.2	
Karat-17	N	38.2	1.1	NR	11.7	1.3	NR
	V	36.3	2.1		9.8	0.7	
Marnoo-14	N	42.0	0.8	R	17.6	0.8	R
	V	36.1	0.9		11.1	0.8	
Regent-06	N	36.3	0.8	R	12.0	0.5	R
	V	31.8	1.2		11.1	0.2	
Regent-17	N	35.4	1.1	R	10.0	0.4	NR
	V	31.9	1.0		9.4	0.4	
Westar-07	N	37.5	2.1	NR	9.4	1.8	NR
	V	35.9	1.2		8.1	0.7	
Global-13	N	52.3	2.0	R	17.5	1.8	R
	V	38.2	1.3		10.0	1.0	
Global-16	N	44.5	2.4	R	16.8	1.2	R
	V	35.0	2.2		10.2	1.0	

* calculated from the difference and standard error of the difference between the V and N treatment.

Table 9. Response (R) and nonresponse (NR) of a range of F1 hybrids of spring B. napus to 0 (N) and 2 weeks at 4° C (V) on days to first open flower (FF) and final leaf number (FLN)

F1 hybrids	Treatment	FF mean	Standard Error	FF response	FLN mean	Standard Error	FLN response
Karat-02 X	N	34.3	1.1	R*	12.0	1.9	NR
Karat-03	V	31.0	1.9		9.7	1.1	
Karat-03 X	N	34.7	2.0	NR	11.3	1.8	NR
Karat-02	V	32.0	1.0		10.0	0.6	
Karat-17 X	N	39.7	1.5	NR	12.2	0.6	NR
Marnoo-14	V	38.8	2.3		12.5	0.8	
Marnoo-14X	N	38.0	1.9	R	10.8	1.9	NR
Karat-17	V	34.2	0.6		9.8	0.6	
Marnoo-14X	N	35.8	1.5	NR	10.0	0.9	NR
Westar-07	V	33.0	1.7		10.3	0.5	
Westar-07X	N	41.2	1.5	NR	14.5	1.4	NR
Marnoo-14	V	38.3	1.5		11.8	1.9	
Regent-06X	N	35.3	1.1	R	10.3	1.1	NR
Regent-17	V	31.0	0.0		10.3	0.0	
Regent-17X	N	36.0	1.9	R	11.7	1.1	R
Regent-06	V	32.0	0.0		10.3	1.1	
Global-16 X	N	44.7	2.0	R	10.0	0.6	NR
Karat-17	V	34.0	0.0		9.2	0.8	
Karat-17 X	N	46.0	1.3	R	10.7	0.8	NR
Global-16	V	35.3	1.2		9.2	0.8	
Global-13 X	N	49.4	1.8	R	11.8	1.3	R
Karat-17	V	37.2	1.9		9.2	0.8	
Karat-17 X	N	48.7	1.2	R	11.0	0.7	R
Global-13	V	36.0	1.2		8.8	1.0	

* calculated from the difference and standard error of the difference between the V and N treatment.

Experiment II- FF

FF results for the reciprocals were not pooled, as Bartlett's test for homogeneity of error variance (Cochran and Cox, 1957) was conducted for each replicate and the error variances were found to be heterogeneous.

For the cross Karat-17 x Marnoo-14, the F1 was in the range of the NR parent, Karat-17, with a difference between N and V of 0.9 days (Table 10). The F1 of the reciprocal cross, Marnoo-14 x Karat-17, had a response that was intermediate between the two parents, with a difference between N and V of 3.8 days.

Characterization of vernalization response for FF of the F2 individuals for Karat-17 x Marnoo-14 indicated that the majority of the F2 was intermediate to the range of the parents (Table 10). The range of difference between N and V for the F2 was 2.0 to 5.9 days. The FF for the majority of F2 individuals for Marnoo-14 x Karat-17 were in the range of the R parent, Marnoo-14, with a difference between N and V that was greater than 6 days.

The responses to vernalization of the reciprocals indicate the possible involvement of significant maternal effects in the inheritance of the response to vernalization. Chi square analysis indicated that more than two genes, for response to vernalization, may be involved in the Karat-17 x Marnoo-14 cross. No ratios were tested for the reciprocal, because all the F2 individuals were responsive. The population from each cross did not have a normal distribution.

Table 10. Distribution and range (days) in segregating populations for Response (R) Intermediate Response (IR) and nonresponse (NR) of spring B. napus on days to first open flower (FF) for the crosses Karat-17 x Marnoo-14 and Marnoo-14 x Karat-17.

Cross	Generation	Range:	NR (0-1.9)	IR (2.0-5.9)	R (6.0->)
Karat-17x Marnoo-14	Karat-17		√		
	Marnoo-14				√
	F1		√		
	F2 individuals		20	104	25
Marnoo-14 x Karat-17	Karat-17		√		
	Marnoo-14				√
	F1			√	
	F2 individuals		0	35	115

Experiment II-FLN

For both Karat-17 x Marnoo-14 and Marnoo-14 x Karat-17, the responses of the F1 generation were in the range of the NR parent, Karat-17, with a difference between N and V that was less than 1.9 leaves (Table 11). The F2 for Karat-17 x Marnoo-14 was distributed across the NR and IR classes. There were 86 F2 individuals characterized as IR and 63 classified as NR. No F2 individuals were characterized as R. The F2 for Marnoo-14 x Karat-17 was distributed across the NR and IR classes, with the majority of F2 individuals in

the NR range, having less than 1.9 leaves difference between N and V. As with the Karat-17 x Marnoo-14 cross, there were no F2 individuals in the R class.

Table 11. Distribution and range (number of leaves) in segregating populations for Response (R) Intermediate Response (IR) and nonresponse (NR) of spring B. napus for final leaf number (FLN) for the crosses Karat-17 x Marnoo-14 and Marnoo-14 x Karat-17.

Cross	Generation	Range:	NR (0-1.9)	IR (2.0-6.5)	R (6.6->)
Karat-17x Marnoo-14	Karat-17		√		
	Marnoo-14				√
	F1		√		
	F2 individuals		63	86	0
Marnoo-14 x Karat-17	Karat-17		√		
	Marnoo-14				√
	F1		√		
	F2 individuals		111	39	0

Chi square analysis indicated that more than two genes were involved in the response to vernalization. Tests for normality indicated that the data for FLN was not normally distributed.

DISCUSSION

The S4 inbred lines and F1 hybrids showed a range in response to vernalization. This is to be expected, given that a range in cultivar response to

vernalization exists in spring B. napus (Thurling and Vijendra Das, 1977; Hodgson, 1978; Myers et al., 1982 and Murphy and Scarth, 1994). However, the range in response of inbred lines developed from the same cultivar indicates that assessment of the vernalization response at the cultivar level may be insufficient. The cultivar Karat had been previously identified as NR using FF (Murphy and Scarth, 1994), yet the S4 line Karat-03 was R. The fact that a range in response to vernalization within a cultivar exists is not surprising, given that canola cultivars are heterogeneous populations (Brandle and McVetty, 1989).

While differences do exist, for FLN, the majority of the F1s were NR. Only 3 of the 12 F1 hybrids examined were R. Where the F1s were R, the range of the response was very limited. Previous research indicates dominance for NR in wheat (Purvis, 1939; Pinthus, 1967; Pugsley, 1972 and Klaimi and Qualset, 1974), B. oleracea var gemmifera (Wellensiek, 1960) and B. napus (Thurling and Vijendra Das, 1979b). The existence of reciprocal differences in the F1 is taken as an indication of cytoplasmic or maternal effects on a character (Allard, 1960). There have been no previous reports indicating cytoplasmic effects on vernalization. As the response of the F1 and F2 individuals appears to be influenced by the response of the female parent, the involvement of maternal effects must be considered.

Previous research in wheat indicates that where dominance for NR occurs in the F1, the response becomes incomplete in later generations (Purvis, 1939;

Pugsley, 1963 and Pinthus, 1967). These three studies looked at days to heading or ear emergence as a measure of relative vernalization response. The incomplete dominance for NR in these trials was attributed to the presence of multiple genes involved in the vernalization response. It would appear that the vernalization response in spring B. napus is conditioned by at least three genes for FF and FLN. The pattern of an NR F1, with segregation for R and NR in later generations that has been identified for wheat appears to also occur in B. napus.

The involvement of cytoplasmic or maternal factors in the response to vernalization can be examined from a physiological perspective. Vernalization involves the production, in the cold, of a floral stimulus which must be translocated to the apical meristem in order to induce the production of flower buds from a previously vegetative meristem (Chouard, 1960). The production of the floral stimulus occurs outside of the meristem; thus any factor that affects its production and translocation would affect the effectiveness of vernalization. The differences that are attributed to maternal effects could be reflect the geographic differences between the parents. Karat was developed as a cultivar for the spring growing season in Northern Sweden, where the breeding priority would be early maturity to avoid fall frost. Marnoo was developed for the cool winter season in Australia, where the crop must mature prior to the heat and drought conditions of summer. Thus, there were distinct growth environment differences and breeding priorities influencing selection during the breeding of both of these cultivars. The analysis of additional cross combinations from different

geographic locations may permit a better understanding of the nature of the difference between the parents.

Plant breeders are interested in physiological requirements such as vernalization, as well as their significance to a breeding program. The timing of key developmental stages such as flowering may vary from year to year, depending on the environment. Thus, for example, temperature conditions at seeding may determine whether the vernalization requirements of responsive cultivars are met, since short periods of cold temperature can occur. This would influence time to first flower and perhaps the success of seed production in field crossing blocks.

In this study, the most responsive inbred line was Global-13, which started to flower under N at 53 days after seeding. The F1 hybrids involving Global-13 began flowering under N at approximately 50 days after seeding. McPherson (1987) studied the growth and development of six canola cultivars, including Global, under a variety of conditions in the field. McPherson (1987) determined that Global was among the latest of the six cultivars studied to reach each stage of the development of the Harper and Berkenkamp (1975) scale. Under early (May 7) and late seeding (May 21), the cultivar Global began flowering in approximately 50 days after seeding in the field and reached maturity under the growing conditions in Manitoba in 1985. The results in the McPherson (1987) study indicate breeding lines such as those from the cultivar Global may mature under western Canadian growing conditions. However,

additional assessment is required, as FF does not determine the duration of the flowering period, or the effect of heat stress during the flowering period.

Inheritance of the Vernalization Response Using Doubled Haploids in spring oilseed rape (Brassica napus L.)

L.A. MURPHY AND R. SCARTH

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada R3T 2N2

Submitted to Crop Science

April 1997

ABSTRACT

The presence of a vernalization requirement can delay floral initiation until the cold requirement of the plant has been satisfied. Breeders attempting to develop early maturing cultivars must understand the inheritance of the vernalization response in order to assess potential impact on their programs. The ability to generate DH lines from oilseed rape (Brassica napus) has lead to the use of doubled haploid populations in genetic studies. The objective of this study was to determine the mode of inheritance of the vernalization response in spring B. napus using doubled haploids. A set of F1-derived DH lines from crosses between spring oilseed rape parent lines were evaluated for their response to vernalization. The use of doubled haploid lines allowed the exposure of the same genotype to two different treatments, vernalization and no vernalization. The vernalization treatment consisted of exposure of germinated seeds to 2 weeks at 4° C. Seeds of the non-vernalized treatment were germinated at 22° C. Comparison between treatments of the final leaf number on the main raceme (FLN) was used to assess the vernalization response. The results indicated that there is a single major gene for nonresponse to vernalization in spring oilseed rape. FLN offers a direct measure of vernalization response, as leaf initiation ceases when floral initiation begins.

Key Words: Vernalization, Brassica napus (spring), haploidy, final leaf number

INTRODUCTION

Early maturity is a major objective of spring oilseed rape (Brassica napus L) breeding programs in Western Canada. In order to develop early maturing oilseed rape lines, plant breeders must have an understanding of the genetic and environmental factors influencing time to maturity. Collins and Wilson (1974) determined that time to floral initiation and time to flowering were the primary determinants of maturity. Any factor which affects either time to floral initiation or time to flowering would have a resulting effect on maturity.

The presence of a vernalization requirement affects plant development by delaying floral initiation until the cold requirement of the plant has been satisfied (Flood and Halloran, 1984). Delays in floral initiation will result in an increase in final leaf number (Quinby and Liang, 1969). Murphy and Scarth (1994) demonstrated that there is a cumulative response to vernalization in spring oilseed rape. In comparisons between treatments, final leaf numbers on the main raceme were reduced with longer exposure to 4° C. Thurling and Vijendra Das (1977) reported the presence of major and minor genes in the determination of days to flowering in a cross between a winter and a spring oilseed rape cultivar.

The development of a successful protocol for the production of microspore-derived doubled haploid (DH) lines in B. napus has allowed the use of DH lines in genetic studies, allowing a considerable time saving in the production of homozygous lines (Ferrie et al., 1994). The homozygous

conditions for all loci in DH lines removes the complexity of intragenic interactions, reducing some of the difficulty in genetic analysis (Collins, 1977). The simpler ratios of the gametic vs the sporophytic generation reduces the size of the populations needed to obtain extreme genotypes (Siebel and Pauls, 1989, Henderson and Pauls, 1992 and Thiagarajah and Stringam, 1993) and class separation is greater (Siebel and Pauls 1989; Van Deynze and Pauls 1994)

Previous studies of the inheritance of vernalization response in B. napus have involved DH lines derived from crosses between winter and spring parental lines. Van Deynze and Pauls (1994) characterized DH lines generated from the F1 of a cross between a winter and a spring line. Based on days to flower, they found two gene differences between the parent lines that were vernalization dependent: a major gene "A", which permitted flowering in less than 62 days without vernalization, and a minor gene "B", which allowed plants to flower without vernalization in under 77 days. The double recessive (aabb) required more than 77 days to flower in the absence of vernalization.

Hoffman et al. (1982) reported the appearance of a winter-type B. napus that required cold for flowering in an otherwise homogeneous population of spring B. napus cultivar Tower. Hoffman et al. (1982) used this line as evidence in support of their hypothesis that there was a higher degree of homozygosity in DH lines, compared to inbred lines of B. napus.

The ability to produce sufficient quantities of genetically identical seed from DH lines allows the assessment of the effects of different vernalizaton

treatments on plant development. The objective of this study was to determine the inheritance of the vernalization response in spring B. napus, using DH lines.

MATERIALS AND METHODS

Plant Material

Murphy and Scarth (1994) characterized B. napus cultivars Global and Marnoo as responsive to vernalization, while Karat was determined to show no response to 2 weeks at 4° C. Brandle and McVetty (1989) developed S4 lines from Global, Karat and Marnoo by using single seed descent. The lines were inbred to S3, then increased in the S4 generation by selfing the S3 plants. F1 hybrids were produced by crossing the S4 parent lines in the greenhouse. The S4 lines selected in this study were characterized by Brandle (1989) as intermediate in days to flower within the sample of S4 lines from each parent cultivar.

The donor plants for microspore culture were F1 hybrids of crosses between individual S4 inbred lines of the canola cultivars Karat (Karat-17), Marnoo (Marnoo-14) and Global (Global-16) and their reciprocals. Ten seeds of each F1 were sown in MetroMix potting medium. The plants were grown in a controlled environment growth room under a 16 hr photoperiod and a 22° C /16° C day/night temperature regime. VHO Grolux wide spectrum and cool white fluorescent lamps supplied a photosynthetic photon flux density of approximately 300 $\mu E\ m^{-2}\ s^{-1}$ at the top of the plant.

Microspore Culture Technique .

Buds 4 to 5 mm in length were removed from the terminal and upper axillary racemes of donor plants that had approximately three open flowers on the terminal raceme. The buds were surface sterilized in a 5.7% hypochlorite solution for 10 min. and used for microspore culture as described by Coventry et al. (1988).

Embryo Culture and Doubled Haploid (DH) Plant Production

Cotyledonary embryos were visible in 7 to 20 days. Modifying the technique used by Chuong and Beversdorf (1985), embryos were transferred directly to solid B5 medium containing 2% sucrose and no hormones. After 4 weeks, plantlets that had developed normal shoots were transferred to solid B5 slant tubes in order to encourage root development. Plantlets were then transplanted into peat pellets and placed in a high humidity chamber. Once the plantlets were established, they were potted and transferred to an environmentally controlled growth room. At flowering, spontaneous diploids, identified by the presence of pollen and normal flower morphology, were bagged to produce selfed seed. Root cuttings were made from haploid plants, characterized by their small, infertile flowers. The cuttings were treated by immersing roots in a solution of 0.05% colchicine for 6 hours with aeration, rinsed and potted in MetroMix. The subsequent fertile flowers produced from the doubled sectors were bagged for selfed seed. Seed of each DH line was grown for a generation of seed increase under controlled environment growth conditions to produce the

seed amounts required for vernalization evaluation. Fifty DH lines were produced from each F1 hybrid.

Vernalization screening.

Seeds were placed on moist coarse acid silica sand, covered and held at 22° C for 24 hours. The vernalization (V) treatment consisted of 2 weeks at 4° C in the dark. Seeds of the nonvernalized (N) treatment were germinated at 22° C for 48 hours prior to the end of the vernalization treatment in order to reach a growth stage similar to that of the vernalized seeds at the end of the V treatment.

Germinated seeds were planted in 2 cm x 2 cm peat pots containing MetroMix potting medium. The plants were grown under a regime of 22° C/ 16° C day/night temperature, with a 20 hr day/ 4 hr night photoperiod. Plants received approximately 300 $\mu E\ m^{-2}\ s^{-1}$ of photosynthetically active radiation at the top of the plant.

Once plants reached the 2.3 stage (Harper and Berkenkamp, 1975), they were transferred to 10 cm peat pots, with 2 plants per pot. Final leaf number (FLN) was determined at appearance of the first flower on the terminal raceme. To facilitate leaf counts, each leaf was punched once with a single hole punch near the leaf margin at full emergence. The resulting 5 mm hole was visible throughout the experiment and was used to determine FLN.

Three replicates of each DH line were arranged under each of three light banks in the growth room and randomized weekly within the three replicates.

Data Analysis

The mean FLN for the two plants in each pot was used for each replicate of each line. The mean and standard error of the mean were used to determine the response to vernalization. Standard errors were derived from the standard deviation and the number of replicates for each DH line-treatment combination. Response to vernalization was calculated as the difference between V and N treatments. DH lines were classified as non-responsive (NR) if there was no significant difference (P=0.05) between the V and N treatment. Lines were considered responsive (R) if there was a significant difference in FLN between N and V treatment (P=0.05). Chi Square analysis was used to compare the observed distribution of the DH population to those predicted by various genetic models.

RESULTS AND DISCUSSION

Initial assessment of the parental lines indicated that there were differences in vernalization response of the three parental lines (Table 12). Global-16 (G16) and Marnoo-14 (M14) were classified as R, while Karat-17 (K17) was NR. The four F1 hybrids, G16 x K17 (GK), K17 x G16 (KG), K17 x M14 (KM) and M14 x K17 (MK) were NR.

Table 12. Characterization of parental lines Global-16 (G), Karat-17 (K) and Marnoo-14 (M) and the F1 hybrids GK, KG, KM and MK as responsive (R) or non-responsive (NR) to vernalization, using final leaf numbers (FLN) after 0 weeks vernalization (N) and 2 weeks at 4°C (V).

Line	Treatment	FLN Mean	Standard Error	Vernalization Response +
G	N	11.9	0.3	R
	V	9.0	0.3	
K	N	10.5	0.6	NR
	V	10.0	0.6	
M	N	12.2	0.3	R
	V	9.9	0.3	
GK F1	N	10.0	0.6	NR
	V	9.2	0.8	
KG F1	N	10.7	0.8	NR
	V	9.2	0.8	
KM F1	N	12.2	0.6	NR
	V	12.5	0.2	
MK F1	N	10.8	0.9	NR
	V	9.8	0.6	

+: Calculated from the difference and standard error of the difference in FLN between N and V treatment;
NR:difference and SE between N and V are non-significant at P=0.05
R: difference and SE between N and V is significant at P=0.05

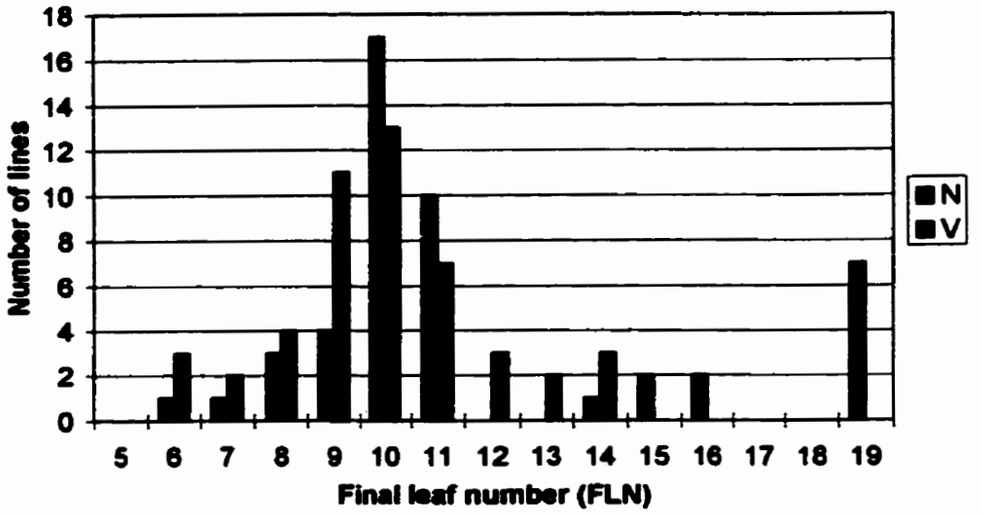
The DH lines derived from each F1 hybrid had a range of FLN (Figures 7 and 8). DH lines from GK and KG ranged from 7.3 leaves to over 19 leaves and from 5.5 to over 19 leaves for MK and KM. Vernalization treatment significantly reduced the FLN in the majority of the DH lines examined. For some DH lines,

the V treatment of 2 weeks at 4° C was insufficient to stimulate floral initiation, resulting in continuing leaf initiation and FLN of greater than 19. In all crosses, DH lines that had more than 19 leaves were designated as 'did not flower'.

Response to vernalization was determined for each of the DH lines from each cross. Each DH line was assigned to the NR or R class, based on the difference between the N and V treatment. Chi square analysis was then performed on the two classes of DH lines from each cross (Table 13). The DH lines from the four crosses examined fit a 1 NR : 1 R gametic ratio. This ratio indicates the involvement of a single major gene with dominance for nonresponse to vernalization. The range of FLN for the DH lines indicates that minor gene alleles are involved in the response as well.

The gene designation LN1 is proposed for the control of the response of FLN to vernalization. The R class would be represented by the homozygote for the recessive alleles (ln1ln1). The NR class would have the genotype LN1_.

a) Global-16 x Karat-17 DH lines



b) Karat-17 x Global-16 DH lines

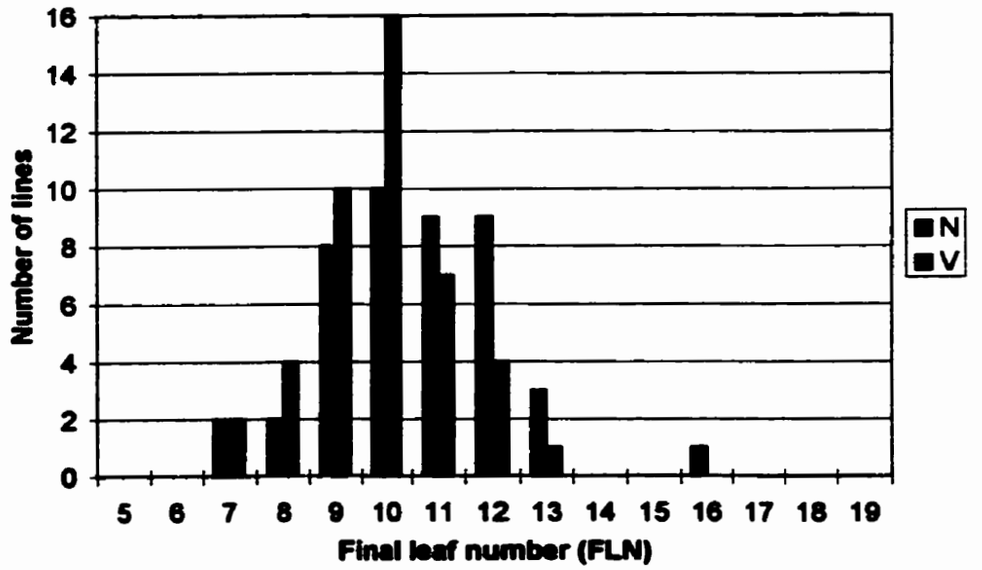
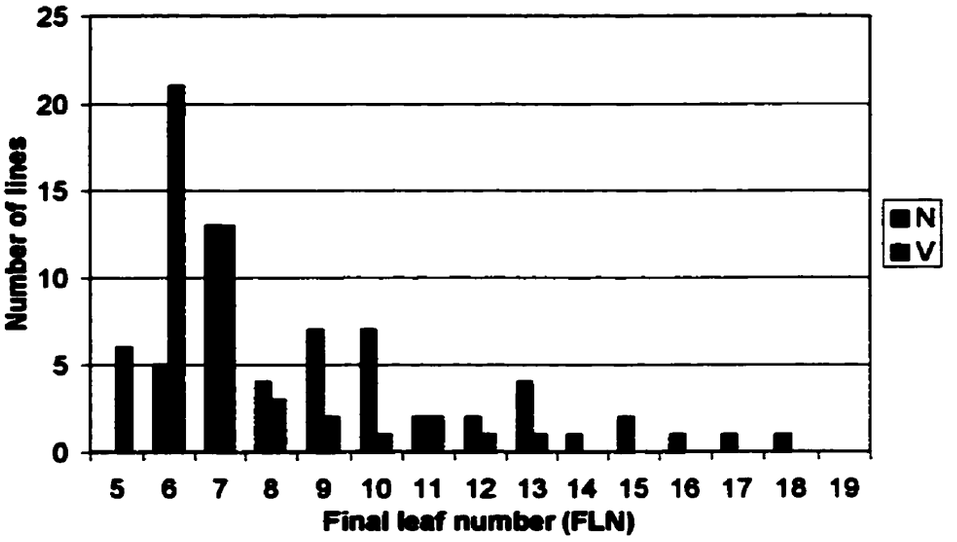


Figure 7. Distribution of F1-derived doubled haploid (DH) lines from crosses between parental lines Global-16 (G) and Karat-17 (K) for final leaf number (FLN) after 0 weeks of vernalization (N) and 2 weeks at 4°C (V).

a) Karat-17 x Marnoo-14 DH lines



b) Marnoo-14 x Karat-17 DH lines

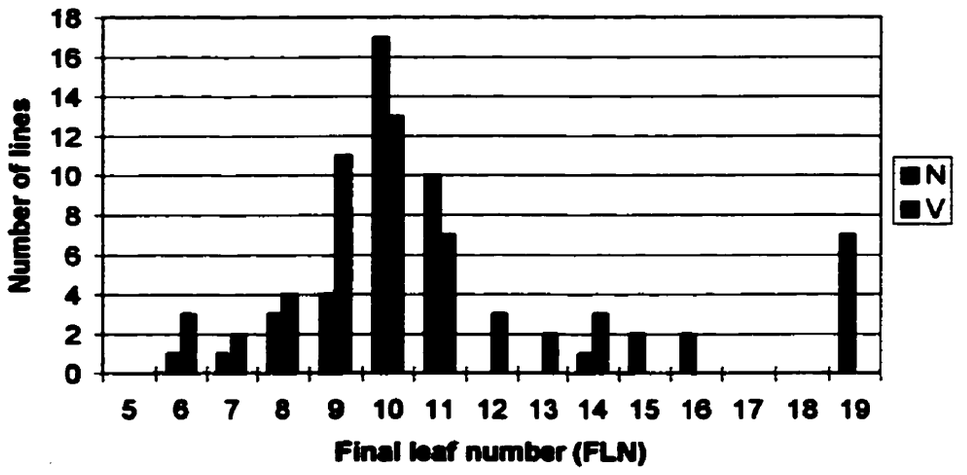


Figure 8. Distribution of F1-derived doubled haploid (DH) lines from crosses between parental lines Karat-17 (K) and Marnoo-14 (M) for final leaf number (FLN) after 0 weeks of vernalization (N) and 2 weeks at 4°C (V).

Table 13. Chi square analysis of segregation for final leaf number (FLN) of F1-derived doubled haploid (DH) lines from crosses between parental lines Global-16 (G), Karat-17 (K) and Marnoo-14 (M) assessed as non-responsive (NR) and responsive (R) after 0 weeks of vernalization (N) and 2 weeks at 4°C (V).

F1 Derived DH lines	Observed NR	Observed R	Ratio Tested	Chi Square Value	Probability
GK	25	24	1:1	0.04	0.95 -0.9
KG	28	22	1:1	0.8	0.5 - 0.1
KM	20	30	1:1	2.0	0.5 - 0.1
MK	27	23	1:1	0.4	0.9 - 0.5

Thurling and Vijendra Das (1979) and Van Deynze and Pauls (1994) indicated a major gene and a minor gene difference between spring and winter oilseed rape. Both of these studies examined days to flower as the criteria for determination of vernalization response. Our study differed in two ways: FLN was used as the criteria for determination of vernalization response and the material was of spring origin only. From a physiological perspective, the use of FLN would appear to offer a more direct measure of vernalization response, as leaves continue to be initiated on the developing terminal apex of the main raceme until floral initiation begins. A delay in initiation, such as would occur in a R genotype under nonvernalizing conditions would result in a higher FLN for the plant.

The parents in this study were identified as intermediate in time of flowering (Brandle, 1989). The fact that some extremely responsive DH lines

were identified is evidence of the accumulation of minor gene alleles enhancing the expression of the major gene allele. Wenzel et al. (1977) believed that the appearance of cold-requiring progeny from spring type plants revealed the occurrence of new gene combinations in the microspore-derived plant progenies. A more likely explanation was offered by Hoffman et al. (1982). They cited the occurrence of a winter type DH line in a population of the spring B. napus cultivar Tower as evidence of more variability in regenerants from different microspores than from the inbred parent. Hoffman et al. (1982) felt that the variants did not represent new variability, but rather were evidence of the higher degree of homozygosity in DH lines, compared to inbred lines of B. napus. The identification of DH lines with more than 19 leaves would be similar to the 'winter type' progeny identified by Hoffman et al. (1982) in Tower.

A spring B. napus cultivar requiring more than 2 weeks at 4°C to begin reproductive development has significance for production in Western Canada, as R lines would only flower under vernalizing conditions. Further characterization of the R lines would be necessary in order to determine whether there is an association with agronomically important traits.

In this study, the V and N treatments were applied to the same genetic material. This provides greater confidence that the response was indeed due to the genotype interacting with the environment provided by the treatment, rather than background heterogeneity, as the genotypes used in each treatment were

identical. The results indicate that DH lines are useful for genetic analyses requiring assessment of contrasting treatments.

An additional advantage of using DH lines is the increased ability to identify desirable combinations of characteristics from smaller populations. Henderson and Pauls (1992) and Thiagarajah and Stringam (1993) indicated that for the introgression of recessive traits such as yellow seed coat into canola quality cultivars, extremely large populations from conventional breeding would have to be assessed in order to obtain one desirable plant. The use of DH lines reduced the size of the population required to obtain extreme genotypes. In this study, 50 or fewer DH lines were examined from each cross, compared with over 150 families, with 50 seeds per family for the conventional genetic analysis (Murphy, 1997).

Siebel and Pauls (1989) and Van Deynze and Pauls (1994) reported that class separation is greater in androgenetic populations, making it easier to distinguish classes than in the F2 population. In this study, the setting of classes for genetic analysis was based on statistical differences between the treatments. The class separation for final leaf number analysis was definitive, as leaves were only counted when the leaf was fully extended. However, the grouping of all lines with greater than 19 leaves may obscure differences between the more responsive lines.

GENERAL DISCUSSION AND CONCLUSIONS

The results of this study indicate that a significant, cumulative response to vernalization exists in spring B. napus. The examination of a range of cultivars, F1 hybrids, S4 inbred lines, F3 families and doubled haploid lines derived from F1 donors, demonstrated a range in response to vernalization from no response, to extremely responsive, or winter type. The fact that a range in response is possible for S4 inbred lines and doubled haploid lines from a spring B. napus cultivar serves to reinforce the hypothesis put forward by Brandle and McVetty (1989) that canola cultivars consist of a mixture of generally homozygous lines. Of particular interest is the observation that a trait such as vernalization response that is conditioned by one major gene, would be maintained within a cultivar intended for spring sowing. Considering the relatively diverse growing region for spring B. napus cultivars, the broad adaptability of canola cultivars may be due in part to selection for diversity among lines within a cultivar population. Thus, the more successful cultivars would be those broadly adapted, capable of responding to a variety of growing conditions. It is probable that as breeders move away from selection for broad adaptability to regional specialization, that the benefits of the capacity to respond to environmental cues such as vernalization will be lost.

One goal of this research was to determine whether response to vernalization was a significant factor in the spring B. napus populations that were assessed in the University of Manitoba canola breeding program as

parental material. However, the identification of the existence of a response was not useful without the ability to quickly and consistently characterize the response of potential breeding material. The use of a 2 week vernalization at 4°C, coupled with exposure to long day conditions of 20 hours day/4 hours night, was sufficient to permit the identification of nonresponsive vs. responsive material. In addition to the screening of plants at flowering, examination of the developing apices of vernalized and nonvernalized material indicates that characterization of the vernalization response may be possible as soon as 20 days after planting, or prior to the 4 leaf stage. The potential savings of time and resources from such an early determination of response to vernalization would permit the prescreening of more material prior to inclusion in a crossing program.

Measurements of FF and FLN provided two methods of determining the response to vernalization. Although there is a significant correlation between the two methods, it is too low to permit the use of one measurement only. From a physiological perspective, the use of FLN would appear to offer a more direct measure of vernalization response, as leaves continue to be initiated on the developing apex until floral initiation is complete. Thus, a delay in initiation related to lack of vernalization would result in a higher FLN for the plant. FF, on the other hand, is influenced by factors other than vernalization. Experimental conditions were maintained as close to uniform throughout the experiment as possible. Such factors as changing light quality or requirements for plant

nutrition could affect the time of appearance of the first open flower. The finding that floral initiation can occur in as few as 20 days after seeding means that confounding factors prior to initiation would be of shorter duration, and potentially less effect, than on FF, which occurred, on average, 30 days after seeding in the controlled environment conditions in this study.

The decision to use either FF or FLN in the assessment of vernalization response would depend on the goals of the breeding program. The necessity for seed vernalization under controlled environment conditions precludes screening in the field. For a large population, FF is visually easier to determine. FLN is more difficult to determine on a large population due to the fact that it requires the inspection of plants on a regular basis, yet it appears to be more closely associated with vernalization. It is possible to determine FLN from leaf scars as plants approach physiological maturity and the leaves have senesced.

The significance of differences in FLN needs to be further assessed, particularly in relation to yield. Tommey and Evans (1992) found that a pre-floral cold treatment of the winter rape cultivar Mikado significantly increased total seed weight in spite of a reduction in the number of leaves and plant size resulting from earlier initiation. Tommey and Evans (1992) found that the highest seed weight was obtained from plants that were intermediate in terms of duration of pre-floral growth and leaf number. Their interpretation of these results was that seed production was restricted by the availability of assimilates

between excessive numbers of branches and flowers in plants with an extended period of pre-floral growth. It is reasonable to assume that increased leaf numbers could provide an increased 'source' of assimilates for the developing seeds in the pods. This could have a corresponding increase in yield potential, providing physiological maturity is achieved before killing fall frosts.

The indication that one major gene is involved in the response is of interest to breeders attempting to incorporate a vernalization requirement or to eliminate it entirely from a breeding population. The fact that S4 inbred and doubled haploid lines with R and NR response exist within the limited population sizes developed in this study would permit the immediate inclusion or exclusion of these lines in a breeding program.

While S4 inbred lines with NR were identified, the use of S4 lines as parents in a breeding program needs to be re-examined. This is particularly true in light of the availability of doubled haploid lines for B. napus. Residual heterozygosity in the S4 inbred lines was identified, which complicated the genetic analysis of segregating generations.

The production of doubled haploids from F1 donor plants resulted in the recovery of R phenotype, or effectively winter type progeny from the spring B. napus parents. In addition to providing evidence of residual heterozygosity in the S4 inbred parent lines, the use of doubled haploids resulted in the recovery of

more genotypes outside the parental populations range of response as compared to the conventional, single seed descent populations.

The single seed descent technique used to produce the S4 inbred lines examined in this study did not produce the same range of variation in genotypes, compared with the doubled haploid lines. Unconscious selection during the single seed descent process may preclude the identification of the extreme genotypes, as only families that successfully set seed were carried to the next generation. Thus, the winter type genotypes identified in the doubled haploid study would not have been carried forward in the conventional population. This difference is sampling may have contributed to the difficulties in characterizing the inheritance of vernalization response using the F2 generation.

As doubled haploid procedure for spring B. napus now has a well-established and successful protocol, its use in a breeding program should be encouraged when the goal is to identify genotypes at the extreme limits of variation. An adapted cultivar, which is a collection of lines, could form the initial selection pool. The advantage of selection out of a cultivar is that the basic agronomic and quality parameters have been assessed. Conventional breeding programs include a component of development of inbred lines, especially in F1 hybrid development and as parental lines in crosses. The results of this study indicate that B. napus breeders should be developing DH lines, rather than S4 inbred lines, for genetic analysis and as a source of variation within an adapted population. The identification of genotypes with extremes in expression, such as

those with winter type vernalization requirements in an otherwise spring type population, was only possible with the DH approach. The reduced size of the DH population as compared to the segregating generation population required to recovery the extreme genotypes produced savings in both labour and resource.

The existence of a significant response to vernalization in spring B. napus would not appear to present any restrictions on the use of geographically diverse germplasm within a canola breeding program if careful evaluation of potential parent material is made. Since a rapid screening method for vernalization is possible at the 4 leaf stage, characterization of response prior to inclusion of new material in a breeding nursery would not present a major undertaking.

The results of this study provide several possibilities for further research. Maturity in plants is affected by environmental response factors, including response to vernalization and response to photoperiod. Given the response to vernalization identified in this study, a survey of photoperiod response in F1 hybrids, S4 inbred lines and doubled haploid lines would be a major priority. Once more information is available on the response to photoperiod, the existence of photoperiod x vernalization interaction could then be evaluated.

Research on the effect of environmental factors on development in spring B. napus is limited, but the results of this study indicate that factors such as vernalization response may have significant impacts on flowering and maturity.

8.0 LITERATURE CITED

ALLARD, R.W. 1960. Principles of plant breeding. John Wiley and Sons, Inc. New York

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

APPELQVIST, L.A. 1972. Rapeseed. In Rapeseed, Elsevier Publishing, New York. eds L.A. Appelqvist and R. Ohlson, pp 1-8.

BARBER, H.N. 1959. Physiological genetics of Pisum. II. The genetics of photoperiodism and vernalisation. Heredity 13:33-66.

BERNIER, G. 1971. Structural and metabolic changes in the shoot apex in transition to flowering. Can. J. Bot. 49:803-819.

BERNIER, G. 1988. The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Mol. Biol. 39:175-219.

BERRY, G.J. and AITKEN, Y. 1979. Effect of photoperiod and temperature on flowering in pea (Pisum sativum). Aust. J. Plant Phys. 6:573-587.

BERRY, G.J.; SALISBURY, P.A. and HALLORAN, G.M. 1980. Expression of vernalization genes in near-isogenic wheat lines: Duration of vernalization period. Ann. Bot (NS) 46:235-241.

BRANDLE, J.E. 1989. Genetic variance, heterosis and combining ability in oilseed rape. PhD Thesis, University of Manitoba

BRANDLE, J.E. and MCVETTY, P.B.E. 1989. Effects of inbreeding and estimates of additive genetic variance within seven summer oilseed rape cultivars. Genome 32:115-119.

BRANDLE, J.E. and MCVETTY, P.B.E. 1990. Geographical diversity, parental selection and heterosis in oilseed rape. Can. J. Plant Sci. 70:935-940.

CAMPBELL, D.C. and KONDRA, Z.P. 1978. A genetic study of growth characters and yield characters of oilseed rape. Euphytica 27:177-183.

CANOLA OIL AND MEAL STANDARDS AND REGULATIONS, 1987. Canadian General Standards Board, Supply and Services Canada

CHARLES-EDWARDS, D.A. 1983. An hypothesis about the control of flowering. Ann Bot 52:105-107.

CHARNE, D.G. and BEVERSDORF, W.D. 1991. Comparisons of agronomic and compositional traits in microspore-derived and conventional populations of spring Brassica napus. GCIRC Eighth International Rapeseed Congress, Saskatoon, Saskatchewan, 1991. D.I. McGregor, editor. pg 64-69.

CHEN, J.L. and BEVERSDORF, W.D. 1990. A comparison of traditional and haploid-derived breeding populations of oilseed rape (Brassica napus L) for fatty acid composition of the seed oil. Euphytica 51:59-65.

CHEN, Z.Z.; SNYDER, S; FAN, ZG and LOH, W.H. 1994. Efficient production of doubled haploid plants through chromosome doubling of isolated microspores in Brassica napus. Plant Breed. 113:217-221.

CHOO, T.M.; REINBERGS, E. and KASHA, K.J. 1985. Use of haploids in breeding barley. Plant Breed. Rev. 3:219-252.

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

CHUONG, P.V. and BEVERSDORF, W.D. 1985. High frequency embryogenesis through isolated microspore culture in Brassica napus L and B. carinata L. Plant Sci. 39:219-226.

COCHRAN, W.G. and COX, G.M. 1957. Experimental Designs. John Wiley and Sons Inc, London.

COLLINS, G.B. 1977. Production and utilization of anther-derived haploids in crop plants. Crop Sci. 17:583-586.

COLLINS, W.J. and WILSON, J.H. 1974. Node of flowering as an index of plant development. Ann. Bot. (NS) 38:175-180.

COVENTRY, J.; KOTT, L. and BEVERSDORF, W.D. 1988. Manual for microspore culture technique for Brassica napus. OAC Publication 0489, University of Guelph, Canada. 35 p.

CRISP, P. and WALKEY, D.G.A. 1974. The use of asceptic meristem culture in cauliflower breeding. Euphytica 23:305-313.

DANIELS, R.W.; SCARISBRICK, D.H. and MAHAMUD, B.S. 1984. Reproductive development of winter oilseed rape, hierarchical structure and its manipulation with plant growth regulators. Asp. Appl. Biol. 6:111-121.

DANIELS, R.W.; SCARISBRICK, D.H. and SMITH, L.J. 1986. Oilseed Rape Physiology. In: Oilseed Rape. Collins, London, pp 83-126.

DAUGHTRY, C.S.T.; COCHRAN, J.C. and HOLLINGER, S.E. 1984. Estimating silking and maturity dates of corn for large areas. Agron. J. 76:421-424.

DAVERN, C.I.; PEAK, J.W. and MORLEY, R.H.W. 1957. The inheritance of flowering time in Trifolium subterraneum L. Aust. J. Agric. Res. 8:121-134.

DAVIDSON, J.L.; CHRISTIAN, K.R.; JONES, D.B. and BREMNER, P.M. 1985. Responses of wheat to vernalization and photoperiod. Aust. J. Agric. Res. 36:347-359.

DOWNEY, R.K. and RAKOW, G.F.W. 1987. Rapeseed and Mustard. In: Principles of Cultivar Development Vol 2. Crop Species. (W.R. Fehr, ed). MacMillan Publishing Company, New York. pp 437-487.

EVANS, L.T. 1959. Flower initiation in Trifolium subterraneum L. Aust. J. Agric. Res. 10:1-15.

FERREIRA, M.E.; WILLIAMS, P.H. and OSBORN, T.C. 1994. RFLP mapping of Brassica napus using doubled haploid lines. Theor. Appl. Genet. 89:615-621.

FERREIRA, M.E.; SATAGOPAN, J.; YANDELL, B.S.; WILLIAMS, P.H. and OSBORN, T.C. 1995. Mapping loci controlling vernalization requirement and flowering time in Brassica napus. Theor. Appl. Genet. 90:727-732.

FERRIE, A.M.R.; PALMER, C.E. and KELLER, W.A. 1994. Biotechnological applications of haploids. In: Biotechnological Applications of Plant Cultures. (PD Shargool and TT Ngo, ed.) CRC Press, Boca Raton. pp 77-90.

FITTER, A.H. and HAY, R.K.M. 1981. Environmental Physiology of Plants. Experimental Botany, Volume 15. Academic Press, New York. 355 pp.

FLOOD, R.G. and HALLORAN, G.M. 1984. The nature and duration of gene action for vernalization response in wheat. Ann. Bot. (NS) 53:363-368.

FLOOD, R.G. and HALLORAN, G.M. 1986. Genetics and physiology of vernalization response in wheat. Adv. in Agron. 39:87-125.

FRIEND, D.J.C. 1985. Brassica. In: CRC Handbook of Flowering. Vol. II. (AH Halevy, ed.). CRC Press, Boca Raton. pp 48-77.

GOWERS, S. and GEMMELL, D.J. 1968. Variation in flowering response to pre-vernalisation growth in Swedes (Brassica napus ssp rapifera). Euphytica 37:275-278.

GREGORY, F.G. and PURVIS, O.N. 1938. Studies in vernalization of cereals. II. The vernalisation of excised mature embryos and of developing ears. Ann. Bot. (NS) 2:237-251.

HALLORAN, G.M. 1975. Genotype differences in photoperiodic sensitivity and vernalization response in wheat. Ann. Bot. (NS) 39:845-851.

HALLORAN, G.M. 1977. Developmental basis of maturity differences in spring wheat. Agron J. 69:899-902.

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

HALSE, N.J. and WEIR, R.N. 1970. Effects of vernalization, photoperiod and temperature on phenological development and spikelet number of Australian wheat. Aust. J. Agric. Res. 21:383-393.

HARPER, F.R. and BERKENKAMP, B. 1975. Revised growth stage key for Brassica campestris and B. napus. Can. J. Plant Sci. 55:657-658.

HENDERSON, C.A.P. and PAULS, K.P. 1992. The use of haploidy to develop plants that express several recessive traits using light-seeded canola (Brassica napus) as an example. Theor. Appl. Genet. 83:476-479.

HOBBS, D.H.; HUME, J.H.; ROLPH, C.E. and COOKE, D.T. 1996. Changes in lipid composition during floral development of Brassica campestris. Phytochem. 42:335-339.

HODGSON, A.S. 1978. Rapeseed adaptation in Northern New South Wales. I. Phenological responses to vernalization, temperature and photoperiod by annual and biennial cultivars of B. campestris, B. napus and wheat cv. Timgalen. Aust. J. Agric. Res. 29:693-670.

HOFFMANN, F.; THOMAS, E. and WENZEL, G. 1982. Anther culture as a breeding tool in rape. II. Progeny analyses of androgenetic lines and induced mutants from haploid cultures. Theor. Appl. Genet. 61:225-232.

JEDEL, P.E. 1994. Inheritance of vernalization response in three populations of spring wheat. Can. J. Plant Sci. 74:753-757.

JEDEL, P.E.; EVANS, L.E. and SCARTH, R. 1986. Vernalization responses of a selected group of spring wheat (Triticum aestivum L.) cultivars. Can. J. Plant Sci. 66:1-9.

JENSEN, C.J. 1986. Haploid induction and production in crop plants. In: Genetic manipulation in Plant Breeding. (W. Horn, ed.) Walter de Gruyter, New York.

KARLSSON, B.H.; SILLS, G.R. and NIENHUIS, J. 1993. Effects of photoperiod and vernalization on the number of leaves at flowering in 32 Arabidopsis thaliana (Brassicaceae) ecotypes. Am. J. Bot. 80:646-648.

KELLER, W.A. and ARMSTRONG, K.C. 1978. High frequency production of microspore-derived plants from Brassica napus anther cultures. Z. Pflanzenzuchtg. 80:100-108.

KELLER, W.A. and STRINGAM, G.R. 1978. Production and utilization of microspore-derived haploid plants. In: Frontiers of Plant Tissue Culture. (T.A. Thorpe, ed.). U of Calgary, pp 113-121.

KELLER, W.A.; ARMSTRONG, K.C. and DELAROCHE, A.I. 1982. The production and utilization of microspore-derived haploids in Brassica crops. In: Plant Cell Culture in Crop Improvement. (K.L. Gilles and B.K. Ben, eds.). Plenum Publishing, pp 169-183.

KING, J.R. and KONDRA, Z.P. 1986. Photoperiod response of spring oilseed rape (Brassica napus L. and B. campestris L.) Field Crops Res. 13:367-373.

KITASHIBA, K.; IWAI, T.; TORIYAMA, K.; WATANABE, M. and HINATA, K. 1996. Identification of genes expressed in the shoot apex of Brassica campestris during floral transition. Sex. Plant Reprod. 9:186-188.

KLAIMI, Y.Y. and QUALSET, C.O. 1974. Genetics of heading time in wheat (Triticum aestivum L.) II. The inheritance of vernalization response. Genetics 76:119-133.

KONDRA, Z.P.; CAMPBELL, D.C. and KING, J.R. 1983. Temperature effects on germination of rapeseed (Brassica napus L. and B. campestris L.) Can. J. Plant Sci. 63:1063-1065.

KUBBA, J.; SMITH, B.M.; OCKENDON, D.J.; SETTER, A.P.; WERNER, C.P. and KEARSEY, M.J. 1989. A comparison of anther culture derived material with single seed descent lines in Brussels sprouts (Brassica oleracea var. gemmifera). Heredity 63:89-95.

LEVY, J. and PETERSON, M.L. 1972. Responses of spring wheats to vernalization and photoperiod. Crop Sci. 12:487-490.

LYSENKO, T.D. 1928. In: CHOUARD, P. 1960. Vernalization and its relations to dormancy. Ann. Rev. Plant Phys. 11:191-238.

MAJOR, D.J. 1980. Photoperiod response characteristics controlling flowering of nine crop species. Can. J. Plant Sci. 60:777-784.

MANDAL, A. 1987. Induction of androgenetic haploids in the breeding materials of winter rape (Brassica napus). Hereditas 106:189-193.

MANUPEERAPAN, T.; DAVIDSON, J.L.; PEARSON, C.J. AND CHRISTIAN, K.R. 1992. Differences in flowering responses of wheat to temperature and photoperiod. Aust. J. Agric. Res. 43:575-584.

McDANIEL, C.N. 1996. Developmental physiology of floral initiation in Nicotiana tabacum L. J. Exp. Bot. 47:465-475.

MCKINNEY, H.H. 1940. Vernalization and the growth-phase concept. Bot. Rev. 6:25-47.

MCKINNEY, H.H. and SANDO, W.J. 1935. Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. J. Agr. Res. 51:621-641.

MCPHERSON, H. 1987. Effects of moisture stress on rapeseed. M Sc thesis, University of Manitoba.

MENDHAM, N.J. and SCOTT, R.K. 1975. The limiting effect of plant size at inflorescence initiation on subsequent growth and yield of oilseed rape (Brassica napus). J. Agric. Sci, Camb. 84:487-502.

MERO, C.E. and HONMA, S. 1985. Inheritance of bolting resistance in an intraspecific Chinese Cabbage x Turnip cross. Hort. Science 20:881-882.

MORRISON, M.J.; MCVETTY, P.B.E. and SHAYKEWICH, C.F. 1989. The determination and verification of a baseline temperature for the growth of 'Westar' summer rape. Can. J. Plant Sci. 69:455-464.

MORRISON, M.J.; MCVETTY, P.B.E. and SCARTH, R. 1990. Effect of row spacing and seeding rates on summer rape in southern Manitoba. Can. J. Plant Sci. 70:127-137.

MOSAAD, M.G.; ORTIZ-FERRARA, G; MUHALAKSHMI, V and FISCHER, R. 1995. Phyllochron response to vernalization and photoperiod in spring wheat. Crop Sci. 35:168-171.

MURFET, I.C. 1977. Environmental interaction and the genetics of flowering. Ann. Rev. Plant Physiol. 28:253-278.

MURPHY, L.A. 1997. Vernalization response in spring oilseed rape (Brassica napus L.) Ph.D. thesis, University of Manitoba

MURPHY, L.A. and SCARTH, R. 1994. Vernalization response in spring oilseed rape (*Brassica napus* L) cultivars. Can. J. Plant Sci. 74:275-277.

MURPHY, L.A. and SCARTH, R. 1997. Inheritance of the vernalization response using doubled haploids in spring oilseed rape (Brassica napus L) submitted to Crop Sci.

MYERS, L.F.; CHRISTIAN, K.R. and KIRCHNER, R.J. 1982. Flowering responses of 48 lines of oilseed rape (Brassica spp) to vernalization and daylength. Aust. J. Agric. Res. 33:927-936.

NAPP-ZINN, K. 1987. Vernalization- Environmental and genetic regulation. In: Manipulation of Flowering (J.G. Atherton, ed). Butterworths, London. pp 123-132.

ORR, A.R. 1978. Inflorescence development in Brassica campestris L. Amer. J. Bot. 65:466-470.

ORR, A.R. 1981. A quantitative study of cellular events in the shoot apical meristem of Brassica campestris (Cruciferae) during transition from vegetative to reproductive condition. Amer. J. Bot. 68:17-23.

ORR, A.R. 1984. Histochemical study of enzyme activity in the shoot apical meristem of Brassica campestris L. during transition to flowering. II. Cytochrome oxidase. Bot. Gaz. 145:308-311.

ORR, A.R. 1985. Histochemical study of enzyme activity in the shoot apical meristem of Brassica campestris L. during transition to flowering. III. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Bot. Gaz. 146:477-482.

ORR, A.R. 1987. Changes in glyceraldehyde-3-phosphate dehydrogenase activity in shoot apical meristems of Brassica campestris during transition to flowering. Amer. J. Bot. 74:1161-1166.

ORR, A.R.; JOHNSON-FLANAGAN, A.M.; KELLER, W.A. and SINGH, J. 1990. Induction of freezing tolerance in microspore-derived embryos of winter Brassica napus . Plant Cell Reports 8:579-581

PARK, S.J.; WALSH, E.J.; REINBERGS, E.; SONG, L.S.P. and KASHA, K. 1976. Field performance of doubled haploid barley lines in comparison with lines developed by the pedigree and single seed descent methods. Can. J. Plant Sci. 56:467-474.

PETERSON, M.L. and BENDIXEN, L.E. 1963. Relationships of gibberellin and auxin to thermal induction of flowering in Lolium temulentum L. Crop Sci. 3:79-82.

PINTHUS, M.J. 1967. Evaluation of winter wheat as a source of high yield potential for the breeding of spring wheat. Euphytica 16:231-251.

POLOWICK, P.L. and SAWHNEY, V.K. 1986. A scanning electron microscope study on the initiation and development of floral organs of Brassica napus (cv. Westar). Amer. J. Bot. 73:254-263.

POWERS, L. 1934. The nature and interaction of genes differentiating habit of growth in a cross between varieties of Triticum vulgare. J. Agr. Res. 49:573-605.

PUGSLEY, A.T. 1963. The inheritance of a vernalization response in Australian spring wheats. Aust. J. Agric. Res. 14:622-627.

PUGSLEY, A.T. 1971. A genetic analysis of the spring-winter habit of growth in wheat. Aust. J. Agric. Res. 22:21-31.

PUGSLEY, A.T. 1972. Additional genes inhibiting winter habit in wheat. Euphytica 21:547-552.

PURVIS, O.N. 1939. Studies in vernalisation of cereals. V. The inheritance of the spring and winter habit in hybrids of Petkus rye. Ann. Bot. (NS) 3:719-729.

PURVIS, O.N. and GREGORY, F.G. 1937. Studies in vernalisation of cereals. I. A comparative study of vernalisation of winter rye by low temperature and by short days. Ann. Bot. (NS) 1:569-591.

QUINBY, J.R. and LIANG, G.H. 1969. Leaf number and duration to floral initiation and flowering of sorghum parents and hybrids. Can. J. Genet. Cytol. 11:275-280.

RAHMAN, M.S. and GLADSTONES, J.S. 1973. Control of lupin flower initiation by vernalization, photoperiod and temperature under controlled environment. Aust. J. Exp. Agric. Anim. Husb. 12:638-645.

ROBERTS, D.W.A. 1990. Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit and height of hardened plants. Genome 33:247-259.

SADIK, S. and OZBUN, J.L. 1967. Histochemical changes in the shoot tip of cauliflower during floral induction. Can. J. Bot. 45:952-959.

SALISBURY, P.A.; BERRY, G.J. and HALLORAN, G.M. 1979. Expression of vernalization genes in near isogenic wheat lines: methods of vernalization. Can. J. Genet. Cytol. 21:429-434.

SALISBURY, P.A.; AITKEN, Y. and HALLORAN, G.M. 1987. Genetic control of flowering time and its component processes in subterranean clover (Trifolium subterraneum L.). Euphytica 36:887-902.

SCARISBRICK, D.H.; ATKINSON, L. and ASARE, E. 1989. Oilseed Rape. Outlook on Agric. 18:152-159.

SCARTH, R.; SEGUIN-SWARTZ, G. and RAKOW, G.F.W. 1991. Application of doubled haploidy to Brassica napus breeding. GCIRC Eighth International Rapeseed Congress, Saskatoon, Saskatchewan, 1991. D.I. McGregor, editor. pg 1449-1453.

SHARP, W.R.; REED, S.M. and EVANS, D.A. 1984. Production and application of haploid plants. In: Crop Breeding- A Contemporary Basis. (P.B. Vose and S.G. Blixt, eds.). Pergamon Press, Toronto, pp 347-381.

SIEBEL, J. and PAULS, K.P. 1989. Inheritance patterns of erucic acid content in populations of Brassica napus microspore-derived spontaneous diploids. Theor. Appl. Genet. 77:489-494.

SMITH, H. 1990. Signal perception, differential expression within multigene families and the molecular basis of phenotypic plasticity. Plant Cell Environ. 13:585-594.

SMITH, L.J. and SCARISBRICK, D.H. 1990. Reproductive development in oilseed rape (Brassica napus cv. Bienvenu). Ann. Appl. Bot. 65:205-212.

SNAPE, J.W.; WRIGHT, A.J. and SIMPSON, E. 1984. Methods for estimating gene numbers for quantitative traits using doubled haploid lines. Theor. Appl. Genet. 67:143-148.

STRINGAM, G.R. and THIAGARAJAH, M.R. 1991. Effectiveness of selection for early flowering in F2 populations of Brassica napus L.- A comparison of doubled haploid and single seed descent methods. GSIRC Eighth International Rapeseed Congress, Saskatoon, Saskatchewan. (DI McGregor, ed) pp 70-75.

STRINGAM, G.R.; DEGENHARDT, D.F.; THIAGARAJAH, M.R. and BANSEL, V.K. 1995. Quantum summer rape. Can J. Plant Sci. 75:903-904.

SYLVESTER-BRADLEY, R. 1985. Revision of a code for stages of development in oilseed rape (Brassica napus L.). Asp. Appl. Biol. 10:395-400.

SYLVESTER-BRADLEY, R.; MAKEPEACE, R.J. and BROAD, H. 1984. A code for stages of development in oilseed rape (Brassica napus L.) Asp. Appl. Biol. 6:398-419.

SYME, J.R. 1968. Ear emergence of Australian, Mexican and European wheats in relation to time of sowing and their response to vernalization and daylength. Aust. J. Exp. Agric. Anim. Husb. 8:578-581.

SYME, J.R. 1973. Quantitative control of flowering time in wheat cultivars by vernalization and photoperiod sensitivities. Aust. J. Agric. Res. 24:657-665.

TEUTONICO, R.A. and OSBORN, T.C. 1995. Mapping loci controlling vernalization requirement in Brassica rapa. Theor. Appl. Genet. 91:1279-1283.

THIAGARAJAH, M.R. and STRINGAM, G.R. 1993. A comparison of genetic segregation in traditional and microspore derived populations of Brassica juncea L. Czern and Coss. Plant Breeding 111:330-334.

THOMAS, B. and VINCE-PRUE, D. 1984. Juvenility, photoperiodism and vernalization. In: Advanced Plant Physiology (M.B. Wilkes, ed.) Pitman Publishing Inc, London, pp 408-436.

THOMAS, E. and WENZEL, G. 1975. Embryogenesis from microspores of Brassica napus. Z. Pflanzenzuchtg. 81:77-81.

THOMAS, P. 1984. Canola Growers Manual. Canola Council of Canada, Winnipeg.

THOMPSON, K.F. 1979. Superior performance of two homozygous diploid lines from naturally occurring polyhaploids in oilseed rape (Brassica napus). Euphytica 28:127-135.

THURLING, N. and KAVEETA, R. 1992. Yield improvement of oilseed rape (Brassica napus L) in a low rainfall environment. I. Utilization of genes for early flowering in primary and secondary gene pools. Aust. J. Agric. Res. 43:609-622.

THURLING, N. and VIJENDRA DAS, L.D. 1977. Variation in the pre-anthesis development of spring rape (Brassica napus L.). Aust. J. Agric. Res. 28:597-607.

THURLING, N. and VIJENDRA DAS, L.D. 1979a. Genetic control of pre-anthesis development of spring rape (Brassica napus L.). I. Diallel analysis of variation in the field. Aust. J. Agric. Res. 30:215-259.

THURLING, N. and VIJENDRA DAS, L.D. 1979b. Genetic control of the pre-anthesis development of spring rape (Brassica napus L.). II. Identification of individual genes controlling development pattern. Aust. J. Agric. Res. 30:261-271.

THURLING, N. and VIJENDRA DAS, L.D. 1979c. The relationship between pre-anthesis development and seed yield in spring rape (Brassica napus L.). Aust. J. Agric. Res. 31:25-36.

TOMMEY, A.M. and EVANS, E.J. 1989. Construction of a key to define flower initiation and floral development in a range of varieties of winter oilseed rape (Brassica napus L). Asp. Appl. Biol. 23:165-172.

TOMMEY, A.M. and EVANS, E.J. 1991. Temperature and daylength control of flower initiation in winter oilseed rape (Brassica napus L). Ann. Appl.Biol. 118:201-208.

TOMMEY, A.M. and EVANS, E.J. 1992. The influence of pre-floral growth and development on the pathway of floral development, dry matter distribution and seed yield in oilseed rape (Brassica napus L). Ann. Appl. Biol. 121:687-696.

TRIONE, E.J. and METZGER, R.J. 1970. Wheat and barley vernalization in a precise temperature gradient. Crop Sci. 10:390-392.

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

VAN DEYNZE, A. and PAULS, K.P. 1994. The inheritance of seed colour and vernalization requirement in Brassica napus using doubled haploid populations. Euphytica 74:77-83.

WALL, P.C. and CARTWRIGHT, P.M. 1974. Effects of photoperiod, temperature and vernalization on the phenology and spikelet numbers of spring wheat. Ann. Appl. Biol. 76:299-309.

WEIBEL, D.E. 1958. Vernalization of immature winter wheat embryos. Agron. J. 50:267-270.

WEINBERGER, P. 1975. Ontogenetic changes in the alcohol-soluble amino acid fraction of the grain, leaves and roots of Triticum aestivum (var. Rideau) following vernalization and seedling growth. Ann. Bot. (NS) 39:767-775.

WEIGAND, C.L.; GERBERMANN, A.H. and CUELLAR, J.A. 1981. Development and yield of hard red winter wheats under semitropical conditions. Agron. J. 73:29-37.

WEIGEL, D. and NILSSON, O. 1995. A developmental switch sufficient for flower initiation in diverse plants. Nature 377:495-500.

WELLENSIEK, S.J. 1960. Annual Brussel Sprouts. Euphytica 9:10-12.

WELLENSIEK, S.J. 1962. Dividing cells as the locus for vernalization. Nature 195:307-308.

WELLENSIEK, S.J. 1964. Dividing cells as the prerequisite for vernalization. Plant Physiol. 39:832-835.

WENZEL, G.; HOFFMAN, F. and THOMAS, E. 1977. Anther culture as a breeding tool in rape. I. Ploidy level and phenotype of androgenetic plants. Z. Pflanzenzuchtg. 78:149-155.

WHELAN, E.D.P. and SCHAALJE, G.B. 1992. Vernalization of embryogenic callus from immature embryos of winter wheat. Crop Sci. 32:78-80.

WORT, D.J. 1941. Phasic development of Marquis spring wheat and Fulhio winter wheat. Bot. Gaz. 102:725-736.

APPENDICES

Appendix A- Means and standard deviations of F3 families for the cross Karat-17 x Marnoo-14.

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
1	N	23	36.1	3.1	10.4	1.0
	V	23	30.8	1.8	8.4	1.2
2	N	19	36.1	2.2	12.5	1.9
	V	24	30.2	1.5	9.7	1.6
3	N	19	33.7	2.7	9.7	1.5
	V	25	33.5	2.0	9.9	0.8
4	N	21	35.7	2.9	11.	1.8
	V	23	34.3	2.4	10.0	1.8
5	N	22	31.7	2.0	10.9	1.8
	V	24	28.5	1.6	8.7	1.1
6	N	24	34.5	1.9	9.8	1.0
	V	25	30.0	1.7	8.5	0.8
7	N	11	35.3	1.7	10.8	1.5
	V	15	31.0	2.0	8.9	1.3
8	N	24	35.7	2.9	10.5	1.1
	V	24	33.4	1.6	10.1	1.2
9	N	24	37.3	2.6	9.5	1.1
	V	24	33.7	2.2	9.0	1.2
10	N	25	33.9	1.7	11.6	1.7
	V	24	32.3	2.8	10.8	2.1
11	N	25	35.0	2.1	10.4	1.8
	V	25	31.2	1.4	9.7	1.2
12	N	24	39.3	4.2	11.3	2.7
	V	25	30.0	2.1	8.5	1.5
13	N	24	33.8	1.7	10.7	1.5
	V	23	28.6	2.3	8.3	1.3
14	N	19	38.2	6.7	13.5	5.9
	V	24	36.8	3.7	12.9	2.2
15	N	25	33.5	2.2	9.8	1.6
	V	24	30.2	2.6	9.5	1.9
16	N	24	35.4	1.8	10.2	1.6
	V	25	33.3	1.9	9.7	1.3
17	N	16	36.0	2.7	12.0	1.8
	V	21	33.8	1.5	11.5	2.0
18	N	20	35.8	2.1	11.6	1.4
	V	20	32.3	2.8	9.6	2.1
19	N	17	34.7	1.7	10.9	2.0
	V	22	31.8	1.2	10.6	1.6
20	N	23	36.7	3.1	11.7	1.3
	V	24	33.7	1.5	12.7	1.7
21	N	22	36.3	2.5	9.8	1.3
	V	22	32.5	1.9	10.1	1.2
22	N	24	32.7	2.1	9.3	0.9
	V	22	29.8	2.0	8.4	0.7

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
23	N	22	34.5	1.2	9.8	1.9
	V	24	33.0	1.2	9.6	1.5
24	N	23	36.8	2.1	10.2	1.6
	V	24	33.0	5.2	10.6	4.8
25	N	20	35.6	1..7	14.4	1.8
	V	25	32.4	1.8	12.6	1.7
26	N	22	36.2	2.4	10.2	1.9
	V	16	33.3	1.9	10.2	1.9
27	N	23	36.9	3.1	13.1	1.5
	V	23	33.2	2.4	12.0	1.4
28	N	23	37.0	2.2	12.2	2.1
	V	22	32.3	2.4	10.5	1.5
29	N	25	36.1	1.3	10.0	1.4
	V	21	31.4	2.0	10.0	1.6
30	N	25	37.3	2.1	11.1	1.6
	V	20	34.7	2.6	10.2	1.7
31	N	25	34.0	1.5	9.4	0.9
	V	24	32.3	2.5	9.1	1.2
32	N	17	35.7	2.2	12.5	2.6
	V	21	33.0	2.3	11.8	1.8
33	N	20	33.7	2.0	9.4	1.0
	V	25	30.6	2.3	8.4	1.2
34	N	25	35.8	2.1	10.4	1.2
	V	24	32.8	1.7	9.9	1.4
35	N	13	39.2	3.0	14.2	3.1
	V	23	31.0	1.3	12.1	1.7
36	N	24	36.6	3.6	10.0	2.1
	V	17	31.5	2.4	9.2	1.6
37	N	17	43.3	2.7	10.4	1.4
	V	21	36.4	1.8	7.2	1.1
38	N	19	39.0	4.0	10.1	2.0
	V	22	33.1	1.8	8.2	1.6
39	N	22	35.7	2.2	8.9	1.3
	V	25	31.8	2.1	7.4	1.4
40	N	23	38.4	2.1	10.9	1.4
	V	24	34.1	2.2	8.4	1.0
41	N	16	40.6	3.8	8.1	1.1
	V	23	34.7	3.0	6.8	0.8
42	N	21	39.6	3.7	9.8	1.6
	V	23	35.0	2.9	8.0	1.1
43	N	24	43.0	3.3	12.7	1.6
	V	20	37.4	2.7	9.6	1.2
44	N	25	35.2	2.6	10.6	1.8
	V	20	34.1	2.9	9.1	1.4
45	N	21	35.3	1.9	10.0	2.2
	V	22	34.6	1.8	9.3	1.6

Family	Vernalization treatment	Number of individuals	FF Mean	FF SD	FLN Mean	FLN SD
46	N	18	43.6	2.2	12.6	1.7
	V	19	42.9	2.6	12.6	1.9
47	N	21	43.5	3.2	13.5	1.8
	V	16	42.4	2.9	12.5	1.7
48	N	16	42.3	2.9	11.0	1.7
	V	15	38.7	3.1	8.7	1.4
49	N	20	42.8	2.0	9.3	1.2
	V	21	40.3	2.9	8.4	1.2
50	N	23	39.3	3.5	8.0	1.2
	V	23	35.4	2.7	7.4	1.2
51	N	25	39.9	2.5	9.9	1.0
	V	25	38.4	2.6	8.9	1.4
52	N	22	41.5	3.1	9.4	1.6
	V	17	38.2	3.0	7.9	1.5
53	N	25	42.2	2.3	10.2	1.2
	V	25	37.0	2.7	9.0	1.5
54	N	22	39.0	1.6	14.3	1.2
	V	24	36.3	1.3	13.2	1.0
55	N	20	42.8	2.5	11.6	1.2
	V	21	40.0	2.8	9.7	1.3
56	N	12	43.6	2.5	14.4	2.2
	V	17	37.6	4.0	10.9	1.6
57	N	19	45.9	1.2	12.3	2.2
	V	15	43.6	1.4	8.2	1.6
58	N	24	35.6	2.8	8.2	1.6
	V	20	35.2	3.0	7.1	1.2
59	N	24	33.5	1.8	7.8	0.8
	V	18	32.4	1.9	12.1	0.8
60	N	23	45.5	2.0	12.1	2.5
	V	17	41.6	2.9	9.2	1.3
61	N	18	38.4	2.2	10.1	1.7
	V	19	37.0	2.9	9.9	1.2
62	N	18	45.1	1.6	11.1	2.0
	V	19	42.1	1.6	10.8	1.6
63	N	16	45.2	1.9	9.6	0.8
	V	16	43.5	2.0	8.9	0.7
64	N	21	39.2	1.7	12.7	1.7
	V	22	38.8	2.5	11.4	1.6
65	N	23	33.9	1.2	10.6	1.1
	V	22	33.3	2.1	9.7	1.0
66	N	16	42.4	2.7	12.8	2.0
	V	17	41.8	3.0	12.8	1.6
67	N	22	43.5	2.1	15.1	1.6
	V	19	42.6	2.6	14.4	1.3
68	N	25	35.9	1.7	10.4	1.3
	V	24	34.2	1.6	8.8	1.4

Family	Vernalization treatment	Number of individuals	FF Mean	FF SD	FLN Mean	FLN SD
69	N	19	36.6	2.6	13.3	1.2
	V	21	32.7	1.7	10.6	1.2
70	N	19	38.3	2.0	12.1	0.6
	V	20	33.4	1.3	9.7	1.0
71	N	23	37.5	3.1	11.2	1.8
	V	25	30.7	1.7	7.8	0.7
72	N	17	39.4	2.5	12.1	0.9
	V	24	32.4	1.6	8.6	0.9
73	N	19	41.2	4.6	12.8	1.7
	V	24	32.5	1.4	9.0	1.2
74	N	21	37.7	2.2	13.5	1.5
	V	23	33.7	1.7	11.0	1.1
75	N	25	37.3	2.4	10.8	0.9
	V	19	32.3	1.2	8.2	0.5
76	N	24	36.7	1.6	11.6	1.7
	V	24	34.4	0.9	9.3	1.0
77	N	19	41.8	2.7	12.1	0.9
	V	25	31.6	1.0	8.2	0.7
78	N	21	37.0	2.1	11.0	1.1
	V	20	35.0	1.4	9.0	0.7
79	N	25	35.9	1.5	10.6	1.3
	V	25	31.3	0.8	8.0	0.6
80	N	20	34.8	1.3	12.7	1.1
	V	23	31.0	1.4	9.2	1.1
81	N	23	35.3	2.0	11.8	1.4
	V	24	31.4	1.2	9.3	1.2
82	N	24	36.2	1.4	10.8	1.1
	V	18	34.2	1.5	9.8	0.9
83	N	22	36.3	2.0	11.8	1.4
	V	24	32.5	1.2	9.0	1.3
84	N	23	36.0	2.4	11.9	1.0
	V	25	32.7	1.2	9.6	1.0
85	N	24	36.0	1.3	13.3	1.2
	V	23	32.5	1.3	9.3	1.3
86	N	24	36.2	2.1	12.2	1.7
	V	23	31.9	1.7	9.6	1.
87	N	24	35.1	1.7	12.4	1.6
	V	25	30.6	1.6	8.5	0.8
88	N	22	39.0	1.8	9.3	1.0
	V	21	33.8	1.0	8.1	0.8
89	N	25	38.0	2.5	12.5	2.1
	V	25	32.8	1.5	8.8	0.9
90	N	25	34.7	1.3	11.2	1.9
	V	25	31.5	1.4	8.8	1.0
91	N	23	35.5	2.0	10.8	1.2
	V	24	32.5	1.7	8.4	0.9

Family	Vernalization treatment	Number of individuals	FF Mean	FF SD	FLN Mean	FLN SD
92	N	25	34.4	1.0	11.1	1.8
	V	23	31.2	1.6	8.4	1.1
93	N	20	37.2	2.5	12.2	1.4
	V	19	33.6	1.7	9.9	1.5
94	N	19	38.3	2.2	11.8	1.2
	V	21	33.6	1.8	8.8	1.2
95	N	23	35.5	1.3	12.7	1.4
	V	25	32.2	1.8	9.3	1.2
96	N	25	34.0	0.8	9.0	1.3
	V	24	30.3	1.3	7.0	0.9
97	N	24	34.8	1.3	11.0	1.3
	V	24	31.3	1.3	8.5	0.8
98	N	16	34.8	1.6	10.8	1.3
	V	21	29.2	1.5	8.0	0.8
99	N	25	33.6	1.2	11.0	1.5
	V	25	28.6	1.2	7.0	0.8
100	N	25	35.5	2.2	13.0	2.0
	V	25	32.2	1.6	10.0	1.0
101	N	24	37.5	1.5	11.4	1.2
	V	20	34.3	0.9	10.0	0.8
102	N	20	35.8	0.8	11.4	0.7
	V	24	31.2	0.9	7.5	0.7
103	N	24	42.4	3.2	12.4	2.1
	V	22	33.3	1.2	8.7	1.0
104	N	25	35.2	1.1	9.5	1.9
	V	23	32.9	1.2	7.4	0.7
105	N	25	34.0	1.1	10.9	1.4
	V	24	31.3	0.8	8.4	0.9
106	N	24	35.3	0.9	10.0	1.3
	V	23	32.0	1.3	8.0	1.1
107	N	17	36.9	1.5	12.4	1.5
	V	20	30.3	2.1	8.3	1.4
108	N	24	36.2	1.6	10.5	1.4
	V	24	33.5	1.2	7.3	0.8
109	N	25	36.9	1.3	11.6	0.8
	V	24	33.3	1.4	8.0	0.9
110	N	24	39.0	2.5	13.5	1.4
	V	24	33.3	1.4	9.9	1.1
111	N	25	37.4	2.2	11.8	1.2
	V	24	31.7	1.7	8.4	1.1
112	N	21	41.9	2.4	12.0	1.8
	V	18	36.1	1.4	9.9	1.5
113	N	25	36.6	1.4	12.4	1.6
	V	22	32.6	1.5	9.3	1.2
114	N	25	36.8	1.7	11.5	1.3
	V	22	32.6	1.5	7.5	1.0

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
115	N	25	39.6	2.2	12.7	1.6
	V	22	33.8	1.3	9.0	1.6
116	N	24	35.3	1.2	12.0	1.7
	V	24	31.0	0.9	8.2	0.9
117	N	24	34.3	0.8	11.1	1.2
	V	24	31.0	0.9	6.9	0.7
118	N	23	36.0	1.5	11.1	1.1
	V	22	32.0	1.3	7.6	0.8
119	N	23	35.2	1.3	11.4	1.1
	V	24	31.0	1.2	8.0	1.0
120	N	18	43.8	2.3	15.4	1.4
	V	15	35.6	1.7	10.7	1.1
121	N	21	36.8	2.2	10.9	1.5
	V	19	31.3	1.9	8.5	0.6
122	N	22	39.2	2.9	12.3	1.4
	V	23	32.8	1.7	9.4	1.5
123	N	19	38.2	2.8	14.3	2.2
	V	25	29.8	2.2	10.0	1.3
124	N	18	38.7	2.8	13.3	1.0
	V	25	29.2	1.7	11.1	1.3
125	N	23	35.9	3.0	12.4	1.7
	V	24	0.7	1.5	10.0	1.3
126	N	18	37.1	3.8	10.7	1.5
	V	23	32.5	1.5	8.0	1.0
127	N	20	39.0	3.0	11.7	1.7
	V	24	31.9	1.4	8.5	1.0
128	N	21	37.7	3.5	10.9	1.8
	V	25	29.8	1.3	8.7	1.4
129	N	25	38.2	2.3	12.6	1.3
	V	24	32.8	1.6	11.0	0.8
130	N	20	39.5	1.9	12.4	1.1
	V	25	32.0	1.7	9.3	0.9
131	N	21	34.5	2.0	10.7	1.5
	V	24	31.0	2.6	8.9	0.9
132	N	20	36.9	2.3	12.9	1.5
	V	21	31.4	1.4	9.8	1.3
133	N	24	36.0	1.9	12.1	1.8
	V	24	30.7	1.7	9.3	1.2
134	N	25	36.1	2.3	12.9	1.8
	V	19	30.8	2.1	10.1	1.1
135	N	19	36.0	2.0	13.6	1.2
	V	23	30.8	5.0	10.7	1.2
136	N	24	35.5	1.8	11.5	1.5
	V	20	30.5	2.5	9.7	0.9
137	N	23	35.1	2.8	11.5	1.9
	V	25	30.9	1.3	9.7	1.1

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
138	N	23	33.6	2.4	11.0	2.1
	V	25	30.1	0.9	10.1	1.0
139	N	21	35.0	0.8	10.0	1.1
	V	24	31.9	2.7	10.4	1.2
140	N	25	33.8	1.4	13.3	1.8
	V	25	27.8	1.4	10.1	1.0
141	N	17	36.9	2.2	12.8	1.8
	V	23	33.2	2.8	10.5	1.8
142	N	24	38.3	3.3	12.0	0.9
	V	23	29.7	1.6	8.9	0.8
143	N	20	38.6	3.3	12.0	1.7
	V	25	31.5	2.0	9.3	0.9
144	N	19	39.1	2.7	12.7	1.5
	V	24	30.0	1.5	9.9	1.1
145	N	20	39.3	1.9	12.3	2.0
	V	25	32.4	1.7	8.4	0.9
146	N	21	34.5	2.0	10.7	1.5
	V	24	31.0	2.6	8.9	0.8
147	N	23	35.1	3.7	11.4	1.9
	V	25	30.9	1.3	9.7	1.1
148	N	23	36.0	1.4	11.0	1.1
	V	22	31.9	1.2	7.6	0.7
149	N	25	38.2	2.2	12.5	1.2
	V	24	32.7	1.6	11.0	0.7
150	N	24	39.0	2.5	13.4	1.4
	V	24	33.2	1.3	11.8	1.1

Appendix B- Means and standard deviations of F3 families for the cross Marnoo-14 X Karat-17.

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
1	N	25	37.1	2.5	11.4	1.2
	V	25	27.7	3.0	8.8	0.8
2	N	25	36.7	1.7	9.5	0.7
	V	25	31.4	1.8	8.2	0.7
3	N	20	38.6	1.5	11.1	0.7
	V	25	27.2	3.3	9.1	1.2
4	N	20	36.4	2.3	12.0	1.2
	V	18	27.3	2.5	10.8	1.5
5	N	16	35.5	2.1	10.3	1.1
	V	20	30.0	2.9	9.1	1.4
6	N	20	38.0	1.7	11.6	2.0
	V	25	31.3	1.0	11.1	1.5
7	N	19	35.6	2.0	10.2	1.2
	V	25	30.7	1.0	8.6	0.8
8	N	25	36.7	2.2	12.4	1.1
	V	21	31.7	1.2	12.0	1.7
9	N	20	34.9	1.0	11.2	1.3
	V	18	30.6	1.4	9.6	1.3
10	N	21	34.9	1.7	12.	1.5
	V	17	29.8	1.5	10.4	1.2
11	N	25	36.7	1.1	12.9	1.6
	V	20	31.3	1.0	10.7	1.1
12	N	21	35.1	1.6	11.9	0.8
	V	20	31.9	1.0	10.2	0.9
13	N	20	34.9	1.3	10.0	0.9
	V	20	30.2	1.3	9.5	0.9
14	N	19	36.3	1.3	9.9	1.0
	V	24	31.0	1.1	9.3	1.0
15	N	24	34.4	0.9	10.5	1.2
	V	23	30.7	1.5	9.5	0.9
16	N	21	34.6	1.7	9.3	1.1
	V	25	31.3	1.2	9.2	1.2
17	N	16	38.4	1.8	9.6	1.2
	V	23	31.0	1.4	8.6	1.0
18	N	20	35.9	1.3	10.6	1.2
	V	24	28.8	1.8	8.3	1.1
19	N	22	33.3	1.6	11.5	0.9
	V	25	26.8	3.6	9.3	1.4
20	N	22	36.8	1.8	10.8	1.0
	V	20	30.7	1.2	10.6	1.1
21	N	23	35.1	2.1	9.5	1.2
	V	24	28.9	0.9	8.3	1.2

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
22	N	22	38.5	2.0	11.1	1.8
	V	25	34.3	0.7	11.0	1.6
23	N	20	34.8	1.1	9.4	0.7
	V	25	30.7	1.2	8.9	1.1
24	N	25	47.8	2.3	11.5	1.2
	V	18	33.1	2.6	9.4	1.3
25	N	17	40.2	2.9	10.6	0.7
	V	16	30.3	1.7	9.9	0.8
26	N	18	47.7	2.5	15.2	2.1
	V	25	37.9	2.6	11.1	1.1
27	N	25	39.3	2.2	9.3	1.1
	V	25	34.5	2.8	9.1	1.2
28	N	23	48.5	1.5	12.5	0.9
	V	20	33.2	2.3	9.6	1.0
29	N	21	46.2	2.4	9.2	0.9
	V	22	38.0	2.1	8.5	0.5
30	N	16	44.3	1.3	9.3	0.7
	V	20	35.4	2.8	8.4	0.9
31	N	24	43.9	2.6	9.2	0.8
	V	24	35.9	1.6	8.6	0.7
32	N	24	47.8	2.5	10.8	1.3
	V	16	34.6	3.4	9.3	0.9
33	N	24	43.2	2.5	9.6	1.0
	V	22	33.4	2.0	8.8	1.0
34	N	23	46.6	2.9	10.5	1.3
	V	16	38.6	4.0	8.6	0.6
35	N	25	46.9	1.9	11.1	1.6
	V	23	36.0	3.0	8.6	0.7
36	N	24	43.8	3.3	11.8	1.5
	V	25	32.2	2.8	8.9	1.3
37	N	25	43.8	2.5	11.2	1.4
	V	21	33.9	2.6	9.2	1.0
38	N	20	48.8	2.4	13.6	2.3
	V	25	48.9	3.9	10.0	1.0
39	N	21	48.9	2.7	12.5	1.5
	V	18	43.8	3.0	9.5	0.7
40	N	24	43.8	3.0	10.4	1.9
	V	25	32.6	2.3	9.0	0.6
41	N	25	44.3	3.6	10.5	1.0
	V	25	36.2	2.6	9.6	1.0
42	N	25	41.1	2.4	9.6	0.7
	V	24	31.7	2.1	8.6	0.9
43	N	18	45.3	2.9	10.7	1.2
	V	24	44.3	2.2	9.9	0.9
44	N	23	44.3	3.8	10.3	1.8
	V	25	33.2	2.7	9.2	0.9

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
45	N	25	48.6	1.9	12.5	1.4
	V	23	34.9	2.5	9.6	1.0
46	N	19	45.5	3.2	11.6	1.7
	V	19	33.1	2.4	9.9	1.4
47	N	20	49.9	2.0	12.5	1.5
	V	23	36.8	2.6	9.9	1.2
48	N	17	34.4	3.3	12.4	1.7
	V	22	34.4	3.0	10.7	1.0
49	N	25	50.8	0.8	11.8	0.8
	V	22	41.1	3.2	10.0	1.2
50	N	16	43.8	4.2	10.9	1.2
	V	24	48.4	2.8	8.9	1.1
51	N	16	48.4	2.8	11.6	1.3
	V	25	34.6	2.4	10.1	1.1
52	N	23	47.0	1.9	10.9	0.9
	V	25	32.3	1.6	8.8	1.1
53	N	20	42.9	3.4	10.0	1.7
	V	21	34.0	2.1	10.0	1.2
54	N	25	35.7	1.4	11.8	1.1
	V	24	28.7	1.8	9.8	0.9
55	N	21	40.1	1.5	11.3	1.0
	V	25	31.8	1.3	10.0	0.9
56	N	23	39.0	1.9	10.0	1.3
	V	24	33.2	1.1	10.8	0.9
57	N	18	40.1	2.6	11.4	1.1
	V	17	32.4	1.0	10.8	1.1
58	N	22	44.4	2.7	13.3	1.4
	V	25	34.4	2.0	12.2	1.0
59	N	25	37.6	2.0	11.9	1.0
	V	23	30.0	1.4	10.6	1.1
60	N	21	38.0	2.2	10.2	1.1
	V	22	33.2	1.2	10.7	0.9
61	N	20	42.2	3.1	10.1	1.1
	V	24	35.4	1.3	10.4	1.1
62	N	25	41.7	2.1	11.3	0.8
	V	25	34.8	2.0	11.1	1.0
63	N	24	33.8	2.2	10.8	1.1
	V	23	33.8	2.0	11.0	1.3
64	N	17	39.4	2.4	11.0	1.1
	V	16	30.5	2.1	10.7	1.5
65	N	25	34.9	0.9	11.0	0.9
	V	19	30.0	1.5	9.2	1.3
66	N	20	37.6	2.9	10.5	0.9
	V	19	31.8	1.0	10.7	0.9
67	N	21	44.1	2.8	11.5	0.9
	V	24	34.2	1.9	11.3	1.2

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
68	N	20	37.7	2.9	11.3	1.0
	V	25	30.5	1.4	10.4	1.3
69	N	22	42.4	3.4	11.6	1.3
	V	24	34.0	1.1	10.8	1.4
70	N	21	41.1	3.4	11.6	1.1
	V	25	31.1	1.0	11.3	1.5
71	N	22	34.9	1.6	11.3	1.0
	V	17	31.4	1.3	10.4	1.1
72	N	20	40.6	1.0	13.9	1.3
	V	22	31.5	1.0	13.0	1.4
73	N	22	44.0	2.8	11.1	0.8
	V	22	35.3	1.7	11.3	0.9
74	N	25	38.5	2.4	12.9	1.1
	V	16	31.7	0.9	12.3	1.1
75	N	17	43.5	4.0	11.1	1.2
	V	20	35.4	1.9	11.0	0.8
76	N	24	39.5	2.4	11.8	1.4
	V	24	32.5	1.1	12.8	1.2
77	N	25	37.5	1.7	11.4	1.0
	V	24	32.8	1.3	10.2	1.5
78	N	25	38.2	2.3	12.5	0.8
	V	25	30.6	1.3	9.8	1.5
79	N	18	40.5	1.5	12.2	0.9
	V	22	33.2	1.0	12.2	1.2
80	N	19	43.6	4.0	11.3	0.7
	V	23	33.4	1.6	11.4	0.9
81	N	25	37.7	1.6	11.6	0.7
	V	24	32.9	1.0	10.8	1.0
82	N	24	35.5	1.8	11.7	0.9
	V	25	30.0	1.4	9.0	1.3
83	N	23	41.9	1.7	11.0	0.6
	V	24	36.8	1.4	12.5	0.8
84	N	25	46.3	3.3	11.4	1.1
	V	24	33.8	1.6	12.0	1.3
85	N	23	44.0	2.9	11.7	0.9
	V	23	35.0	1.8	11.5	1.1
86	N	20	41.0	1.8	11.2	1.0
	V	23	33.4	1.0	12.3	1.2
87	N	19	35.1	1.2	10.6	0.7
	V	17	27.3	0.9	8.6	0.6
88	N	25	34.5	1.4	10.5	0.8
	V	25	28.8	0.9	8.9	0.7
89	N	25	35.5	7.8	10.7	0.8
	V	17	29.2	0.8	9.2	0.6
90	N	25	36.4	2.1	10.8	1.0
	V	24	29.2	1.4	9.9	0.9

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
91	N	23	36.3	1.8	12.9	0.8
	V	18	27.4	1.2	9.7	0.8
92	N	21	36.4	2.1	12.0	0.9
	V	22	29.1	1.5	9.7	0.8
93	N	25	41.1	2.2	11.4	0.8
	V	23	30.6	1.3	9.9	0.5
94	N	25	37.1	2.6	12.5	1.2
	V	25	29.7	1.4	9.9	0.9
95	N	18	41.4	1.8	10.6	0.8
	V	19	32.7	1.3	9.9	0.7
96	N	24	39.8	2.6	10.8	0.4
	V	24	30.9	1.3	10.0	0.7
97	N	25	44.2	4.3	10.8	1.2
	V	24	35.3	4.3	10.6	1.2
98	N	25	37.6	2.6	12.3	0.9
	V	24	29.3	0.8	9.8	0.8
99	N	25	38.7	1.8	11.6	0.9
	V	25	30.4	2.1	10.4	1.1
100	N	19	38.7	2.6	11.2	0.7
	V	17	30.6	1.3	10.4	1.1
101	N	23	37.4	1.8	10.8	0.7
	V	24	29.4	0.8	10.1	0.8
102	N	18	39.7	3.3	11.9	0.8
	V	24	28.4	1.1	9.7	1.0
103	N	18	41.8	2.0	11.6	0.7
	V	24	29.2	1.2	10.5	0.9
104	N	25	34.6	1.6	11.3	1.1
	V	18	30.1	1.7	9.6	0.7
105	N	19	37.1	2.0	9.6	0.5
	V	21	31.7	1.2	8.6	0.5
106	N	22	39.5	1.3	10.2	0.9
	V	20	30.1	109	8.7	0.5
107	N	22	38.6	1.8	10.0	1.0
	V	25	28.8	1.3	7.9	0.4
108	N	24	35.8	1.4	9.2	0.6
	V	24	28.9	0.9	8.0	0.3
109	N	17	35.3	2.1	10.5	1.0
	V	19	28.7	1.9	8.3	0.6
110	N	21	38.7	1.1	11.0	1.1
	V	23	30.2	1.6	8.6	0.5
111	N	22	35.4	1.6	10.2	1.0
	V	21	28.6	1.1	7.6	1.0
112	N	22	36.4	1.0	9.4	0.6
	V	19	29.6	0.5	8.1	0.2
113	N	23	38.7	1.2	11.2	0.6
	V	21	30.8	2.4	7.9	0.9

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
114	N	16	38.1	1.2	10.4	0.5
	V	24	27.9	0.9	7.6	0.5
115	N	19	40.1	1.6	10.3	0.5
	V	23	32.3	1.2	8.8	0.5
116	N	17	39.0	1.3	10.6	0.5
	V	23	32.4	1.1	9.0	0.4
117	N	21	38.7	1.8	9.9	0.4
	V	22	30.2	2.1	8.1	0.9
118	N	22	38.0	1.8	10.7	0.9
	V	22	27.6	0.7	7.5	0.5
119	N	22	38.4	1.8	11.1	1.1
	V	22	28.3	1.0	7.7	0.5
120	N	21	38.4	1.7	10.2	0.7
	V	23	28.9	1.2	8.0	0.5
121	N	25	40.6	0.9	10.2	0.4
	V	24	35.3	2.0	9.8	1.0
122	N	23	39.7	1.4	10.9	0.9
	V	19	32.6	1.4	9.1	0.5
123	N	24	41.2	1.0	10.2	0.4
	V	24	34.3	1.6	9.2	0.5
124	N	22	37.7	1.7	10.0	0.8
	V	24	30.4	1.7	8.4	0.7
125	N	22	37.9	1.9	11.0	0.5
	V	23	29.6	1.4	8.2	0.4
126	N	22	37.7	2.0	10.9	0.8
	V	22	29.6	1.7	8.4	0.5
127	N	22	42.5	1.1	11.6	0.3
	V	23	32.0	0.7	9.8	0.3
128	N	22	42.4	1.1	11.5	0.3
	V	24	33.4	0.6	10.2	1.1
129	N	25	39.0	2.4	10.3	0.9
	V	25	33.6	0.9	10.3	1.1
130	N	25	40.3	1.8	11.1	0.6
	V	25	33.0	0.4	10.5	0.4
131	N	25	37.1	2.2	9.6	0.8
	V	25	31.7	0.8	9.6	0.8
132	N	25	39.5	0.9	10.2	0.8
	V	25	30.1	2.1	8.7	0.9
133	N	24	38.6	1.1	10.0	0.5
	V	25	28.8	1.4	7.9	0.5
134	N	25	35.8	1.6	9.2	0.9
	V	25	28.9	1.2	8.0	0.9
135	N	25	35.3	3.2	10.5	1.1
	V	25	28.7	2.3	8.3	0.9
136	N	24	38.7	0.9	11.0	0.9
	V	25	30.2	1.5	8.6	0.8

Family	Vernalization treatment	Number of Individuals	FF Mean	FF SD	FLN Mean	FLN SD
137	N	23	35.5	1.8	10.2	1.1
	V	24	28.6	1.1	7.6	1.5
138	N	24	36.4	1.2	9.5	0.9
	V	24	29.6	0.8	8.1	0.9
139	N	25	38.7	2.5	11.2	1.5
	V	24	30.8	3.2	7.9	1.6
140	N	23	36.1	2.1	10.4	0.9
	V	24	27.9	3.2	7.6	0.9
141	N	25	40.1	3.3	10.3	1.2
	V	25	32.3	0.9	8.8	0.8
142	N	24	39.0	2.4	10.6	1.1
	V	24	32.4	1.2	9.0	1.0
143	N	25	38.7	2.9	9.9	1.0
	V	25	30.2	2.9	8.1	1.2
144	N	25	38.0	3.2	10.7	1.1
	V	25	27.6	1.5	7.5	0.8
145	N	25	38.5	2.4	11.1	1.2
	V	25	28.3	1.4	7.7	0.8
146	N	25	38.4	2.6	10.2	1.0
	V	25	28.9	2.1	8.0	0.6
147	N	25	40.6	3.1	10.2	1.1
	V	25	35.3	2.5	9.8	1.2
148	N	24	39.7	3.5	10.9	1.8
	V	25	32.6	2.1	9.1	0.8
149	N	24	41.2	2.8	10.2	1.1
	V	24	34.3	2.3	9.2	0.8
150	N	25	37.7	2.6	10.0	1.2
	V	24	30.4	2.2	8.5	0.9

Appendix C- Means and standard deviations for DH lines derived from the F1 of a cross between Karat-17 x Marnoo-14.(DNF = did not flower)

Family	Vernalization treatment	FLN Mean	FLN SD
KM01	N	15.0	1.7
	V	12.5	1.3
KM02	N	10.8	0.3
	V	11.2	0.8
KM03	N	14.8	0.3
	V	7.5	0.5
KM04	N	11.7	0.8
	V	9.8	0.8
KM05	N	13.7	1.0
	V	11.3	2.1
KM06	N	11.5	5.8
	V	7.5	0.5
KM07	N	10.7	0.6
	V	5.8	0.3
KM08	N	13.2	1.0
	V	5.8	0.3
KM09	N	10.5	1.5
	V	8.0	1.7
KM10	N	9.3	1.6
	V	7.0	0
KM11	N	12.5	1.0
	V	7.5	1.3
KM12	N	16.0	0.0
	V	14.0	0.9
KM13	N	9.5	3.0
	V	6.2	1.0
KM14	N	6.3	0.6
	V	6.5	0.5
KM15	N	8.0	1.0
	V	6.8	0.8
KM16	N	8.2	0.3
	V	7.5	0.3
KM17	N	10.5	0.5
	V	8.8	1.3
KM18	N	7.0	1.0
	V	6.5	0
KM19	N	7.0	0
	V	6.7	0.3
KM20	N	9.0	1.3
	V	7.8	0.3
KM21	N	9.7	4.6
	V	6.5	0.5
KM22	N	DNF	
	V	10.2	0.6
KM23	N	8.8	1.4
	V	7.5	0.5

Family	Vernalization treatment	FLN Mean	FLN SD
KM24	N	15.2	1.9
	V	7.7	0.6
KM25	N	7.7	0.8
	V	7.7	0.6
KM26	N	9.0	1.0
	V	6.8	0.3
KM27	N	6.7	0.3
	V	5.5	0.5
KM28	N	10.2	0.5
	V	7.0	0.9
KM29	N	7.5	1.3
	V	6.2	0.3
KM30	N	13.8	1.0
	V	7.7	1.0
KM31	N	7.0	0
	V	6.7	0.8
KM32	N	7.5	0.0
	V	6.3	0.6
KM33	N	9.8	0.8
	V	8.3	0.3
KM34	N	6.5	0.0
	V	6.0	0
KM35	N	9.0	1.5
	V	6.5	0.5
KM36	N	18.5	0.5
	V	9.8	1.6
KM37	N	6.8	0.3
	V	5.5	0.5
KM38	N	7.0	0.0
	V	7.2	0.3
KM39	N	7.3	0.6
	V	6.2	0.3
KM40	N	17.5	0.9
	V	6.0	0.0
KM41	N	6.8	0.3
	V	6.2	0.3
KM42	N	7.5	1.3
	V	6.7	0.6
KM43	N	13.7	2.8
	V	6.0	0.0
KM44	N	12.5	0.5
	V	6.7	0.3
KM45	N	7.0	0.0
	V	5.3	0.3
KM46	N	7.5	0.5
	V	6.3	1.2
KM47	N	7.0	0.0
	V	5.8	0.8

Family	Vernalization treatment	FLN Mean	FLN SD
KM48	N	7.2	0.3
	V	6.2	0.3
KM49	N	10.7	1.2
	V	5.8	0.3
KM50	N	8.2	1.6
	V	6.2	0.3

Appendix D- Means and standard deviations for DH lines derived from the F1 of a cross between Marnoo-14 X Karat-17. (DNF = did not flower)

Family	Vernalization treatment	FLN Mean	FLN SD
MK01	N	6.5	0.9
	V	6.3	1.1
MK02	N	DNF	
	V	DNF	
MK03	N	9.5	0.5
	V	8.7	1.2
MK04	N	16.8	0.3
	V	11.7	0.8
MK05	N	8.8	0.3
	V	6.7	1.3
MK06	N	7.3	0.6
	V	6.3	0.3
MK07	N	11.7	0.6
	V	10.3	0.6
MK08	N	DNF	
	V	DNF	
MK09	N	9.7	0.3
	V	9.3	1.2
MK10	N	10.3	0.6
	V	10.0	0.5
MK11	N	11.8	0.3
	V	10.7	0.3
MK12	N	15.0	0.0
	V	10.5	1.3
MK13	N	8.3	0.8
	V	9.7	2.3
MK14	N	19.8	0.8
	V	8.7	0.8
MK15	N	14.3	0.4
	V	7.8	1.3
MK16	N	20.3	1.9
	V	8.8	2.4
MK17	N	9.8	0.8
	V	10.3	0.3
MK18	N	10.0	0.0
	V	10.0	0.0
MK19	N	8.2	0.3
	V	7.7	0.6
MK20	N	10.0	0.0
	V	10.0	0.0
MK21	N	10.3	0.6
	V	10.7	1.1

Family	Vernalization treatment	FLN Mean	FLN SD
MK22	N	10.3	0.6
	V	11.0	0.6
MK23	N	10.3	1.1
	V	11.8	0.8
MK24	N	15.3	3.4
	V	12.7	1.1
MK25	N	16.0	3.2
	V	13.5	0.9
MK26	N	DNF	
	V	13.5	0.9
MK27	N	DNF	
	V	14.5	2.3
MK28	N	DNF	
	V	14.0	1.9
MK29	N	DNF	
	V	13.8	0.8
MK30	N	11.0	0.9
	V	9.7	1.1
MK31	N	11.3	1.1
	V	12.8	1.9
MK32	N	10.5	0.9
	V	11.2	0.6
MK33	N	10.5	0.9
	V	10.8	3.0
MK34	N	11.5	0.9
	V	12.3	1.1
MK35	N	11.2	1.5
	V	11.2	0.5
MK36	N	11.3	1.1
	V	11.7	0.5
MK37	N	11.2	1.5
	V	10.8	1.5
MK38	N	10.7	0.6
	V	11.3	1.1
MK39	N	DNF	
	V	12.3	2.1
MK40	N	10.5	0.9
	V	11.8	1.9
MK41	N	10.7	0.8
	V	11.8	0.3
MK42	N	10.7	1.2
	V	9.2	1.0
MK43	N	10.0	0.0
	V	9.5	0.5
MK44	N	10.0	0.9
	V	9.7	1.2

Family	Vernalization treatment	FLN Mean	FLN SD
MK45	N	9.7	1.2
	V	10.0	1.7
MK46	N	9.8	0.4
	V	10.7	0.8
MK47	N	10.2	0.3
	V	8.7	0.6
MK48	N	9.7	0.6
	V	10.0	0.0
MK49	N	9.5	0.0
	V	10.3	0.3
MK50	N	9.5	0.5
	V	9.8	0.3

Appendix E- Means and standard deviations for DH lines derived from the F1 of a cross between Global-16 x Karat-17 (DNF = did not flower)

Family	Vernalization treatment	FLN Mean	FLN SD
GK01	N	DNF	
	V	DNF	
GK02	N	DNF	
	V	10.5	0.9
GK03	N	11.5	1.3
	V	9.5	2.7
GK04	N	11.8	2.5
	V	8.5	1.3
GK05	N	12.0	0.0
	V	12.0	1.9
GK06	N	12.2	1.5
	V	10.3	1.1
GK07	N	11.7	0.6
	V	9.2	2.7
GK08	N	11.7	1.1
	V	11.7	2.9
GK09	N	11.3	1.1
	V	12.0	0.0
GK10	N	10.7	1.1
	V	10.3	0.8
GK11	N	11.2	0.6
	V	13.0	1.9
GK12	N	11.7	1.5
	V	11.2	0.6
GK13	N	12.3	1.1
	V	10.0	3.8
GK14	N	11.8	0.6
	V	10.2	3.8
GK15	N	11.3	1.1
	V	10.5	2.5
GK16	N	11.8	0.6
	V	11.8	3.8
GK17	N	11.8	1.5
	V	12.8	0.6
GK18	N	11.3	1.1
	V	12.8	1.5
GK19	N	10.3	1.1
	V	11.2	1.9
GK20	N	11.3	0.6
	V	10.8	1.5
GK21	N	11.3	1.1
	V	11.2	0.6
GK22	N	12.2	0.6
	V	11.3	1.1
GK23	N	11.5	1.0
	V	10.5	1.7

Family	Vernalization treatment	FLN Mean	FLN SD
GK24	N	DNF	
	V	12.0	2.5
GK25	N	11.7	1.5
	V	12.2	0.6
GK26	N	12.0	1.9
	V	9.2	0.6
GK27	N	12.0	1.9
	V	11.7	2.5
GK28	N	12.0	1.0
	V	11.7	1.9
GK29	N	12.7	1.1
	V	11.2	2.7
GK30	N	13.0	1.9
	V	12.0	0.0
GK31	N	11.5	1.0
	V	10.0	0.0
GK32	N	13.3	1.5
	V	9.3	1.1
GK33	N	11.8	0.6
	V	9.8	1.5
GK34	N	11.0	1.9
	V	11.3	1.5
GK35	N	12.5	1.7
	V	11.0	1.9
GK36	N	11.5	1.0
	V	8.2	0.6
GK37	N	11.3	0.6
	V	8.3	0.6
GK38	N	11.0	0.0
	V	11.0	0.0
GK39	N	11.2	0.6
	V	10.3	3.0
GK40	N	11.0	0.0
	V	10.7	0.6
GK41	N	11.3	0.6
	V	11.0	0.0
GK42	N	11.0	0.0
	V	9.5	2.5
GK43	N	11.5	1.7
	V	10.2	3.0
GK44	N	10.8	0.6
	V	9.0	1.0
GK45	N	11.0	1.9
	V	11.5	1.0
GK46	N	11.0	0.0
	V	11.8	0.6
GK47	N	11.3	1.1
	V	10.3	2.3

Appendix F- Means and standard deviations for DH lines derived from the F1 of a cross between Karat-17 x Global-16 (DNF = did not flower)

Family	Vernalization treatment	FLN Mean	FLN SD
KG01	N	9.7	1.9
	V	10.0	3.4
KG02	N	9.3	1.1
	V	9.7	0.6
KG03	N	DNF	
	V	DNF	
KG04	N	DNF	
	V	DNF	
KG05	N	DNF	
	V	DNF	
KG06	N	DNF	
	V	DNF	
KG07	N	DNF	
	V	DNF	
KG08	N	11.7	1.1
	V	10.2	0.6
KG09	N	11.0	0.0
	V	10.5	1.7
KG10	N	10.0	1.9
	V	9.5	1.7
KG11	N	10.5	1.0
	V	9.8	3.0
KG12	N	10.0	0.0
	V	8.5	1.0
KG13	N	8.8	3.6
	V	9.2	0.6
KG14	N	9.5	1.3
	V	8.3	2.7
KG15	N	DNF	
	V	10.7	1.1
KG16	N	10.0	0.0
	V	10.0	0.0
KG17	N	9.8	0.6
	V	10.5	1.0
KG18	N	11.8	0.6
	V	10.8	0.6
KG19	N	10.0	0.0
	V	9.3	2.7
KG20	N	12.3	0.6
	V	12.5	1.0
KG21	N	10.0	0.0
	V	9.7	2.9
KG22	N	10.5	1.0
	V	10.2	2.7
KG23	N	9.7	1.1
	V	7.5	1.0

Family	Vernalization treatment	FLN Mean	FLN SD
KG24	N	9.8	0.6
	V	8.2	3.0
KG25	N	9.5	1.0
	V	10.0	2.5
KG26	N	11.8	3.0
	V	9.3	1.1
KG27	N	11.5	1.7
	V	9.3	1.1
KG28	N	12.3	1.5
	V	10.3	1.1
KG29	N	12.3	0.6
	V	11.3	1.1
KG30	N	11.5	1.0
	V	10.8	0.6
KG31	N	13.0	0.0
	V	11.7	1.1
KG32	N	12.5	2.5
	V	11.2	0.6
KG33	N	11.0	0.0
	V	10.7	1.1
KG34	N	11.7	1.9
	V	11.3	0.6
KG35	N	12.3	2.3
	V	11.3	1.1
KG36	N	10.2	0.6
	V	10.3	1.5
KG37	N	12.2	0.6
	V	12.3	2.2
KG38	N	11.7	2.3
	V	11.2	0.6
KG39	N	7.3	1.1
	V	7.3	0.6
KG40	N	12.5	1.6
	V	10.2	0.6
KG41	N	9.7	1.1
	V	9.2	3.6
KG42	N	12.3	1.9
	V	10.7	2.9
KG43	N	10.3	1.1
	V	10.3	0.6
KG44	N	12.7	1.1
	V	12.0	0.0
KG45	N	13.2	1.5
	V	12.0	0.0
KG46	N	8.7	1.1
	V	9.8	2.5
KG47	N	13.8	0.6
	V	11.5	1.0

Family	Vernalization treatment	FLN Mean	FLN SD
KG48	N	7.7	2.3
	V	8.0	0.0
KG49	N	DNF	
	V	DNF	
KG50	N	16.0	1.7
	V	13.8	2.7