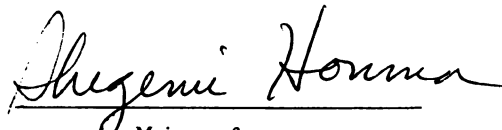This is to certify that the

thesis entitled

A DIALLEL ANALYSIS OF BOLT RESISTANT
GERMPLASM IN SEVERAL BRASSICA SPECIES
AND THE INHERITANCE OF LATERAL
SUPPRESSION AND LEAF NUMBER IN BROCCOLI
(Brassica oleracea L., Italica group)

presented by

Katherine A. Keyes

has been accepted towards fulfillment
of the requirements for

Masters degree in Horticulture

Major professor

Date September 4, 1987

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A THESIS

Submitted to

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

MASTER OF SCIENCE

Department of Horticulture
1987

ABSTRACT

A DIALLEL ANALYSIS OF BOLT RESISTANT GERMPLASM IN SEVERAL BRASSICA SPECIES AND THE INHERITANCE OF LATERAL SUPPRESSION AND LEAF NUMBER IN BROCCOLI (Brassica oleracea L. Italica group)

By

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A combining ability analysis for bolting response was performed on data from an incomplete diallel cross among 12 parents representing seven species. Significance for all contrasts and effects was observed. Results suggest diversity among parents and that bolt resistance will respond to selection. The Hakuran group was found to contribute the greatest amount of bolt resistance to its progeny.

Studies were conducted to determine the inheritance of lateral suppression and leaf number in broccoli. In reciprocal crosses 83-857-2 X "Spartan Early" lateral suppression was found to be dominant over non-suppression. A 9:7 recessive suppressor model is proposed. Correlation studies suggest no relationship between lateral suppression and other characters studied.

F_2 data from the cross between 83-866-1 X "Spartan Early" showed that leaf number was controlled by one or two major loci and modifiers, with few leaves being dominant over many leaves.

To my parents, Janet (in memory) and Norman Keyes,
for their love and support all through my life.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PART I: A DIALLEL ANALYSIS OF
BOLT RESISTANT GERMPLASM IN
SEVERAL BRASSICA SPECIES

INTRODUCTION

Brassica species, like many biennial plants, require vernalization in order to flower. Vernalization induces the change from the vegetative to the reproductive phase in response to low temperatures. The length of cold period required varies among species and between cultivars.

As Chinese cabbage (Brassica campestris L. Pekinensis group) becomes more popular in the western world, a longer production season is desirable. At present, Chinese cabbage is grown as a late summer crop because cool spring temperatures cause premature flowerstalk formation (bolting) which makes the crop unmarketable.

Cultural practices, such as use of protective row covers and adjustment of planting dates may be employed to reduce losses due to bolting. Row covers are costly in terms of time, labor, and capital. A more permanent and satisfactory solution to the problem is the development of bolt-resistant cultivars. Thus the production season could be extended into early summer.

Other Brassica species are known to have slower bolting characteristics, that is a longer vernalization requirement. Since other traits, such as disease resistance, have been transferred through interspecific hybridization, it is possible that genes for bolting resistance could be transferred to commercial Chinese cabbage cultivars. The objective of this study was to examine several Brassica

1

species and progeny of interspecific crosses to determine their vernalization requirements for possible use as sources of bolt resistance in addition to those already reported.

LITERATURE REVIEW

Vernalization

Many biennial plant species have an obligate cold requirement in order to flower. The length of cold period varies among species and between cultivars. This phenomenon, called vernalization, induces the change from vegetative to reproductive growth in response to low temperatures .

Gassner (cited by Purvis, 29) was the first to examine low temperature effects on the promotion of flowering of winter cereals in 1918. Other crops requiring vernalization are celery, beets, and members of the genus Brassica.

This literature review will focus on vernalization aspects of Brassica species, in particular Chinese cabbage (B. campestris L. Pekinensis group). A distinction must be made between regular flowering in response to vernalization and bolting, the latter being commonly referred to as premature flower stalk formation. In Chinese cabbage, "bolting" refers to the formation of a flower stalk before a marketable head is produced. Other closely related Brassica species have "later bolting" characteristics.

The effectiveness of the low temperature treatment depends on temperature, the length of exposure, and the age or size of the plant at the time of flowering (5,9,35). Some investigators have studied the interaction of photoperiod and low temperature on flowering. Each of these catagories will be discussed in detail.

3

The exact physiological effects of vernalization are not known, but two schools of thought have emerged. The first is that the vernalization treatment, per se, does not induce flowering, but only sensitizes the plant to the appropriate daylength and/or temperature conditions (29). After vernalization, no flower primordia are formed, but the plant is "ripe to flower"-- that is all the prerequisites to flowering have occurred but the last step (24,38). Exposure to low temperatures hastens the capacity to flower, but shows up as an after effect. It is only a preparatory phase, not a true inductive effect (3).

The other school of thought is that the period of cold temperatures is an inductive process, and the subsequent environmental conditions only hasten or delay flower stalk development. This occurred in cabbage plants (B. oleracea L. Capitata group) (21), and in recent experiments with Chinese cabbage (7,37).

Numerous investigators have tried to determine the interaction of photoperiod and the vernalization treatment. Chroboczek (4) confirmed that table beets (Beta vulgaris) are "long day plants" (L.D.) by shortening the time for seed stalk formation by artificially extending the daylight following vernalization. A combination of low temperatures (10-15 C) and long days (15+ hrs.) is most effective in obtaining a high seed yield in beet. He also noted that beets respond better to the cold treatment when they are lighted than if they are in darkness (5).

Lorenz (16) reported that Chinese cabbage flowered more rapidly under a 16 hr. day than an 8 hr. day. Short day length appears to have a negative effect on flowering because it remained vegetative longest when grown under short days (8 hrs. light) with relatively high temperatures (above 24 C) (14,16). Premature bolting and flowering in Chinese cabbage was delayed or prevented when plants were raised under S.D. (10 hrs.) for 3-4 weeks before transplanting into natural long days (18 hrs.) (22).

However, Vander Meer and Van Dam showed that daylength had limited influence on Chinese cabbage compared to low temperatures and genotype (1,37). Similarly, Elers and Wiebe (7) found that with constant low temperatures, no photoperiodic effect was noticable in Chinese cabbage. They concluded that Chinese cabbage requires cold for flower initiation, and long days stabilize the vernalization effects.

As stated previously, the effect of photoperiod can only be studied in relation to temperature and the duration of vernalization. Response to these factors has been studied in many cold-requiring plants. In table beets, plants held at 4.4 C to 10 C for 30 days induced flowering (under long days) (5). The most effective condition to induce bolting in celery is 2 weeks or more at 15.5 C or lower (34). Collard (Brassica oleracea Acephala group) cv. "Vates" has similar requirements of 5 weeks at 6 C for floral induction (26). Lorenz observed the most rapid flowering of Chinese cabbage

occurs when plants are held at 5 C for 2 weeks under long days, and followed by warmer temperatures.

It is generally agreed that, depending on the temperature, the longer the plants are exposed to cold, the faster they flower (2,4,6,11,14,23,26,36,42). Yamasaki (42) developed a general formula to predict flowering in Chinese cabbage: (13 C-X)Y = 87 C, where X is the temperature below 13 C, and Y is the number of days with minimum temperature below 13 C. When the sum of the equation equals or exceeds 87 C, bolting will occur. Sensitivity to low temperature varies widely among cultivars (9,10,23).

High temperatures immediately following the cold period may nullify the effects or "devernalize" the plants. For example, temperatures of 21-27 C prevented table beets from flowering (4,5). If Chinese cabbage and Japanese radish (<u>Raphanus sativus</u> L. var. <u>acanthiformis</u> Makino) are not fully vernalized, they will not complete the successive stages of floral initiation (6). Purvis and Gregory (30) found that discontinuous low temperature treatments on winter rye elicited less response than continuous exposure, with the same total time exposed. This was shown only with short individual periods at low and high temperature. Plants are more stable to heat exposure the longer they are vernalized. After 8 weeks of cold, high temperatures (35 C) did not have a devernalizing effect.

The apical meristem is the site of perception of the cold treatment for most vernalization-requiring plants.

Chroboczek (4) demonstrated, that by cooling beet meristems with cold water through a rubber tubing wound around the growing point he could induce flowering. Wellensiek (40), in _Lunaris biennis_, concluded that vernalization took place only where there is actively dividing tissue (mitoses). Purvis also regenerated fragments of winter rye embryos that contained the shoot apex (28).

Another factor to consider in the vernalization effects is the stage of the plant that is sensitive to cold treatment. Chroboczek (5), in a study on table beets, concluded that it was the age, not the size of the plant, that determines susceptibility to cold temperatures. Species such as Chinese cabbage, Japanese radish and _Brassica napus_ may be vernalized as germinating seeds (7,23,31). Eguchi et al. (6), found that 60- day-old seedlings of Chinese cabbage were more sensitive to the chilling temperatures than 2-day-old seedlings. Flower stalk development and flowering were accelerated by low temperatures in older seedlings as compared to young ones. However, Guttormsen and Moe (9) found that increased plant age (1-3 weeks) at the start of vernalization delayed bolting in certain cultivars. Other species such as table beets, cabbage, and collards must be vernalized at the "green-plant stage" or after they have completed a juvenile phase (37,38). Collard cv. "Vates" must reach a stem diameter of 4.0 mm to be sensitive to low temperatures (2). Cabbage plants must attain a certain size before they will bolt (21).

Genotype also plays a role in the premature seeding of beets and other biennials, but the environment determines whether the bolting factors will be expressed. Two plants of the same cultivar may respond differently under different environmental conditions. It is impossible to determine the mode of inheritance of bolt resistance without consideration of the environmental conditions (5). Purvis asserted that in cold requiring plants, the vernalization treatment is a physiological compensation for the absence of "flowering genes" (29).

The number and action of the genes involved varies for the species in question. In Petkus rye (Secale cereale L.), the difference between winter and summer types is conditioned by one major gene, with the winter habit being recessive. It is possible to have a population of either habit through the selection of the desirable genotype (24). Van Heel determined that bolting in sugar beets (Beta vulgaris L.) is recessive (36). Bouwkamp and Honma found easy-bolting in celery (Apium graveolens Dulce group) to be dominant to later-bolting, and the response was controlled by one major gene (Vr) (1). The tendency of cabbage to bolt is recessive (33). In an intraspecific cross of Chinese cabbage X turnip, bolting appeared to be controlled by 2 major additive genes. There is an apparent association between bolting response and the turnip phenotype (20). Similarly, results of an interspecific cross between kale and Chinese cabbage suggest that a few major additive genes condition bolting response

(18). Bolting in Chinese cabbage appears to be controlled by 4 major additive genes with modifiers controlling the degree of resistance (19). "LB-7", a hybrid between Chinese cabbage and kale, has the genotype $v_1 v_1 v_2 v_2$ and bolts after 3 weeks of vernalization (19).

MATERIALS AND METHODS

Parent Material

Chinese cabbage

Chinese cabbage (**Brassica** **campestris** L. Pekinensis group) is a head forming vegetable with a biennial habit. The foliage is light green in color. Its large leaves are tender and veiny with wide petioles and undulating margins.

Two cultivars were used in this study, " Wong Bok" and "Mandarin". Wong Bok forms a short barrel shaped head and is a late season standard cultivar. Seed was obtained from Takii Seed Co., Kyoto, Japan. Mandarin, a small headed, early summer variety was obtained from the National Seed Storage Laboratory, Fort Collins, CO. Both cultivars bolted after 2 weeks of vernalization.

Turnip Rape

Brassica **napus** Oliefera L.F.F. Fibie group cv. "Siberian" is a winterhardy biennial. It is a leafy plant with blue-green coarse foliage with dentate margins. Seeds were obtained from the Institute of Plant Genetics, Warsaw, Poland. Siberian turnip rape has a vernalization requirement of at least 6 weeks.

Pak choi

Pak choi or Bok choy (**Brassica** **campestris** **L.** Chinensis group) is a leafy non-heading biennial. It has large leaves with wide petioles, prominent veins and smooth margins.

This cultivar "Su-e-ma" (translated "Later of April"), was obtained from Guan Zhong Yang from the Peoples Republic of China. It can be induced to bolt after 3 weeks of 5 C temperatures.

Chikale

The line "LB-7" is a late bolting line which was developed from an F_4 population derived from an interspecific cross between Siberian kale (Brassica napus) and Chinese cabbage cv. Mandarin . This cross was first made by Honma and Heecht (12) and further selected by Mero and Honma (19). The foliage has a shape intermediate between Chinese cabbage and kale, but is more similar to Chinese cabbage in texture and color and does form a head. LB-7 is reported to bolt after 4 weeks of vernalization (19).

Stubble turnip

"Taronda Zelder", a tetraploid variety of stubble turnip (B. campestris Rapa group) was obtained from the Institute for Horticultural Plant Breeding, Wageningen, The Netherlands. The root of Taronda Zelder is small, cylindrical and purple. The leaves are dark green, narrow and coarse with wavy margins. This cold hardy plant is used for fodder in other parts of the world. Its vernalization requirement is 4 weeks.

Hakuran

Hakuran is an artificially synthesized B. napus (25,42).

It is an amphidiploid developed from an interspecific cross between Chinese cabbage and cabbage (B. oleracea) which showed heading characteristics. According to Yamagishi and Takayanagi (42), Hakuran can be easily crossed with Chinese cabbage and may be useful as a bridge species in other Brassica vegetables. The foliage is medium green in color. Its texture is intermediate between Chinese cabbage and cabbage, more coarse than Chinese cabbage, but not as waxy as cabbage. Leaves are semi-rugose and have semi-undulate margins.

Tyfon

Tyfon is a synthesized variety developed from a cross between stubble turnip (B. rapa) and Chinese cabbage (B. campestris Pekinensis group). It is currently grown as a fodder crop and also as greens in the U.S. (41). The foliage is medium green in color and has coarse texture resembling the turnip. The large leaves have serrate margins and long, thin petioles. It is comparable to rape and stubble turnip for cold hardiness. Seeds were obtained from the Pacific Seed Production Co., Albany, OR.

Vernalization Procedure

All vernalizations in this study were conducted in a vernalization room held at 5 C $\pm$1. Plants were exposed to 12 hours of light/day with banks of fluorescent lamps (approximately 20 Wm^{-2}). Upon removal from the cooler, plants were kept in a 20 C greenhouse with 14 hours of light/day

supplied by G.E. High Intensity Discharge lamps with 1000 watt multivapor bulbs (approximately 24 Wm^{-2}).

Yamasaki (43) proposed a formula for predicting floral induction of Chinese cabbage, where 13 C is the critical temperature: (13 C -X)Y = 87 C, where X is the temperature below 13 C and Y is the number of days with minimum temperature below 13 C. When the sum of the equation is greater than or equal to 87 C, flowering is induced.

This formula proved to be adequate under the vernalization procedures previously described since Mandarin and Wong Bok did not bolt after 1 week, but did bolt after 2 weeks at 5 C in the vernalization room. Following Yamasaki's formula, 1 week of vernalizaton only adds to 56 C which is insufficient to induce bolting, but 2 weeks at 5 C equals 112 C which is sufficient to induce flowering.

Screening Parental Material

In November 1983, 4 week old seedlings of 18 potential parent lines were planted in a greenhouse ground bed. The bed was fertilized prior to planting with .013 kg/m^2 of ammonium nitrate and .065 kg/m^2 of sulfur.

Based on observations of phenotypic desirability and uniformity, 12 parental lines representing 7 species and species hybrids were selected to be used as parents in a diallel study (Table 1). On February 15,1984, 2 plants from each selected parental line were transplanted into large pots and vernalized for 6 weeks to obtain selfed seed to be used

Table 1. Parental lines used in diallel study

Parent Number	Cultivar	Species
1	Siberian	Turnip rape
2	Tyfon	(Chinese cabbage x Stubble Turnip)
3	Hakuran (Takii Seed)	(Chinese cabbage x cabbage)
4	Taronda Zelder	Stubble Turnip
5	Hakuran (Mikado Seed)	(Chinese cabbage x cabbage
6	Hakuran Strain "20 Self"	"
7	Hakuran Strain "36 Self"	"
8	Hakuran Strain "43 Self"	"
9	Mandarin	Chinese cabbage
10	LB-7	(Chinese cabbage x Kale)
11	Su-e-ma	Pak-Choi
12	Wong Bok	Chinese cabbage

in future experiments.

In the spring of 1984, a preliminary experiment was conducted to determine the vernalization requirements for the 12 parental lines. The treatments were 1,2,3,4,5,6 weeks of cold treatment at 5 C. None of the parental lines bolted after 1 week of vernalization. Siberian rape (2) required at least 6 weeks to bolt, the Chinese cabbage lines 9 (Mandarin) and 12 (Wong Bok) bolted after 2 weeks of cold and the rest were intermediate. Due to poor germination, the parental lines 4 ("Taranda Zelder") and 8 (Hakuran Strain 43) were not included in this phase of the study.

Hybridization

Crosses were made during the fall and winter of 1984-85. Pollinations were made by emasculating the buds, brushing pollen of the male parent on the stigmatic surface of the female parent, then covering with glassine bags until the siliques began to develop. Many of the crosses failed to produce viable seed after numerous pollinations (Figure 1), probably due to incompatibility and differences in chromosome number. Due to the many unsuccessful crosses, the design became an incomplete diallel.

Vernalizaton Experiment

The F_1 progeny were planted in vermiculite at planned intervals beginning September 6, 1985. They were transplanted 7-10 days later into No. 24 PVC trays filled with a mixture

Figure 1. Seed yield of crosses obtained in the incomplete diallel cross.

	1	2	3	4	5	6	7	8	9	10	11	12
Siberian					35				65	30		
Tyfon				15								
Hakur.(T)									30	10	25	
Tar. Zel.		20			11				20		15	
Hakur.(M)			21						35		40	
Hakur.(20)					40				20		30	
Hakur.(36)					45				20	15	30	
Hakur.(43)					25						35	
Mand.			30		20					70	100+	100+
LB-7			20						100+		60	
Su-e-ma			40		60				200+	25		
Wong Bok			20		10				40	40	60	

of equal parts of peat, perlite, and vermiculite. The experiment was a completely randomized design with 3 replications. There was a maximum of 72 plants of each line. Standard cultural practices for Chinese cabbage were employed.

Vernalization treatments for most of the F_1's were estimated based on the cold requirements of the susceptible parent, the mid-parent value, and 1 week beyond the bolt resistant parent. For F_1's with few seeds (less than 20) only the mid-parent treatment was used (Table 2).

All treatments were removed from the vernalization room on November 15, 1985 and placed in a greenhouse to observe bolting response. The greenhouse temperature was kept at 15.5 C for 2 weeks to stabilize the plants and to prevent devernalization, and subsequently raised to 20 C for the remainder of the experiment. Supplemental lighting of 14 hours/day was provided by HID lamps.

Data on the number of bolted plants was recorded every 2-3 days for 6 weeks beginning December 2,1985. Plants were considered to have bolted when flower buds were visible in the apex (17). At the conclusion of the experiment, all remaining plants were cut in half longitudinally to determine if the apex had begun to elongate, but not yet shown visible bud (Figure 2). An elongated apex indicates floral induction according to the criteria defined by Mero and Honma (17).

Data Analysis

TABLE 2 Vernalization treatments selected for parents and hybrids

Pedigree (female, male)	No. Weeks at 5 C								
	0	2	21/2	3	31/2	4	41/2	5	6
1 1	x						x		x
2 2	x	x	x	x		x	x	x	
2 4			x						
3 3	x			x	x	x		x	
3 5					x				
3 9				x					
3 10					x				
3 11					x				
3 12				x					
4 2				x					
4 4	x	x		x	x	x			
5 1									x
5 4						x			
5 5	x	x		x	x	x			
5 6					x			x	
5 7					x	x			
5 8					x			x	
5 9			x						
5 11				x		x			
5 12			x						
6 6	x			x	x	x		x	
7 7	x			x	x	x		x	
8 8	x			x	x	x			
9 1		x				x	x		x
9 3				x					
9 4				x					
9 5			x						
9 6				x					
9 7			x						
9 9	x	x	x	x	x	x			
10 1				x				x	x
10 3					x				
10 7						x			
10 9		x				x			
10 10	x	x	x	x	x	x	x		

TABLE 2   (cont'd)

Pedigree (female, male)	0	2	21/2	3	31/2	4	41/2	5	6
10 11				x		x			
10 12		x	x			x			
11 3					x			x	
11 4			x						
11 5				x	x	x			
11 6					x			x	
11 7				x		x			
11 8				x		x		x	
11 9		x	x	x		x			
11 10				x	x	x			
11 11	x	x	x	x	x	x			
11 12			x			x			
12 9	x	x	x	x	x				
12 12	x	x	x	x	x	x			

1   Turnip rape
2   Tyfon
3   Hakuran (T)
4   Stubble turnip
5   Hakuran (M)
6   Hakuran "20 Self"
7   Hakuran "36 Self"
8   Hakuran "43 Self"
9   Mandarin
10   LB-7
11   Su-e-ma
12   Wong Bok

Figure 2. Stages in seedstalk development.
Left, not bolting; center, bolting;
right, bolting with visible bud,
growing points outlined for ease of
identification
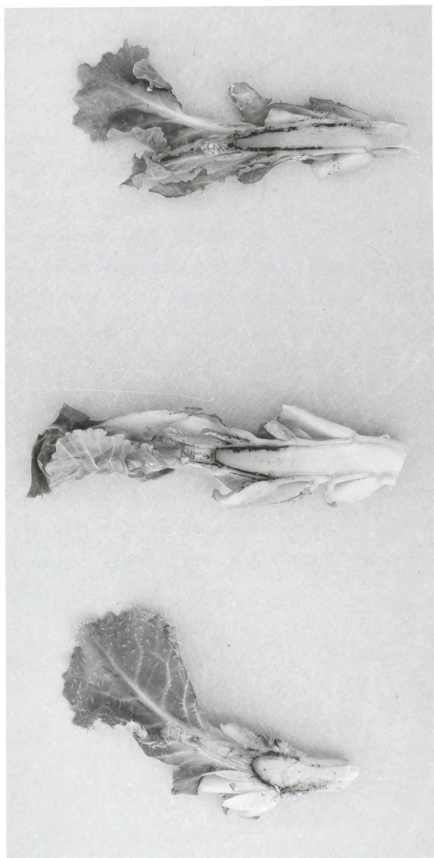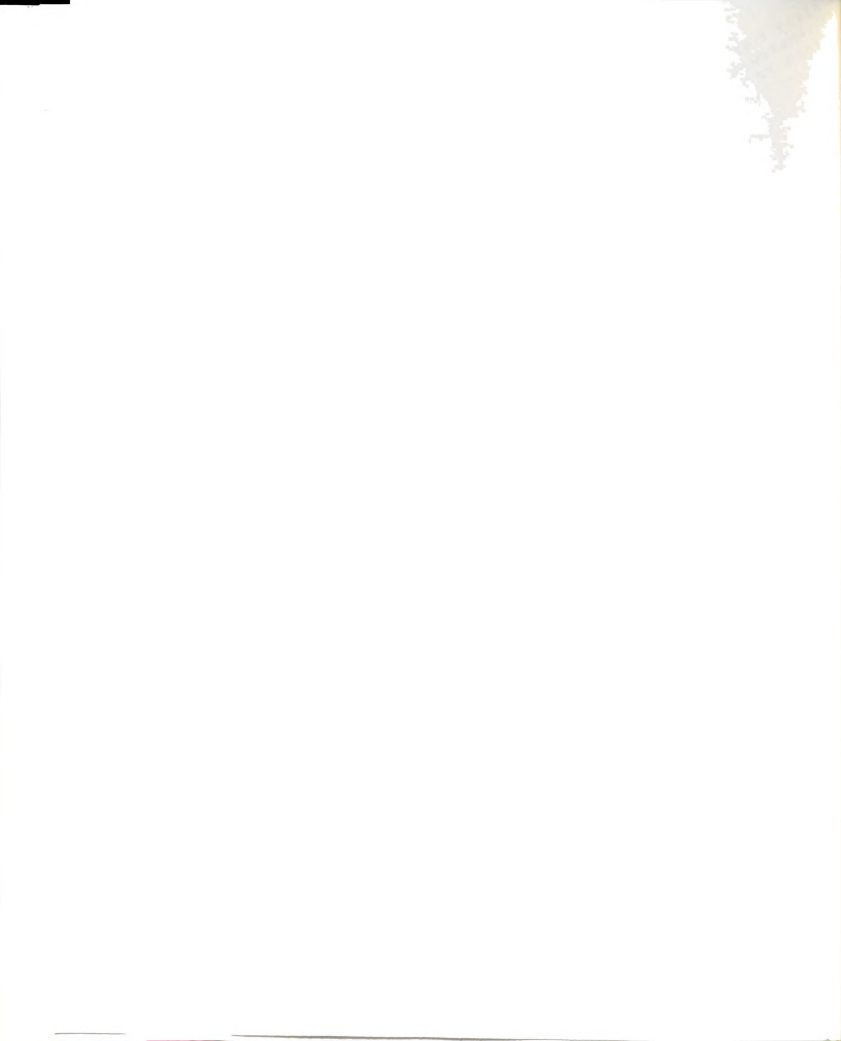
The minimum vernalization requirement for each cross was determined by the number of weeks at which at least 50% of the plants in each treatment bolted. Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) Version 9. Additional analysis was performed using tables and formulae given in Steele and Torrie (32) and Little and Hills (15).

A combining ability analysis of the data was performed according to a modification of the fixed effects, full diallel model (method 1, model 1) described by Griffing (8). The fixed effects model limits statistical inference to the population used in the study. Parameters of the model were adjusted for parents versus crosses (PVC) and parental differences among and within species (PA and PW respectively) using orthogonal comparisons.

Combining abilities were computed according to Griffings method (8), modified to extract general combining abilities for among parental species (GA) and within (GW). The model is as follows:

$$Y_{(ij)(kl)m} = u + (G_i + g_{ij}) + (G_k + g_{kl}) + s_{ijkl} + e_{ijklm}$$

where:

$Y_{(ij)(kl)m}$ = the value of the mth observation of the cross between the jth (l^{th}) parent of the ith (k^{th}) species.

u = the grand mean of the population

G_i = general combining ability effect for the ith (k^{th}) species

$$\sum_{i}^{\rho} n_i G_i = 0$$

g_{ij} = the GCA effect of the jth (l^{th}) parent of the ith (k^{th}) species

$$\sum_{1}^{\rho} g_{(ij)} = 0$$

s_{ijkl} = specific combining ability effects associated with the cross between the jth and l^{th} parent of the ith and k^{th} species

e_{ijklm} = random error, $n \sim NID$ $(0, \sigma^2)$

The effects were estimated using the least squares method and performed by the New Regression subroutine of SPSS.9.

The effects of unequal numbers of progeny and missing crosses resulted in a loss of orthogonality and balance in the design. The equations for computing the individual dependent effects are thus :

$$GA_7 = -1/5 \ (GA_1 + GA_2 + GA_3 + GA_4 + GA_5 + 2GA_6)$$
$$-GW_{75} = GW_{71} + GW_{72} + GW_{73} + GW_{74}$$
$$GW_{61} = -GW_{62}$$

The effects of GW were assigned 5 df which may be distributed as 1 df for the contrast between the two Chinese cabbage parents and 4 df for contrasts between the 5 Hakuran parents. Therefore, two dependent effects were computed from these 5

df. The effect for within Chinese cabbage has been designated GW 62 and the Hakuran effect is GW 75. All contrasts are explained in Table 3.

The variances due to PVC, PA, PW, GA, GW and reciprocal effects were estimated from the mean squares computed as each group of individual effects was entered stepwise into the computations. Each effect was compared for significance by the F test using the residual mean square as the error term. Differences between estimates of individual effects were statistically compared using Student's t test.

Among species means were arranged in an order of magnitude and adjacent means were compared using Fisher's (protected) LSD test adjusted for unequal replications. The formula given by Steele and Torrie (32) is thus:

$$LSD = t \sqrt{(s^2/r_1) + (s^2/r_2)}, \text{ where } r_1 \neq r_2.$$

TABLE 3   Definitions of contrasts in diallel model

PVC = Effect of parents versus F$_1$ hybrids

Orthogonal contrasts of parents among species

PA 1 = Hakuran vs. other parents
PA 2 = Chinese cabbage vs. other non-Hakuran parents
PA 3 = turnip rape, Tyfon, stubble turnip, Chikale vs. Pak-choi
PA 4 = turnip rape, Tyfon, stubble turnip vs. Chikale
PA 5 = turnip rape, Tyfon vs. stubble turnip
PA 6 = turnip rape vs. Tyfon

Orthogonal contrasts of parents within species

PW 1 = Chinese cabbage parent 9 vs. Ch. cabbage 12
PW 2 = Hakuran parents 3, 5, 6, 7, vs. 8
PW 3 =   "   "   3, 5, 6 vs. 7
PW 4 =   "   "   3, 5 vs. 6
PW 5 =   "   "   3 vs. 5

General combining ability effects among parental species

GA 1 = Effect of turnip rape (parent 1)
GA 2 =   "   " Tyfon (parent 2)
GA 3 =   "   " stubble turnip (parent 4)
GA 4 =   "   " Chikale (parent 10)
GA 5 =   "   " Pak-choi (parent 11)
GA 6 =   "   " Chinese cabbage (parents 9, 12)
GA 7 =   "   " Hakuran species (parents 3, 5, 6, 7, 8)

General combining ability effects within parental species

GW 61 = Effect of Chinese cabbage parent 9
GW 62 = Effect of Chinese cabbage parent 12
GW 71 = Effect of Hakuran parent 3
GW 72 =   "   " Hakuran parent 5
GW 73 =   "   " Hakuran parent 6
GW 74 =   "   " Hakuran parent 7
GW 75 =   "   " Hakuran parent 8

RESULTS AND DISCUSSION

The minimum vernalization requirement for each of the 12 parents and their F_1's are listed in Table 4. Parent 1 (Siberian rape) had the greatest vernalization requirement of 6 weeks and parents 9 ("Mandarin"), 10 ("LB-7"), 11 ("Su-e-ma") and 12 ("Wong Bok") had the least requirements of two weeks. The vernalizaton time of 2 weeks for "LB-7" disagrees with that reported by Mero and Honma (19). It is possible that the population was still segregating in the F_4 generation. Analysis of variance was performed to test for significance of individual parental and combining ability effects (Table 5). Results show that all effects except parents versus crosses (PVC) were highly significant (p = 0.01). Although the variance of PVC was initially non-significant, it became highly significant as more effects were added to the model.

Each of the parental contrasts was significant, suggesting that there are some genetic differences among and within parental species. The significance of the GCA values among and within species also suggests that bolting resistance would be amiable to selection.

The significance of the specific combining ability (SCA) effects suggests that some hybrid combinations can be expected to perform better or worse than the mean performance of the lines involved. Although highly significant SCA effects were observed (Appendix A-1), estimates of individual

TABLE 4. MINIMUM VERNALIZATION REQUIREMENTS FOR PARENTAL LINES AND F1 HYBRIDS

Pedigree	No. Weeks	Pedigree	No. Weeks
1 1	6.0	9 5	2 5
2 2	2.0	9 6	3.0
2 4	3.0	9 7	2.5
3 3	3.0	9 9	2.0
3 5	3.5	9 10	2.0
3 9	3.0	9 11	2.0
3 10	3.5	9 12	3.0
3 11	3.5	10 1	4.5
3 12	3.0	10 3	3.5
4 2	3.0	10 7	4.0
4 4	4.0	10 9	2.0-2.5
5 1	6.0	10 10	2.0
5 4	4.0	10 11	3.0
5 5	2.5	10 12	2.0
5 6	3.5	11 3	3.5
5 7	3.5	11 4	2.5
5 8	3.5	11 5	3.0
5 9	2.5	11 6	3.5
5 11	3.0-4.0	11 7	3.0
5 12	2.5	11 8	3.0
6 6	3.0	11 9	2.0
7 7	4.0	11 10	3.0-3.5
8 8	3.0	11 11	2.0
9 1	4.0	11 12	2.5
9 3	3.0	12 9	2.0
9 4	3.0	12 12	2.0

1. Siberian
2. Tyfon
3. Hakuran (Takii Seed)
4. Taronda Zelder
5. Hakuran (Mikado Seed)
6. Hakuran Strain "20 Self"
7. Hakuran Strain "36 Self"
8. Hakuran Strain "43 Self"
9. Mandarin
10. LB-7
11. Su-e-ma
12. Wong Bok

Table 5. Analysis of variance of combining ability effects

Source	df	Sum of squares	Mean squares
Parents versus crosses	1	.03625	.03625
Parental contrasts among species	6	.58563	.09761**
Parental contrasts within species	5	.03773	.00755**
General combining ability among parental species	6	.63304	.10551**
General combining ability within parental species	5	.04846	.00969**
Specific combining ability effects	19	.15295	.00850**
Reciprocal effects	9	.05830	.00648**
Error	113	.08420	.00075

** = significant at the 1% level

effects were not used in the analysis due to the confounding of the SCA and GCA effects. In Griffing's analysis (8), the SCA effects are only valid when all possible crosses are represented. Because of the incompleteness of this diallel design, each parent was not represented in the same number of crosses. Since SCA effects are expressed as the average deviation of the cross from its value predicted by the GCA effects of its parents. This unequal number of crosses involving each parent would bias the results of the SCA effects. Therefore, no hypothesis about gene action could be inferred. Since high ratios of GCA to SCA sums of squares were obtained for both among species and among + within species, GCA effects appear to be more important than SCA effects in influencing bolt resistance.

Since the among parental species effect was significant by the F test, a mean separation using Fisher's (protected) LSD test for unequal replications was performed. Results show all among species means were significantly different from each other except Tyfon, Chikale, Pakchoi, and Chinese cabbage (Table 6).

Estimates of individual effects are shown in Table 7. The effect of parents versus crosses was highly significant, suggesting genetic differences between the parents and when used in hybrid combinations.

All of the GCA effects (Table 3) for among species were significantly different from each other (p = .01) except Tyfon and LB-7 (GA 2 vs. GA 4) suggesting that these two

Table 6. Mean separation test for minimum vernalization
requirements among parental species

Species	Mean minimum vernalization requirement (weeks)
turnip rape	6.00^{z} a
stubble turnip	4.00 b
Hakuran	2.95 c
Chikale	2.00 d
Tyfon	2.00 d
pak choi	2.00 d
Chinese cabbage	2.00 d

[z]Means within a column with the same letter are not
different (1%) using a protected LSD test

Table 7. Estimates of individual effects for bolt resistance

Effect	Estimate	Effect	Estimate
Grand Mean	.31772**		
PVC	- .00636**	GA1	.14028**
PA1	.02579**	GA2	.05035 * a
PA2	- .09221**	GA3	.02200
PA3	- .17361**	GA4	.05017** a
PA4	- .28935**	GA5	.03979**
PA5	.04630	GA6	.07391**
PA6	-1.11110**	GA7	- .02517**
PW1	.00000	GW61	1.02093
		GW62	.02093
PW2	.00139	GW71	- .02256**
		GW72	.02491**
PW3	.03009**	GW73	- .01192
		GW74	- .36336E-03
PW4	.01852**	GW75	.00993
PW5	- .05556**		
R 39	.15689E-14	R 511	- .01190 *
R 310	0	R 910	.01667**
		R 911	0
R 311	.66343E-15	R 912	- .08333**
R 59	.00741	R 1011	.00794

PVC = Parents versus crosses   GA = GCA among parent
                                         species

PA = Parents among species   GW = GCA within parent
                                         species

PW = Parents witnin species   R = Reciprocal effects
                                      (female, male)

**, * = significant at the 1% and 5% levels resspectively

    a = not significantly different at the 5% level by
        student's t.

species have similar bolting responses. The GCA effect among species were significant at the 1% level except GA 3 (stubble turnip). Since GCA effects of a parent are computed as the mean deviation of its progeny from the grand mean, the non-significant GCA effect of stubble turnip (GA 3) may be due to the similarity of the mean bolting response of stubble turnip and its progeny to the grand mean . It may also be due to similar bolting response of the crosses involving stubble turnip or due to a limited number of F_1's.

Since the minimum vernalization requirement was computed as the reciprocal of the number of weeks to 50% bolting, the lower GCA value indicates those parents which would be expected to contribute the greatest amount of bolt resistance to their progeny.

The Hakuran parents as a group exhibited the lowest GCA effect for bolting response and would probably be the best sources of greater bolt resistance. Turnip rape (parent 1) exhibited the highest GCA value suggesting that although it has the greatest vernalization requirement, it does not uniformly transmit this character to its progeny. This may due to interspecific incompatibility (18) since it yielded a limited number of seeds.

Examination of the GCA effects within species revealed only those of Hakuran parents 3 (GW 71) and 5 (GW 72) were significant. This is in agreement with the GA effect that Hakuran most readily transmits bolt resistance to its progeny. Based on the GA and GW effects, parent 3 exhibited

the lowest values and should therefore be considered before parent 5 in a breeding program.

Significant reciprocal cross differences were noted for crosses between parents 5 and 11, 9 and 10, and 9 and 12. These differences might be explained by accidental selfing or to possible cytoplasmic effects.

SUMMARY AND CONCLUSIONS

A combining ability analysis was performed on measurements of vernalization requirements from an incomplete diallel cross. Twelve parental lines representing 7 species were examined. Use of the fixed effects model limits the conclusions to the parental lines used in this study.

The analysis of variance detected highly significant variation among parents and hybrids. Genetic variation was observed both among and within species. Highly significant effects among and within species suggest that selection for bolt resistance would be effective. Although significant SCA effects were found, individual estimates could not be used in the interpretation due to confounding of GCA and SCA effects. The high ratio of GCA to SCA sums of squares for both among and among + within species suggests that a large proportion of the genetic variation is probably due to general combining ability effects.

The Hakuran species and in particular parent 3 exhibited the lowest GCA value. For breeding purposes, parent 3 would be the best choice for greater bolt resistance. Since cabbage generally requires approximately 8 weeks of vernalization to induce flowering (27), it is possible that the higher degree of bolt resistance in Hakuran may have come from the contribution of cabbage in the genetic make-up of the hybrid.

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PART II: INHERITANCE OF LATERAL SUPPRESSION
        AND LEAF NUMBER IN BROCCOLI
    (Brassica oleracea L. Italica group)

INTRODUCTION

Broccoli (<u>Brassica</u> <u>oleracea</u> L. Italica Group) is an ancient crop that originated in the Mediterranean area about 2500 years ago (5). From the eastern Mediterranean, broccoli was introduced to Italy where the crop became quite diverse (8). Many present day varieties have been developed from selections made in Italy over the past 2000 years. In the last 300 years, Danish and English horticulturists have done much to improve this crop through selection (5). Broccoli did not become widely grown in the United States until the early 1900's (5,8).

The nomenclature of this plant has become confusing because the general term "broccoli" refers to several plant types in this genus (5). The term "sprouting broccoli" refers to the branching habit where the young edible inflorescences are called sprouts. This is the normal habit of broccoli. The form of broccoli called calabrese is the main type grown in the United States. It is the only vegetable in the Italica Group that has been extensively developed through breeding (8).

A single-head type broccoli is desirable for a once over mechanical harvest used in commercial production. The yield per plant would be greater if all of the plant's photosynthates were concentrated in the central head and not diverted into production of lateral shoots (32).

the main head develops would be advantageous to the home gardener and would allow for more than one harvest.

Correlations between the number of axillary shoots and number of leaves produced have been observed in tobacco (<u>Nicotiana</u> <u>tabacum</u> L.) (16) and brussel sprouts (<u>Brassica</u> <u>oleracea</u> L. Gemmifera group) (11). A study was therefore conducted to see if such a relationship exists in broccoli (<u>Brassica</u> <u>oleracea</u> L. Italica group).

The objectives of this study were:

1. To determine the inheritance of lateral suppression

2. To determine the inheritance of leaf number

3. To ascertain the relationship between leaf number and lateral shoot production.

LITERATURE REVIEW

I. Axillary Shoots

An axillary shoot in broccoli occurs where a bract subtends a rudimentary floral shoot, a branchlet of the peduncle (5). According to Pressman et al. (23), the smaller inflorescences on side shoots are more desirable for fresh export in Europe so greater numbers per plant are preferred. Yukihiro and Hirose (32) assert that the yield of broccoli depends on apical dominance and the production of one large head.

Pressman et al. (23) studied the effect of removing the apical bud on the production of lateral shoots. Elongation of lateral shoots generally starts at the maturation of the terminal inflorescence (head). After the head is harvested, the laterals rapidly elongate and button. Pinching the apical bud of young plants stimulate elongation of the lateral shoots . The plants were pinched at three stages of development. Pinching at the later stages of development significantly increased the number of shoots The number of shoots varied from 6 (control) to 10 (pinched at stage 3).

Takahashi and Yazawa (28) observed that lateral shoots appeared at almost the same time the terminal bud was differentiated. The time of flower bud differentiation on the lateral shoot was independent of the shoot's size and order of appearance.

Yukihiro and Hirose (32) investigated the effects of growth regulators on lateral branching in broccoli. Certain chemicals did affect the number of side shoots. They concluded that despite the influence of external factors, branching habit is primarily genetically controlled.

The inheritance of lateral shoot formation or branching has been elucidated for several crops. Yarnell (31) describes a completely dominant gene $\underline{Ax}$ for axil sprouts in cabbage (<u>Brassica</u> <u>oleracea</u> L. Capitata group). Akratanakul and Baggett (1) also studied the inheritance of axillary heading in cabbage. This trait was highly heritable in the broad sense, but narrow sense heritability was low, indicating dominance effects. The F_2 population showed a skewed frequency distribution for low axillary heading tendency. This supported their hypothesis that the character is recessive and controlled by one or a few genes.

In Brussel sprouts (<u>Brassica</u> <u>oleracea</u> L. Gemmifera group), the final node number and rate of production are highly heritable. In a half diallel study of 10 parents, the traits were controlled by additive genes (11). In crosses between Brussel sprouts and other Brassica species observations for axillary head production were made. In cabbage x Brussel sprouts and Brussel sprouts x broccoli, the F_2 populations showed continuous variation for the ability to form heads in the leaf axils, indicating more than a monohybrid segregation (12).

In the case of Indian mustard (<u>Brassica</u> <u>juncea</u>),

partial dominance controlled the number of primary branches. Heritiability estimates were moderate for this character (6).

There have been extensive studies on the inheritance of branching in sunflower (<u>Helianthus annuus</u> L.). Branching is an atavistic trait in this crop. In a cross between a cultivated type and a wild type, the F_2 generation exhibited a 9:7 unbranched : branched ratio which is characteristic of a dihybrid trait. Nonbranching is dominant (15). Hockett (9) reported that branching at the top of the plant is controlled by a single dominant gene in cultivated species and in the wild type by duplicate dominant genes. Putt (24) described a different kind of branching habit than Hockett reported. In this new type, the character is controlled by one recessive gene " <u>b</u>".

Hockett and Knowles (10) devised a classification system for the different branching types. Four genes were identified from crosses made between plants of the various classifications: $\underline{Br}_1$, dominant for top branching, $\underline{Br}_2$ and $\underline{Br}_3$ - duplicate dominant genes in the wild type, and $\underline{b}_2$ and $\underline{b}_3$, recessive which give a fully branched habit when homozygous for both genes.

Matsuda and Sato (16,17,18) conducted a series of experiments on tobacco (<u>Nicotiana tabacum</u> L.) and sucker production. They found significant differences among varieties for the number of suckers. In an experiment with 2 varietal crosses (low x high sucker production), estimates of additive and non-additive genetic effects were highly
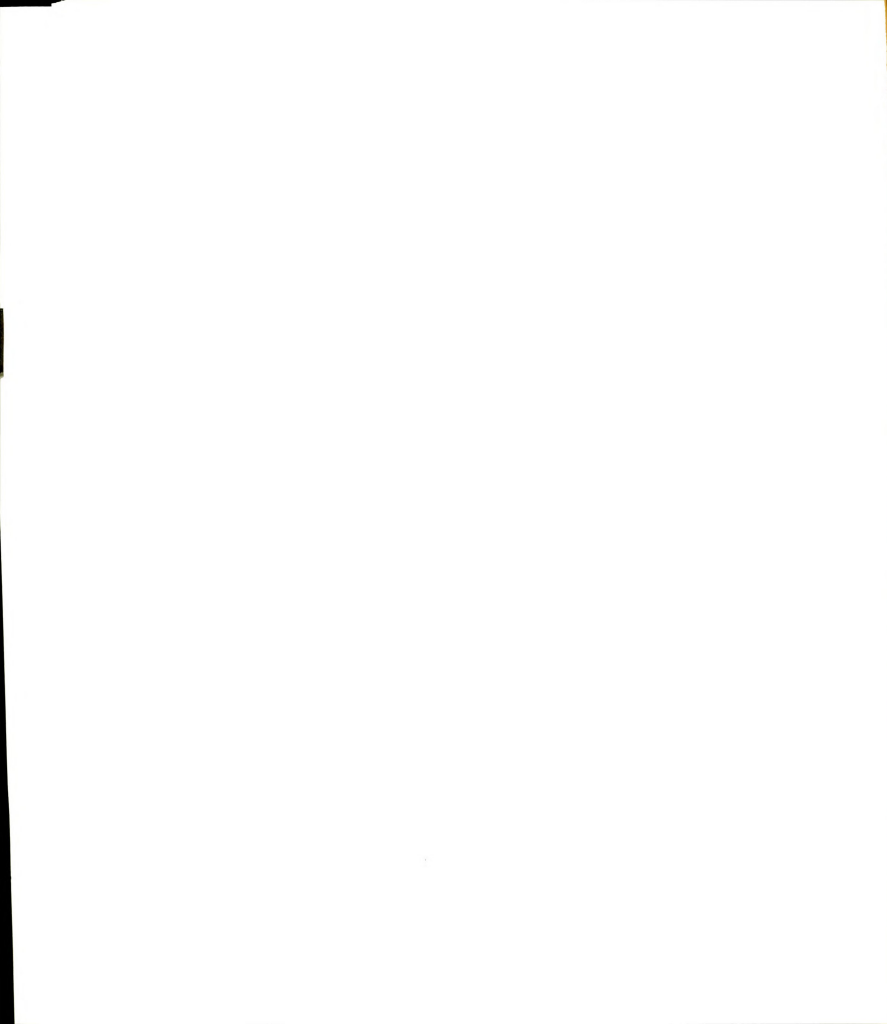
significant. They proposed that the number of ground suckers is controlled by 3 genetic factors for one of the crosses and 6 factors for the other cross.

Almost all tomato plants (<u>Lycopersicon esculentum</u>) produce lateral side shoots profusely. However, there are two isogenic lines of the variety "Craigella" which show inhibition of lateral side shoot development of varying degrees. "Craigella Blind" (<u>blbl</u>) initiate bud primordia at most nodes, but these either fail to develop or show supressed outgrowth compared to normal. Tucker (30) describes the "Craigella Lateral Suppressor" (<u>ls</u>) found by Malayer and Guard as plants that do not initiate axillary bud primordia at all during the vegetative phase.

II. Leaf Number

The inheritance of leaf number has been studied in several crops. In cabbage, Summers and Honma (26) found few non-wrapper leaves was dominant in crosses between 2 smooth green cabbage lines. However, many leaves was dominant in crosses between smooth green x red cabbage. Larger heritability estimates were obtained for green x green crosses than red x green (.67 vs .50). In crosses between smooth green x green savoy, the number of non-wrapper leaves was influenced by planting time.

Swarup and Sharma (27) also studied cabbage, but reported that the additive x additive epistatic component was higher than the other gene effects, although the additive x

dominance effects were also highly significant. The heritability estimates were low, giving a mean value of 0.052%.

Prasad and Prasad (22) studied several characteristics in radish (Raphanus sativus L.). Leaf number gave a heritability estimate of 81.34% and showed a low genetic advance (17.17%). This combination indicates that dominance or epistasis are the major effects.

The same authors (21) investigated 21 varieties of carrot (Daucus carota Linn.) and found both heritability and genotypic coefficient of variation to be high, but genetic advance was low due to minimum variation. This indicates that selection for this trait would not be beneficial.

In a study of Indian mustard (Brassica juncea (L.) Czern & Coss.), Chauhan and Singh (6) concluded the number of leaves was governed mainly by genes exhibiting dominance effects. The degree of dominance indicated overdominance. Both heritability and genetic advance were low, 12.28 and 0.88%, respectively.

Inheritance of leaf number in spring rape (Brassica napus L.) appears to be determined by 1 gene or a small number of major genes. Broad sense heritability ranged from 58.05%-93.5% over 3 planting dates. Dominance and non-allelic interactions were significant (29).

A diallel analysis of four varieties of tobacco leaf number revealed that additive gene action was the major part of total genetic variation, but there appears to be some

dominance influence, possibly overdominance. It is likely that few leaves is dominant over many leaves (17).

In a 6 x 6 diallel study with corn (Zea mays L.), leaf number showed evidence of partial dominance, but additive effects accounted for a high proportion of the total variation. Both broad and narrow sense heritabilities were high, 97.1 and 95.3% respectively (3).

Dangi and Paroda (7) studied the gene effects for ten quantitative characters of five hybrids of sorghum (Sorghum bicolor L.) They found the number of leaves per plant was governed mainly by dominance as well as epistatic components.

Burton (4) conducted a biparental study of pearl millet (Pennisetum glaucum L.) using two female and three male parents. Few leaves per stem showed slight dominance over many leaves in crosses involving 1 parent, but the reverse was true in crosses involving the other parent. There appeared to be both additive and multiplicative effects with 2-13.6 effective factors involved. The heritability for most crosses was quite low.

Phul, Nanda and Gill (20) also studied pearl millet in a 9 x 9 diallel experiment. The estimates of average degree of dominance and narrow sense heritability showed the importance of additive gene effects in the inheritance of leaf number.

MATERIALS AND METHODS

Parent Material

Three inbred lines of broccoli were used for the present study. The parent exhibiting lateral shoots (normal) was an inbred derived from the commercial cultivar "Spartan Early". This cultivar was developed at Michigan State University and was selected from the cultivar "Green Mountain" for early maturity, uniformity and general horticultural characteristics. The plants are short and compact, the foliage is light bluish green and produces abundant lateral shoots (9). The parent exhibiting suppression of lateral shoots was 83-857, an inbred selection from the Michigan State University breeding program. This line has a normal growth habit and an average maturity date. It is derived from a single F_8 plant selected from the intercrossing of two F_2 lines derived from the cross "Early Fuji" cabbage x "Self Blanche" cauliflower: $[(E.F. x S.B.)F_2 x (E.F. x S.B.)F_2] F_8$. This line was selected from a segregating population resistant to black rot (<u>Xanthomonas campestris</u>). This line produces a central head and no lateral shoots, however it will produce lateral side shoots several months after decapitation of the central head.

A third inbred line, 83-866 was used for the leaf number study. Line 83-866 is the F_4 progeny from the cross between "Spartan Early" and an F_5 line derived from intercrossing two F_2 lines from the cross "Early Fuji" cabbage x "Self Blanche"

cauliflower: $\{[(E.F. \ x \ S.B.)F_2 \ x \ (E.F. \ x \ S.B.)F_2]F_5 \ x \ S.E.\}$ F_4. This line also exhibited normal growth habits and average maturity date and produces fewer than three shoots per plant.

Hybridization

Mature plants of "Spartan Early", 83-857, and 83-866 were transplanted from the field to the greenhouse in summer of 1983 to obtain selfed seed. In January 1984, the S_1 seed from each of these lines were sown in a mixture of equal parts of peat, perlite and vermiculite in No. 24 PVC trays and transplanted to 15 cm. clay pots at 3-4 true leaves.

Reciprocal crosses were made between the lateral shoot suppressor and normal parents. A cross was made between "Spartan Early" and 83-866 using two full sib lines for the leaf number study. Hybridization was made by emasculating the flower bud, brushing pollen from the male parent on the stigmatic surface of the seed parent, then covering with a glassine bag until the siliques began to develop. Each of the crosses produced a limited number of seeds, and the resulting F_1 plants were fertile.

The F_2 populations were produced in the greenhouse in the spring of 1985 either by hand pollination or shaking the F_1's. Plants were kept apart to prevent outcrossing.

Although backcrosses were attempted, all populations obtained were too small to be used in the genetic analysis. Reduced population size was due to the central head of the F_1

plant flowering for only a brief period of time.

Field Experiment

In the summer and fall of 1985, various populations representing the two crosses were grown simultaneously in the field to study the inheritance of lateral shoot suppression and leaf number. Seeds of the F_2, BC and parents were sown in flats of vermiculite in the greenhouse on July 27, 1985. When the plants were 2 weeks old they were transplanted into No. 24 PVC trays filled with a mixture of equal parts of peat, perlite and vermiculite. The trays were placed under shade cloth in a greenhouse maintained at 21 C. After two weeks the shade cloth was removed and plants were exposed to 4 hours of supplemental lighting (G.E. High Intensity Discharge Lamps with 1000 watt multivapor bulbs approximately 24 Wm^{-2}).

The seedlings were placed in a lath house for five days prior to field transplanting at the Michigan State University Horticulture Research Center on September 3 and 4, 1985. The experiment was a randomized complete block design with 3 replications. Each replicate included a maximum of 60 F_2 plants of each cross for the lateral suppressor study, 50 F_2's for the leaf number study, 15 plants of each parent, and as many backcross plants as were available.

The spacing within rows was 46 cm. and between rows was 91 cm. At transplanting, all plants were watered with 120 ml of a starter solution and insecticide (Diazinon). The plot

was irrigated, sprayed and cultivated as necessary. A preplant application of 897 kg per hectare of 19-19-19 fertilizer was made and two sidedressings of ammonium nitrate at 56 kg actual N per hectare was applied every two weeks after transplanting. A postemergence herbicide (Dacthal) at 10 kg per hectare was applied 3 days after transplanting.

Data

Data on shoot and leaf number were recorded when each plant exhibited a head approximately 2.5 cm. in diameter, at which time axillary shoots were visible. The experiment was terminated when all plants had been harvested or at killing frost. Each plant was pulled up or cut off at soil level and shoots and leaves were counted for the appropriate crosses. Plants were also classified for glossy foliage.

Data Analysis - Axillary Shoots

The F_2 data were analyzed by the analysis of counts method given in Little and Hills (14). The F_2 families were statistically compared using a Chi-square test for independence to see if reciprocal crosses could be pooled. Correlation analysis was also performed by SPSS (Statistical Package for the Social Sciences). Population data were analyzed by a Chi-square goodness of fit to test the genetic hypothesis.

Data Analysis -Leaf Number

Means, variances and standard deviations were obtained from individual plant data and calculated using SPSS.9. Population means were statistically compared by use of a modified Students t test which accounts for 2 populations with unequal variances. The formula given by Steel and Torrie is thus:

$$t' = \frac{y1 - y2}{s_{y1-y2}} \qquad \text{where } s_{y1-y2} = \sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}$$

$$\text{and effective df} = \frac{(s^2/n1 + s^2/n2)^2}{[(s^2/n_1)^2/n_1 - 1] + [(s^2/n_2)^2/n_2 - 1]}$$

Tests for normality of the non-segregating populations were conducted as outlined by Leonard (13), utilizing the probability integral table given in Steel and Torrie (25). Population data was analyzed by a Chi-square goodness of fit to compare the observed and theoretical ratios.

RESULTS AND DISCUSSION

Shoots

The F_1 plants exhibited the lateral suppressor characteristic. A total of 335 F_2 plants were rated for the presence of lateral shoots. Results of the Chi-Square test for independence of the F_2 population suggest that reciprocal crosses "Spartan Early" X 83-857-2 and 83-857-2 X "Spartan Early" were similar (p = .95-.90) and therefore the data were pooled.

The number of shoots varied from 0 to 17 in the F_2 lines (Figure 1), however there was a greater proportion of plants in the lateral suppressor class than the normal class. Based on the segregation ratio of 173 lateral suppressor plants : 162 normal, there appears to be dominance toward the lateral suppressor character.

The observed F_2 ratio suggests a dihybrid, 9:7 duplicate recessive model. The Chi-square test suggests a good fit to this hypothesis (Table 1). $\underline{Ns}_1\_\underline{Ns}_2\_$ conditions lateral suppression.

TABLE 1. CHI-SQUARE TEST FOR GOODNESS OF FIT FOR POOLED RECIPROCAL CROSSES OF "SPARTAN EARLY" X 83-857-2, BASED ON A TWO GENE, 9:7 SEGREGATION RATIO FOR LATERAL SUPPRESSION

Generation	Observed ratio	Theoretical ratio	Chi-sq.	P
Pooled F_2	173 : 162	188 : 147	2.55	.50-.10

Figure 1. Broccoli stalks with leaves removed.
Left, lateral suppressor parent 83-857-1;
Right, non-lateral suppressor parent
Spartan Early.

Each gene is epistatic to the other such that recessive homozygosity at either or both loci conditions the presence of lateral shoots. The proposed genotype of 83-857-2 (P_1) is $Ns_1 Ns_1 Ns_2 Ns_2$ (lateral suppressor) and "Spartan Early" (P_2) is $ns_1 ns_1 ns_2 ns_2$ (normal). The proposed genotypes and phenotypes of the F_2 generation are shown below :

1 $Ns_1Ns_11Ns_2Ns_2$		
2 $Ns_1Ns_1Ns_2ns_2$	9 Lateral	
2 $Ns_1 ns_1 Ns_2 Ns_2$	suppressor	
4 $Ns_1 ns_1 Ns_2 ns_2$		

1 $Ns_1 Ns_1 ns_2 ns_2$		
2 $Ns_1 ns_1 ns_2 ns_2$		
2 $ns_1 ns_1 Ns_2 ns_2$	7 non-	
1 $ns_1 ns_1 Ns_2 Ns_2$	suppressor	
1 $ns_1 ns_1 ns_2 ns_2$		

Luczkiewicz (15) reported a similar case of 9:7, two gene epistasis for the inheritance of branching in sunflower. Non-branching was dominant to branching.

The hypothesis of inheritance for lateral suppression in broccoli is not similar with that proposed by Yarnell (31) in cabbage. He identified a single completely dominant gene for axil sprouts. Akratanakul and Baggett (1), however, found the opposite to be true in cabbage. They suggested that the character is recessive and controlled by one or a few genes and their suggestion is supported by a skewed F_2 distribution toward low axillary heading.

Hodgkin (11) found that in crosses between Brussel sprouts and other Brassica species, the F_2 showed continuous variation for axillary heading.

Although this study is concerned with the presence or

absence of lateral shoots, a range in number of lateral shoots was noted for the normal phenotypes and suggests the presence of modifiers.

Correlations

Although a number of plants with glossy foliage were observed in the F_2 population, the correlation coefficient (r = .02) suggested no relationship between glossy foliage and lateral suppression. There was no correlation between lateral suppression and number of leaves (r = .03).

Leaf Number

The mean number of leaves for parent 83-866-1 was 21.500 and 16.128 for parent "Spartan Early". The two parental lines were significantly different (Student's t test p < .01). The two full sib F_2 populations were homogeneous (p> .50) and therefore only the pooled data are shown. The frequency distributions for all generations are presented in Table 2.

Since the F_2 distribution is skewed toward low leaf number and its mean is close to that of the low leaf number parent, there appears to be dominance toward low leaf number. The F_2 was partitioned into low and high leaf number between 18 and 19 leaves. This dividing point is the arithmetic mean between the two parents. With this class as the separation point between the two phenotypes, the 90 low leaf : 22 high leaf ratio conformed to either a 3:1, 13:3, or 12:4

Table 2. Frequency distribution of leaf number for the cross 83-866-1 x "Spartan Early"

| Generation	No. of plants	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Mean
83-866-1 (P$_1$)	4								1	-	1	-	1	-	-	-	1	21.500 ± 3.42
Spartan Early (P$_2$)	39			1	-	3	8	7	8	2	4	2	1	-	1	-	1	16.128 ± 2.87
866-1 x Spartan Early (F$_2$)	112	1	-	5	7	13	28	19	17	11	5	4	1	1				16.821 ± 2.09

segregation pattern (Figure 2). The Chi-square test shows a good fit to these models (Table 3).

TABLE 3. CHI-SQUARE TEST FOR GOODNESS OF FIT FOR THE CROSS 83-866-1 X "SPARTAN EARLY" BASED ON THE OBSERVED 90:22 SEGREGATION RATIO FOR LEAF NUMBER

Generation	Genetic model	Theoretical ratio	Chi-sq.	P
Pooled F2	3 : 1	84 : 28	1.440	.25-.10
	13 : 3	91 : 21	0.015	.95-.90
	12 : 4	84 : 28	1.440	.25-.10

In this cross the following hypothesis is proposed: One or two major genes with heterozygous modifiers determine leaf number. Studies in several Brassica species support the hypothesis of dominance for few leaf number which is controlled by a small number of genes. Summers and Honma (26) observed dominance for few non-wrapper leaves in crosses between two smooth green leafed cabbages. Chauhan and Singh (6) concluded the number of leaves in Indian mustard (B. juncea L. Czern and Coss.) was governed mainly by dominance effects and possibly overdominance. Inheritance of leaf number in spring rape (B. napus L.) appeared to be determined by one or a small number of major genes (30).

Figure 2.   Frequency curves for leaf number in the
            broccoli cross 83-866-1 x Spartan Early.

Leaf Number
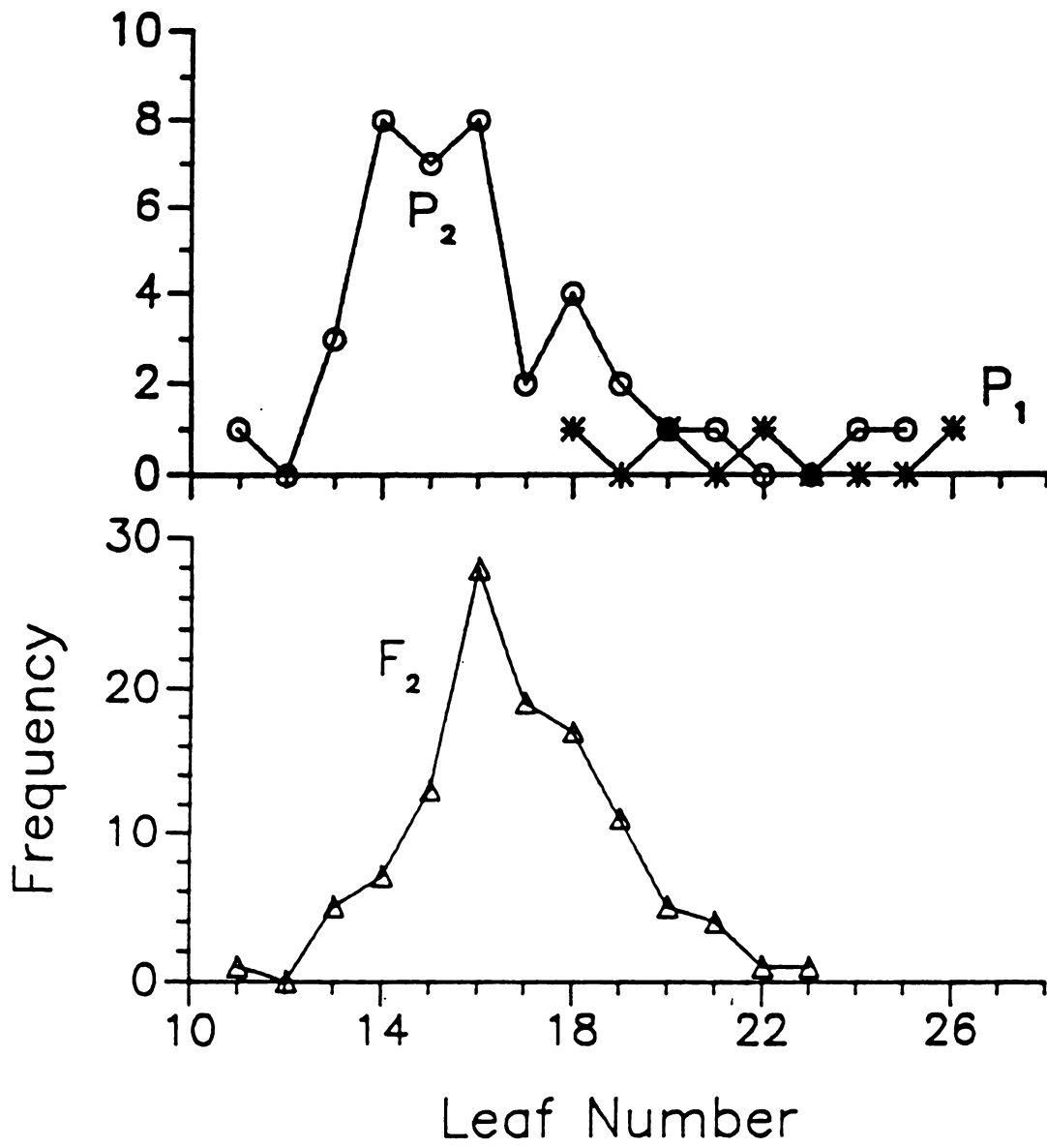
SUMMARY AND CONCLUSIONS

Shoots

Data from the pooled F_2 populations between 83-857 and "Spartan Early" were examined to determine the mode of inheritance of lateral suppression character. Dominance for lateral suppression was noted. A model of two epistatic genes was proposed where P_1 ("Spartan Early") is designated as $\underline{Ns_1}\ \underline{Ns_1}\ \underline{Ns_2}\ \underline{Ns_2}$ and parent (83-857) is designated as $\underline{ns_1}\ \underline{ns_1}\ \underline{ns_2}\ \underline{ns_2}$.

Inability to obtain sufficient backcross populations was due to the short flowering period of the lateral suppressor parent since the absence of secondary shoots did not provide flowers to make backcross pollinations. Estimates of heritability and genetic advance were not calculated since backcross data were not available.

Tucker (31) suggested that inhibition of axillary bud formation in lateral suppressor type tomato plants may be due to an accumulation of indole acetic acid which suppresses bud and shoot development. The inhibition may be controlled by the levels of abscissic acid and cytokinin since lateral suppressor tomatoes had lower levels of these hormones than normal plants.

Perhaps if the hormone levels of each plant in this experiment could have been measured, there may be evidence to support this hypothesis. It is possible that the two gene system to control lateral suppression in broccoli may be part

of a more complex biochemical mechanism.

Leaf Number

Leaf number was found to be controlled by one or two major epistatic genes and modifiers. Dominance was noted for few leaves.

Ratios obtained by partitioning the F_2 generation into the two phenotypes based on the arithmetic mean of the two parents conformed to a 3:1, 13:3, or 12:4 ratio. Estimates of heritability and expected gain from selection were not calculated due to the absence of the backcross generations.

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APPENDIX

Table A-1. Estimates of individual SCA effects for bolt
resistance

Effect	Estimate	Effect	Estimate
SCA 1 5	.00221	SCA 5 9	-.01439
SCA 7 9	.05903**	SCA 5 11	-.04690**
SCA 5 8	-.00378	SCA 9 11	.06038**
SCA 3 10	-.02171	SCA 6 9	.00392
SCA 5 6	.01807	SCA 10 11	-.07749**
SCA 3 5	.02871	SCA 1 9	-.00955
SCA 3 9	-.01877	SCA 7 10	-.07963**
SCA 5 7	.00651	SCA 4 9	-.04449*
SCA 3 11	-.01134	SCA 9 10	.01185
SCA 4 5	-.03273 *		

SCA = Specific combining ability (female, male)

**, * = Significant at the 1% and 5% level respectively.