FLOWERING AND SEED PRODUCTION OF CABBAGE FOR THE TROPICS

BY

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LIST OF ABBREVIATIONS

5-azacytidine	5-azaC
6-benzyladenine	BA
Absorbance at 645 nanometre	A_{645}
Absorbance at 663 nanometre	A_{663}
Auxin (indole acetic acid)	IAA
Cell membrane thermostability	CMT
Centimetre	cm
Chlorophyll fluorescence	CF
Coefficient of velocity of germination	CV
Conductance values for control tubes	C
Conductance values for treatment tubes	S
Controlled environment room	CER
Correlation coefficient	R
Cultivar	cv
Days after transplanting	DAT
Days	d
Day neutral plants	DNP
Days after transplanting	DAT
Days to flowering-after-sowing	DFS
Days to flowering-after-transplanting	DFAT
Degree Celsius	^{o}C
Day degree (Celsius)	°Cd
Deoxyribonucleic acid	DNA
Degrees of freedom	df
Et alii (and so on)	*et al*
Fisher probability	*P*
Food and Agriculture Organization	FAO
Flowering stalk	FS
Gibberellic acid	GA_3 or GA
Gibberellins	GAs
Glasshouse	GH
Gram	g
Gram per litre	$g\ l^{-l}$
Hour	h
Initial fluorescence	Fo
International Seed Testing Association	ISTA
Kaurenoic acid hydroxylase	KAH
Kilogram	kg
KiloPascal	kPa.
LD	Long day
Length of most recent developed leaf	LMRL
Litre	l
Long day plant	LDP
Maximum fluorescence	Fm
Micromole per metre square per second	$\mu mol.m^{-2}s^{-1}$

Millilitre	ml
Millimetre	mm
Minute	min
Milli Molar	mM
Mole per litre	mol l^{-1}
Mega Pascal	MPa
Main stalk	MS
Nitrogen	N
Nanometre	nm
Non-stressed at temperatures between 23º-26ºC	T1
Non-vernalized	Nvn
Number	#
Part per million	ppm
Percentage	%
Photo systems I	PSI
Photo systems II	PSII
Polyethylene glycol (molecular weight 6000)	PEG 6000
Regression Coefficient	R^2
Relative injury	RI
Short day	SD
Standard error of the mean	SE
Second	s
Standard error of the difference	SED
Short day plant	SDP
Species	*Sp*
Square centimetre	cm^2
Stressed at 30º-45ºC for three hours	T2
United Kingdom	UK
United States	US
Variable fluorescence	Fv
Variety	var
Vernalized	Vn
Warwick-Horticultural Research Institute	Warwick-HRI
Messenger ribonucleic acid	mRNA

Genes

APETALA 1	*AP1*
CONSTANS	*CO*
FLOWERING LOCUS C	*FLC*
FLOWERING LOCUS T,	*FT*
FLOWERING TIME GENE	*FWA*
FRIGIDA	*FRI*
GIGANTEA	*GI*
LEAFY	*LFY*
LUMINIDEPENDENS	*LD*
PHOTOPERIOD INDEPENDENT EARLY FLOWERING 1	*PIE1*
SUPPRESSOR OF EXPRESSION OF CONSTANS 1	*SOCI*
VERNALIZATION 2	*VRN2*

ABSTRACT

This thesis presents experiments to determine if cabbage flowering and seed production can be achieved in tropical conditions by selection of genotype and by chemical and physical treatments applied to seeds and to plants. Part one determines whether cabbage seed produced under tropical temperatures (20^{o}-30^{o}C) is viable and selects suitable lines and methods for further investigations. There was an indication that cabbage can flower and produce viable and vigorous seeds in the cooler part of the tropics provided that green plants are vernalized. Chlorophyll fluorescence parameters, especially variable fluorescence (Fv) and the ratio between Fv and maximum fluorescence (Fm), were found to be a good method for screening cabbage for heat tolerance. It was also found that cabbage seed can be primed and vernalized at the same time without adversely affecting the viability and vigour. The second part of the work was to determine whether there are alternatives to plant vernalization that can induce flowering and lead to less expensive cabbage seed production in the tropics. Seed vernalization and gibberellic acid (GA_3) promoted early flowering of lines 'HRI 011446' and 'HRI 006556'. For line 'HRI 006556', not only did additional supply of N reduce days to flowering of plants raised from medium with elevated sucrose (3%) but also GA_3 treatment could be substituted for *in vitro* cold treatment to promote early flowering. The combined effects of seed vernalization and GA_3 induced flowering in cabbage plants of line 'HRI 009617' developed from seeds or ratoons and potential flowering plants were easily generated from flowering stalks through vegetative propagation methods.

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CHAPTER ONE: GENERAL INTRODUCTION

1.1 BACKGROUND INFORMATION

Cabbage (*Brassica oleraceae* L. var. *capitata*.) is one of the most popular 'exotic' vegetables in tropical countries like Ghana. It is a high value crop because there is a demand from most expatriate staff, restaurants, hotels and a large section of the population, especially in the cities and urban areas. The crop is also popular because cabbages are easy to cultivate, durable in the market place, have high nutritive value (Dickson and Wallace, 1986; Norman, 1992) and there is increasing evidence that their consumption is associated with reduced incidence of some types of cancer (Chiang *et al*., 1993; Rungapamestry *et al*., 2006).

Almost all the seeds for cabbages grown in tropical countries are imported and the countries spend substantial amounts on importation of seeds of exotic vegetables. For example, in Ghana, 588,000 US dollars was spent on importation of exotic seeds in 2002 as compared with 118,000 US dollars in 1999 (Eurotrace, 2004). Seeds of cabbage are very expensive and this causes considerable constraint to its large scale production since few farmers can afford the high prices. The seeds are imported because cabbage requires low temperatures to induce flowering (Nieuwhof, 1969; Yamaguchi, 1983), a phenomenon known as vernalization, but the average annual temperature in Ghana, even at high altitudes, is about 24^{o}C. Despite these challenges, some scientists have advocated locally produced seeds in the tropics (George, 1984; Buttenschon, 1985) citing reasons such as the shortage

of foreign exchange, the dangers of importing poorly adapted lines, the fact that germination is often poor and the potential introduction of seed-borne diseases which are not prevalent in the countries.

1.2 OBJECTIVE OF THE STUDY

The objective of this study is to determine if flowering and seed production of cabbage can be achieved in the tropics by selection of genotype and by chemical and physical treatments applied to seeds and to plants.

1.3 THE CROP

Cabbage was one of the first brassicas to be domesticated. Prior to its domestication and use as food, it was used mainly for medicinal purposes for ailments such as gout, diarrhoea, deafness and headache (Rubatzky and Yamaguchi, 1997). Cabbage growth with regards to temperature exhibits a pattern common to leafy crops whereby foliage growth follows a sigmoid curve with most of the phenological development occurring in the later stages of vegetative growth. It is also known that cabbages have a broad temperature adaptation (Ghosh and Madhavi, 1998; Rubatzky and Yamaguchi, 1997) but vegetative growth is optimum between 15^{o} and 20^{o}C and that temperature greater than 25^{o}C adversely affects head density and shape. The authors further noted that, young cabbage plants with stems less than 6 mm diameter are more tolerant of low and high temperatures than mature plants and, when appropriately acclimated, most plants

tolerate frost and even freezing. Rubatzky and Yamaguchi (1997) found that non-hardened cabbage seedlings can be injured at –2°C but hardened ones are not injured at –6°C.

It has been known for some time that cabbage contains at least 11 glucosinolates and some glucosinolate-derived products such as isothiocyanate and indoles which are responsible for the anti-cancer property of the crop (Quiros, 1987; Dickson and Eckenrode, 1980). Nutritionally, cabbage is a good source of vitamin A and possesses moderate amounts of vitamin C and the B-complex vitamins. It also contains high levels of potassium and some calcium and phosphorus (Ghosh and Madhavi, 1998).

The agronomy and genetics of cabbage have been described in detail (Rubatzky and Yamaguchi, 1997; Yamaguchi, 1983; Dickson and Wallace 1986; Nieuwhof, 1969). The salient points are:

- In temperate regions, cabbage seed production normally begins with planting in the summer followed by the half-grown plants over-wintering, flowering in the spring and maturation and harvest of the seeds in summer.
- Cabbage requires an abundant supply of water and as a result typically grows best on soils with a high clay and organic matter content. The soil water level should be maintained at about 80 % field capacity. The pH should be 6.0 to 6.5 for optimum growth (George 1999; Ghosh and Madhavi, 1998).
- Organic manure and NPK fertilizer should be applied before transplanting. Nitrogen applied during the establishment of cabbage plants leads to softening of tissues and an increased susceptibility to frost. When plants show signs of

head formation, an application of about 220 kg N / ha is recommended (Tindal, 1983; Yamaguchi, 1983; George, 1999).

- Cabbage plants are raised from seed sown in beds in the field or in containers in a greenhouse. The seed requirement is 500-700 g / ha for transplanted seedlings. The seedlings are transplanted 30-45 days later in the field in rows 107 cm apart with 40 cm between plants to produce a population of 3,645 to 4,050 plants per hectare (Nieuwhof, 1969; Tindal, 1983).
- The major diseases of cabbage are downy mildew (*Perenospora parasitica*) and alternaria (*Alternaria brassicae, A. brassisicola*). Others are black rot (*Xanthomonas campestris*), club root (*Plasmodiophora brassicae*), fusarium yellows (*Fusarium oxysponum*) and blackleg (*Phoma lingam*). Control of some of these diseases is achieved by spraying 3-5 times with appropriate fungicides (Messiaen, 1992; Ghosh and Madhavi, 1998).
- Cabbage is susceptible to many pests. Cabbage white fly (*Aleyrodes proletella*), aphids (*Brevicoryne brassicae*), Cabbage looper (*Trichoplusia ni*) and caterpillars (*Plulella xylostella*) are serious insect pests that can be controlled with appropriate insecticides. Root knot nematode (*Meloidogyne sp*) is another pest of cabbage which can be controlled by rotation and soil fumigation (Messiaen, 1992).
- The diploid chromosome number, 2n, is 18. The genome is denoted cc and the cytoplasm is C (Dickson and Wallace, 1986; Tindal, 1983).

1.4 VERNALIZATION

Vernalization has been defined as the exposure of a germinating seed or young plant to a prolonged low temperature to induce flowering in the adult plant (Sheldon *et al.*, 2000a). The term was originally applied to treatments given to imbibed seeds or seedling plants but later it was extended to include cold treatments, which have similar effects when applied to plants during later stages of development (Roberts and Summerfield, 1987). Some species will not flower without vernalization. In others, vernalization advances the time of flowering (Dennis *et al.*, 1996). The duration of the low temperature period within which vernalization can occur, and the range of effective temperatures, vary between species and even between lines of the same species (Bernier *et al.*, 1981). For most species, the optimum inductive range is 1^{o} to 10^{o}C and the low temperature must be maintained for 1-3 months. Depending on the species, either the imbibed seed or growing plants can be vernalized (Dennis *et al.*, 1996). Vernalization of seed usually requires slow growth of the germinating seeds during low temperature treatment, obtained by soaking seeds and maintaining their water content above 50% of their dry weight (Chouard, 1960; Purvis, 1961). Vernalization is regarded as a natural adaptation ensuring that flowering occurs only after winter, in order for flowers and seeds to develop under favourable conditions (Lin *et al.*, 2005; Sheldon *et al.*, 2006). Thus, some temperate species use this physiological phenomenon as a means of survival from the cold in winter.

It has been shown in a number of cases that it is the stem apex which is the sensitive region for the vernalization stimulus and that the apex has to reach required maturity before the cold treatment is effective (Street and Opik, 1984; Ito *et al.,* 1966; Fernandez *et al*., 1997, Bernier, 1988). Vernalization of excised shoot tips of cabbage and carrots (*Daucas carota* var. sativa) had been successfully used to demonstrate the perceptive role of the shoot apex (Lang, 1965), however, in peas (*Pisum sativum*) and a few other species, it is reported to be perceived by leaves (Bernier and Perilleux, 2005), and Wellensiek (1964) also observed that mitotically dividing cells in any part of the plant can respond to vernalization. This was confirmed when root and leaf cuttings of *Lunaria biennis,* which were mitotically active at the time of vernalization, regenerated into flowering shoots, whereas cuttings from fully grown leaves exposed to vernalization regenerated into vegetative shoots (Wellensiek, 1964). The observation that mitotically active imbibed seeds can respond to vernalization, whereas dry seeds cannot, further indicates that cell division is necessary for vernalization (Bernier *et al.,* 1981). It has also been reported that isolated cells (originating from different locations of the mother plants) and excised buds and embryo in tissue culture can also perceive vernalization especially when external supply of sugar is available (Metzger *et al*., 1992; Dixon, 2006). The thermo-induced state is inherited through successive mitotic divisions but it is not passed through meiosis (Lang, 1965). This implies that the progeny from seed of vernalized plants require a vernalization treatment to induce flowering.

High temperature (25°-40°C) before or after the vernalization period may delay or even stop flower induction as well as flower development. These plant reactions are called anti-vernalization and devernalization respectively (Dennis *et al.*, 1996; Aditya and Fordham, 1995; Napp-Zinn, 1973). This means that the vernalizing effect of low temperature is reversible in some species by exposure to such high temperatures. Although high temperature may lead to devernalization in the early stages of chilling, the vernalized condition is usually extremely stable once established (Vince-Prue, 1975). Devernalization temperatures are usually in the range of 20°-40°C. These effects are known for many vegetable crops (Wiebe and Liebig, 1989; Lang, 1965) and have been well studied by Heide (1970) for cabbage as reported by Wiebe *et al.* (1992). Heide found that 15°C caused devernalization, whereas 12°C resulted in additional vernalization. Therefore it could be expected that, in the field, devernalization will occur when warm daytime temperatures alternate with low temperatures during the spring in temperate regions. This does not occur, probably because the fully vernalized condition reached after winter cold is stabilized (Heide, 1970). This assertion is confirmed in a recent finding (Corbesier and Coupland, 2006) that the vernalization pathway involves *VRN* genes, which ensure the stabilization of the cold condition after return to the warm temperature.

1.4.1 Requirements in brassicas

Brassicas originated in temperate regions where they are grown as biennials. They require chilling temperatures (4°-10°C) for 5-8 weeks for flower induction

(Nieuwhof, 1969; Yamaguchi, 1983). The lower the temperature within this range, the shorter the time required. When green plants are vernalized, the older and larger the plants the shorter the period of exposure to low temperature required for effective vernalization (Heide, 1970) as reported by Friend (1985). Heide (1970) found out that temperature requirements for vernalization could not be separated from those of flower initiation. Vernalization may be needed both for inflorescence initiation in cabbage and for the full development of the inflorescence (Kagawa, 1956). Some lines of cabbages have obligate vernalization requirements and will stay vegetative for a number of years when grown continuously at high temperatures. For instance, Friend (1985) reported that cabbage plants which were kept in a warm greenhouse for 2 years did not flower but produced a branched growth habit with six heads and were more than 2m tall.

Friend (1985) also observed that some brassicas have a preferential vernalization requirement and will eventually flower at high temperatures although flowering is enhanced by low temperature treatments. For vernalization to take place, brassicas require a period of exposure to low temperature, either as germinating seeds (Nakamura and Hattori, 1961) or after a period of vegetative growth (Ito and Saito, 1961). It has also been reported that a head cabbage needs to reach a certain developmental stage (7 to 9 leaves or when the stem diameter reaches 5-6 mm) before it becomes sensitive to low temperature (Ito *et al.*, 1966; Friend, 1985; Lin *et al*., 2005).

1.4.2 Physiological explanation of vernalization

There are several theories attempting to explain the mechanism of vernalization (Dennis *et al.,* 1996; Teroaka, 1992, Lee *et al.*, 1993). From work on *Arabidopsis thaliana* (hereafter called *Arabidopsis*), it has been suggested that there are two pathways for flowering. The first pathway (called vernalization-independent) is involved with the supply of carbohydrate to the apex while the other pathway (called vernalization-dependent) is concerned with gibberellic acid biosynthesis in the shoot apex (Bernier, 1988; Koornneef *et al.,* 1991; Martinez-Zapatar and Somerville, 1990). Dennis *et al*. (1996) supported this theory and also noted that non-vernalized plants are blocked in gibberellic acid synthesis in the apex and that this block is released by vernalization. They further reported that the *Arabidopsis* mutants *pgm, fca, fve, fpa, fy* and *ld* were said to undergo a vernalization independent pathway whereas *ga1-3* mutant undergoes a vernalization dependent pathway. In their hypothesised pathway of flowering (Figure 1.1), Dennis *et al.* (1996) were not certain whether irradiance, cytokinin and gibberellic acid (GA) influence the vernalization independent pathway or whether carbohydrate influenced the vernalization dependent pathway. However, they identified kaurenoic acid hydroxylase (KAH), an enzyme that catalyses an early step in GA biosynthesis, as the main enzyme responsible for flower promotion in the vernalization dependent pathway. Thus, vernalization results in expression of KAH in the shoot apex and the synthesis of bioactive GAs which promote flowering.

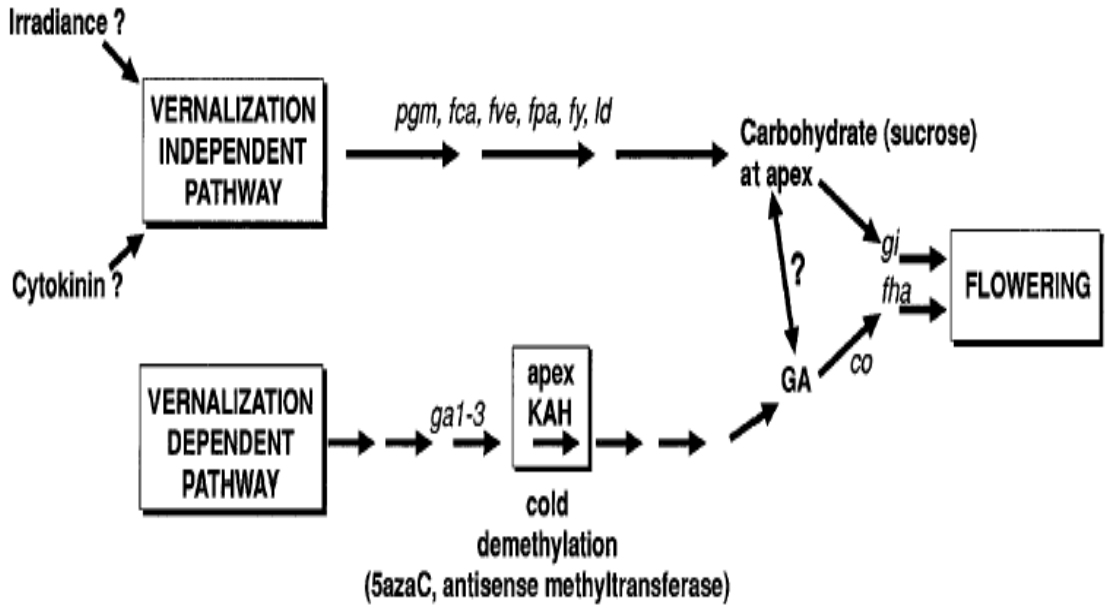


FIGURE 1.1: Pathways to flowering. Two pathways, a vernalization dependent and a vernalization independent pathway are shown. The step postulated to be blocked by methylation is indicated. The *Arabidopsis* mutants are in italics.

(Source: Dennis *et al.*, 1996).

Recently, four main flowering promotive pathways have been proposed in *Arabidopsis*: the 'photoperiodic', 'autonomous', 'vernalization' and 'GA' pathways (Corbesier and Coupland, 2006) and each normally involves different gene(s). It appears that these pathways (Figure 1.2) interact in a complex manner and that all the genes involved are connected to special floral genes, usually referred to as floral integrators (*FLOWERING LOCUS T, FT,* and *SUPPRESSOR OF EXPRESSION OF CONSTANS 1, SOCI*), the activities of which promote expression of *APETALA 1* (*AP1*) and *LEAFY* (*LFY*) genes involve in floral initiation (Corbesier and Coupland, 2005). Apart from these four main pathways,

light quality and changes in ambient temperature were also identified to strongly influence flowering time.

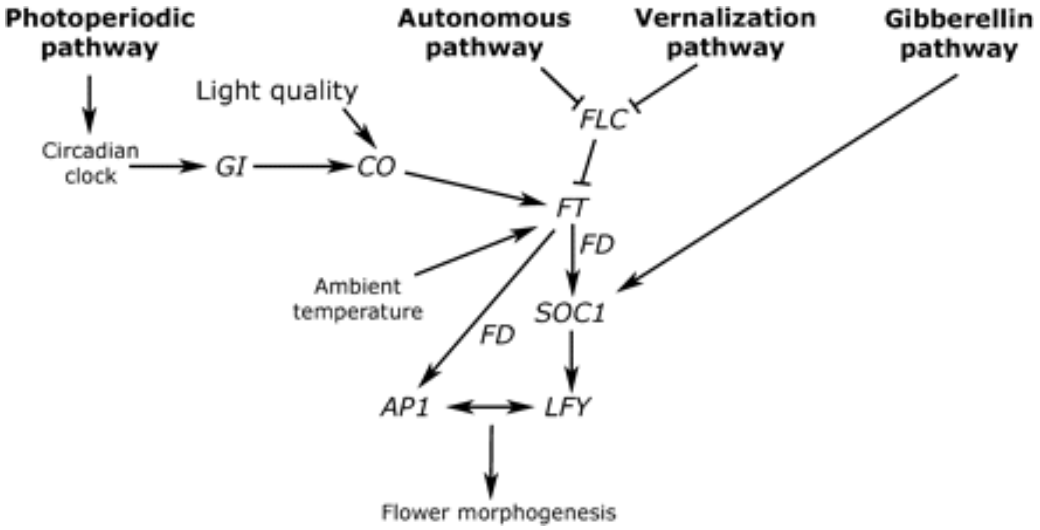


FIGURE 1.2: Four flowering pathways controlling flowering time in *Arabidopsis thaliana*. The photoperiod pathways promotes flowering under LDs. The transcription of the GI and CO genes is regulated by circadian clock, whereas light quality regulates CO protein abundance. The autonomous pathway negatively regulates the abundance of the mRNA of the floral repressor FLC. FLC mRNA abundance is also repressed by vernalization independently of the autonomous pathway. Finally, gibberellin promotes flowering of Arabidopsis, particularly under SDs. Genes are in italics and the full names are given in the list of abbreviation. (Source: Corbesier and Coupland, 2006)

Sucrose has been identified as the carbohydrate most probably involved in vernalization. Changes in the carbohydrate content of shoot tips, leaves and roots of strawberry (*Fragaria ananassa* cv. Kordestan) have been investigated as a function of treatments inducing flowering and the most abundant soluble sugar, in all the organs tested, was sucrose (Eshghi and Tafazoli, 2006). Increased levels of

soluble sugars in the apical bud of *Cheiranthus cheiri* L., a cold requiring plant, were detected in response to vernalization (Diomaiuto, 1988). Friend *et al*. (1984), in an experiment involving direct addition of sucrose to a medium in which seeds of *Brassica rapa* L. cv. Ceres were grown in sterile culture, concluded that sucrose may be an important controlling factor determining floral initiation in brassicas. Atherton *et al.* (1987) also confirmed that application of sucrose to the shoot tip of intact plants could partially replace the low temperature stimulus when sucrose solutions (50-100 mol l^{-1}) were supplied four times at intervals of five days to young leaves of cauliflower at the vicinity of the apical dome using a hypodermic syringe. Further, Roldan *et al.* (1999) reported that when the apex was in contact with sucrose, late flowering mutants of *Arabidopsis* were induced to flower early in the dark also suggesting that transport of photosynthate (sucrose) to the apex was important for flowering. Earlier, carbohydrate was known to be important in flower induction (Grainger, 1964) and a correlation between flower induction and an increased concentration of soluble carbohydrates in the apex has been found in broccoli (*Brassica oleracea* var. *botrytis*) (Fontes and Ozbun, 1972), suggesting that, under some conditions, floral initiation may be dependent on carbohydrates. Chilling during vernalization was found to suppress leaf initiation and leaf growth at the shoot apex, which consequently allowed increased availability of dry matter at the apical point (dome), thereby permitting its use for development to flower initiation. Sachs and Hackett (1969) suggested that reproductive development requires more energy than vegetative growth and postulated that flower initiation is as a result of increased assimilate supply to the apex. These results clearly

showed that carbohydrates, especially sucrose, may be a very important factor for flowering in cabbage and the effect of sucrose supply on flowering will be investigated in this study. Apart from sucrose, other substances such as nitrate, glutamine and cytokinins have recently been identified as floral signals in plants such as *Sinapis alba* (Bernier and Perilleux, 2005) and these should also be of interest to researchers.

1.4.3 Molecular studies of vernalization

Work on vernalization at the molecular level has been extensively reviewed (Corbesier and Coupland 2006; Bernier and Perilleux, 2005; Nocker, 2001; Sheldon *et al.,* 2000b). It has been reported that many genes have been identified to be involved in the vernalization requirements of different species (Boss *et al,* 2004; Kinet, 1993; Napp-Zinn, 1987). The fact that, in many species, a number of genes are involved suggests that several substances are implicated in the control of flowering. Genetic analysis of late and early flowering *Arabidopsis* ecotypes identified two major loci determining flowering time: *FRIGIDA* (*FRI*) on chromosome 4 and *FLOWERING LOCUS C (FLC*) on chromosome 5 (Sheldon *et al*., 2000a; Lee *et al*., 1993; Koorneef *et al.,* 1998). *FRI* and another gene, *PHOTOPERIOD INDEPENDENT EARLY FLOWERING 1 (PIE1)* (Noh and Amasino, 2003; Michaels and Amasino, 2000; Michaels *et al*., 2004) were subsequently identified as promoting the expression of *FLC*.

The duration of vernalization has been shown to be proportional to the degree of down-regulation of *FLC* (Sheldon *et al*., 2000a) which suggests that *FLC* is main

determining gene for vernalization. In fact, it has been identified as a major repressor of flowering in the vernalization pathway in *Arabidopsis* (Lin *et al.*, 2005; Finnegan *et al*., 2005). It has been recently explained that vernalization pathway involves the function of a special vernalization gene, *VRN*, which is necessary for the stability of *FLC* repression after cold treatment to ensure flower initiation after plants return to warm temperature (Corbesier and Coupland, 2006). *FLC* is expressed mainly in the shoot apical meristem and roots, but is absent from the inflorescence apex. It seems that the expression of *FLC mRNA* is not significantly decreased as the plant proceeds through the vegetative phase, suggesting that repression of flowering by *FLC* cannot be overcome by developmental progression (Sheldon *et al*., 1999). The cloning of *FRI* has been reported; this gene encodes a protein that does not exhibit significant sequence identity to any other protein of known function (Johnson *et al.*, 2000). In addition to *FRI* and *FLC,* about 80 loci that influence flowering time have been identified by a mutational approach (Levy and Dean, 1998). Genes such as *LD, FCA, FY, FLK, FPA, FL and VRN2* have been shown to repress *FLC* expression (Wood *et al*., 2006; Simpson, 2004; Sheldon *et al.*, 2000a, 1999).

It is not only known that cell division is a pre-requisite for vernalization to occur in plants and seeds but also that the stability of the vernalized state can be achieved through mitosis (Wellensiek, 1964). A possible reason given for this observation is the covalent modification of DNA cytosine methylation. Brock and Davidson (1994) and Burn *et al.* (1993) were among the first scientists to provide

evidence for the involvement of DNA methylation in the vernalization response. They found that the promotion of flowering by extended cold (vernalization) in wheat and *Arabidopsis*, respectively, could be partially substituted by exposure of plants to the ribonucleotide analogue 5-azacytidine (5-azaC). Treatment with this compound was reported to result in demethylation of DNA. However, flowering was reportedly promoted only in lines that are known to respond strongly to vernalization (Burn *et al.,* 1993). Thus, the ability of 5-azaC to partly replace cold treatment for flower promotion seemed to suggest that it has an effect on the vernalization pathway. It was therefore hypothesised by Finnegan (1998) that vernalization results in the selective demethylation and the transcriptional activation of floral-promotive genes. Similarly, a combined effect of vernalization and 5-azaC treatment caused a substantial reduction in methylated cytosine (i.e. reduced the methylation of DNA) in winter wheat (*Triticum aestivum* L.) cultivar, Martonvasari 15 (Horvath *et al.,* 2003). Although the 5-azaC treatment reduced the methylation of the DNA in unvernalized plants, the treatment was not sufficient to induce flowering in wheat. Genger *et al.* (2003) confirmed that demethylation of DNA decreased *FLC* expression in the vernalized responsive mutants of *Arabidopsis*, but was not associated with a promotion of flowering. In some lines, it delayed flowering. This opposing effect of demethylation was attributed to another gene, *FWA*, which was activated in response to demethylation. However, Finnegan *et al*. (2005) observed later that DNA methylation is not part of the vernalization pathway of *Arabidopsis.*

As in *Arabidopsis*, other *Brassica* species such as cabbage rely on vernalization to promote flowering (Friend, 1985). Osborn *et al*. (1997) reported that vernalization-responsive flowering time loci of *Brassica* species segregate as two major quantitative trait loci that are co-linear with the regions of *FRI* and *FLC* in the *Arabidopsis* genome. Further work has also shown that several *FLC* homologues have been identified in *Brassica* species such as *B. napus* (Tadage *et al.,* 2001) and *B. oleraceae* (Scranz *et al*., 2002). In addition, Tadage *et al*. (2001) were able to modify the flowering time in *B. napus* through genetic manipulation of *FLC*. These results revealed that *FRI* and *FLC* genes were very important in the control of flowering time through vernalization in other *Brassica* species.

1.5 OTHER FACTORS AFFECTING FLOWERING

1.5.1 Environmental

The vernalization and flowering responses may be influenced by environmental factors other than temperature. One of the most important factors is light, especially photoperiod and irradiance. Photoperiodism is the response of plants to the relative lengths of the daily light and dark periods. Photoperiod flowering responses are divided into five groups (Thomas and Vince-Prue, 1997): short day plants (SDP, flowering when length of the dark period exceeds a critical length); long day plants (LDP, flowering when length of the dark period is shorter than a critical length); day neutral plants (DNP, flower irrespective of day/night length); intermediate day plants (flower only when the day length is neither too long or

short); and amphiphotoperiodic day plants (dual-day length requiring plants, require SD and LD in a sequence). An earlier experiment by Garner and Allard (1931) with alternating light and dark periods of equal duration from 15 seconds to 12 hours indicated that, whereas LDP flowered rapidly under short light-dark cycles, SDP remained vegetative unless they received long uninterrupted dark periods. It has therefore been established that the dark period plays a central role in a plant's response to photoperiodism and that, whereas temperature during the photoperiod has little effect, the temperature during the dark period has a marked effect on the flowering response (Hamner and Bonner, 1938). However, it was shown that even SDP needed some light for flowering. Day length is usually believed to be effectively perceived by leaves but, in absence of leaves, there are indications that it can also be perceived by the stem (Bernier and Perilleux, 2005; Havelange and Bernier, 1991). In some species, however, very young leaves or excised apices grown *in vitro* are sensitive to photoperiodic treatments (Francis, 1987). These observations revealed that a direct response of the stems and shoot tips to photoperiod could occur, but do not challenge the fact that leaves are the main site of day length perception.

There is ample evidence that photoperiodism normally works with other factors to induce flowering. It has been shown, in some cases, that under long days, flowering is induced when the temperature, water availability or mineral nutrition are reduced (Kinet, 1993). There is also evidence that decreasing temperatures progressively nullify the day length requirement of absolute long day or short day

plants (Bernier *et al.,* 1981), although the reverse has been reported for *Godetia quadrivulnera* which behaves as a day neutral plant at 20^{o}-24^{o}C and as an absolute long day plant at lower temperatures (Halevy and Weiss, 1991). Within species, the photoperiod requirements may vary between cultivars as was shown in ornamental *Helianthus annuus* L., sunflower (Yanez *et al*., 2004), *Brassica rapa* L. var. *Rapifera,* turnip (Takahashi *et al*., 1994) *and Brassica rapa* L. var. *Pekinensis,* chinese cabbage (Suge, 1984). Species of *Brassica* have either long day or day neutral photoperiod responses. The long day response may be either obligate, where the plants remain vegetative when maintained under constant short days (Friend, 1985), or preferential, as in Chinese cabbage where short days delay, but do not prevent, flowering. The day neutral response is found especially in rosette plants that require vernalization, such as head cabbage (Tindal, 1983). However some cabbages were reported to be LDP after vernalization (Kagawa, 1962).

It is also known that irradiance affects flower formation especially in autogamous flowering plants. Irradiance was found to affect earliness of flowering of many herbaceous ornamental plants (Mattson and Erwin, 2005; Warner and Erwin, 2001; Zhang *et al.*, 1996). For instance, Warner and Erwin (2001) showed that *Hibiscus surattensis* L. and *H. trionum* L. flowered developmentally earlier (fewer leaves below the first flower) as irradiance increased from ambient daylight to day light plus continuous 100 µmolm^{-2}s^{-1} high pressure sodium lighting. Low light conditions delay floral transition in tomato while, in tobacco, it may prevent

flowering when the temperature is too high (Bernier *et al.*, 1981).

There is also much evidence that nitrogen nutrition plays an important role in flowering of many plants (Ma *et al*., 1997; Jeuffroy and Sebillotte, 1997; Xu *et al.*, 2001). Jeuffroy and Sebillotte (1997) found that early and prolonged nitrogen starvation prematurely stopped the progression of flowering in pea, while Xu *et al.* (2001) reported that gradually increasing the total N concentration with the progressing physiological stages from 3 to 9 mM increased the total set of flowers and fruits of sweet pepper (*Capsicum annuum* L). Similar findings have been reported earlier for some cold requiring plants. For example, Colder and Cooper (1961) reported that *Dactylis glomerata* lose their need for low temperature in the presence of high nitrogen levels. Shortage of nitrogen also delayed curd initiation and maturity in the cauliflower (Parkinson, 1952 cited by Hand, 1988). It is probable that where nitrogen deficiency retards floral initiation it is due to a reduction of the level of metabolites at the stem apex. Recent evidence revealed that reduced N- compounds are among the floral signals translocated from the leaves to stem apex to cause events that are specifically related to the induction of flowering (Bernier and Perilleux, 2005). Nitrogen nutrition in relation to flowering of cabbage will therefore be investigated in this study.

It has been found that vegetative propagation methods have promoted flowering in some crops, e.g. induction of cabbage flowers using ratoons (MnZava and Msikita,

1988). *In vitro* flowering has also been reported for some crops. Tang *et al.* (1983) were the first to report *in vitro* flowering in cassava (*Manihot esculenta* Crantz), without hormones in the culture medium. *In vitro* inflorescences have been developed through inflorescence culture in *Morus alba* (Naik and Latha, 1996**)** and *Bambusa vulgaris* (Nadguada *et al*., 1990). Other researchers have also reported *in vitro* flowering from callus-derived plantlets of *Papaya somnifera* and *Saccharum officinarum* (sugar cane) (Yoshikawa and Furuya, 1983; Virupakshi *et al*., 2002) and somatic embryogenesis and *in vitro* flowering in *Brassica nigra* (Mehta *et al*., 1993). Virupakshi *et al.* (2002*)* found that *in vitro* flowering occurred with sucrose concentration of 30 g l^{-1}. In this study, an attempt will be made to investigate the possibility of vernalizing cabbage plants *in vitro* as an alternative to the normal plant vernalization.

1.5.2 Plant growth regulators

There are several reports that endogenous hormones, particularly gibberellins, are involved in cold-induced stem elongation and flowering in plants (Chen *et al.*, 2003; Kings and Evans, 2003; Mander *et al.*, 1991; Zeevaart, 1983), however, the role of plant growth regulators in floral induction has been a controversial issue, mostly because experiments with different species have produced conflicting results (Bernier, 1988). The isolation by Went in 1928 of a plant hormone, which controlled extension growth and meristematic activity, as reported by Evans (1969), stimulated the search for a flowering hormone. Later, Chailakhyan (1936) proposed that the flowering process was under the control of a long distance factor

called florigen. Bernier (1988) further suggested that the floral transition involves a multifactorial controlling system including different growth regulators and other substances acting either simultaneously or sequentially to trigger different steps. The recent identification of some hormones and some metabolites as long distance floral signals and the fact that all were not of equal importance in all the species studied (Corbesier and Coupland, 2006; Bernier and Perilleux, 2005) supported this 'mutifactorial control hypothesis'.

The role of gibberellins (GAs) in the control of flowering has been reviewed extensively (Metzger, 1990; Kinet, 1993). It has been found that GAs stimulate flower production in tulip, *Tulipa gesneriana* var. Cassini (Hanks, 1985; Kurtar and Ayan, 2005), *Cordyline terminalis* (L) Kunth and various ornamental aroids, which are photoperiodically neutral and do not respond to the cold (Halevy, 1990). In contrast, GA inhibits flower initiation in several perennial angiosperms, particularly fruit trees and woody angiosperms (Davenport, 1990). Kinet (1993) also reported that one of the most consistent effects of GAs is hastening the floral transition in terms of time from sowing.

As for many plants, there are conflicting reports on effect of GA application on flowering of brassicas. Wittwer and Bukovac (1957) showed that GA treatments of brassicas stimulated flowering even under short days and also promoted earlier flowering in unvernalized cabbage lines (Brunswick and Sugar Loaf) at 100-200 ppm with 8 foliage sprays at intervals of one week. Kahangi and Waithaka (1981)

also showed that GA_3 promoted earlier flowering of cabbage (kale cv Collards), but failed in cv Thousand Headed. Hamano *et al.* (2002) explained that although GA hastened flower bud development and increased stem elongation, it did not participate in inducing flowering in *B. oleraceae* var. capitata, at least in some cultivars. GA_3-treated rutabaga [*B. napus* subsp. *rapifera* (Metzeg.) Sinsk] failed to flower when it was not chilled (Ali and Machado, 1982). It seems that the biologically active GA_1 is the main growth-effective GA responsible for stem elongation and the subsequent flowering of brassicas. Both vernalization at 10°C and exogenous application of GA_3 increased the levels of endogenous GA_1 in cauliflower (Guo *et al*., 2004) indicating that it is likely to be a causal factor in inflorescence stalk elongation and flowering.

Low doses of auxin are required for flower initiation to occur, but inhibition occurs at high levels (Bernier, 1988). The auxin indole acetic acid (IAA) inhibited and delayed flowering in *Pharbitis nil* and *Lens culinaris* respectively (Wijayanti *et al.,* 1997; Naeem *et al.,* 2004). *In vitro* studies also indicate that auxin included in the medium is essential, although an increased level inhibits flower formation in most experimental systems (Dickens and van Stadens, 1988). Therefore, the mode of action of auxin, either to promote or inhibit flowering, is still debatable, although the inhibitory effect of applied supraoptimal auxin concentrations has received much attention (Evans, 1969) and de Zeeuw (1955) confirmed that IAA inhibits flowering but hastens the end of the juvenile phase to low temperature vernalization in Brussels sprout.

Reports indicating that cytokinins are involved in the control of floral transition differ with species. While they are reported to promote flowering in some species (Bernier *et al*., 2002; He and Loh, 2002, Corbesier *et al.*, 2003), in others there is no evidence that they affect flowering (Bernier *et al*., 1981; Wang *et al*., 1997). This discrepancy may be due to the fact that cytokinins' effects are highly dependent on the dosage. However, in most situations, exogenous cytokinins have a promotive effect (Dennis *et al.*, 1996; Bernier *et al.*, 1990; Bernier, 1988). Sotta *et al.* (1992) again showed that cytokinin levels in the shoot apex of *Sinapis sp.* usually increased during floral evocation thus giving evidence that the hormone was involved in flowering. Chailakyan *et al.* (1988) observed that, under non-inductive long days, cytokinin present in the culture medium induced flowering whereas it inhibited flowering under short days. The cytokinin, 6-benzyladenine (BA) has been reported to promote *in vitro* flowering in *Bambusa arundinacea (Retz)* (Joshi and Rajini, 1997) and *Kniphofia leucocephala* (Baijnath) (Taylor *et al*., 2005). The latter authors also found that the effect of cytokinins was dose-dependent, with high BA inhibiting flower formation. The more recent evidence (Bernier *et al*., 2002; Bernier and Perilleux, 2005) revealed that cytokinin of the isopentenyladenine type actually promoted flowering in *Sinapis alba.*

Like cytokinins, the effect of exogenous ethylene depends on the species (Bernier, 1988; Halevy, 1990). Treatment of bulbs with ethylene stimulates flowering in some species (Botha *et al.*, 1998; Imanishi *et al*., 1992). Imanishi *et al*. (1992) found that when bulbs of tulip were stored at 20^{o}C for 0-6 weeks ethylene

increased the number that flowered but reduced the flowering period. In a range of geophytes and bromeliads, promotion usually occurs since flowering in these plants is stimulated by ethylene or ethephon application (Halevy, 1986). However, the use of ethylene to promote flowering should be handled with care because of the adverse effect it may have after flowering. The post harvest quality of many flowering plants is reduced by the ethylene treatment as it is known to cause premature wilting, colour fading and abscission of flower petals (Jones *et al*., 2001; Clark *et al*., 1997).

1.6 JUVENILITY

The interval between germination of a seed and the ability of the seedling to respond to an environmental signal inducing flowering is referred to as the juvenile phase (Friend, 1985). The phenomenon of juvenility (Wareing and Frydman, 1976; Wareing, 1987; Hackett, 1985) and the relationship of juvenility to flowering (Zimmerman, 1972) have been extensively reviewed. Most plants exhibit juvenility, in the sense that following germination there is normally a vegetative phase during which flower initiation does not occur. This phenomenon is seen in both day-neutral species and in species in which flower initiation is regulated by day length (Wareing, 1987).

Like most plants with long day and chilling requirements for flowering, brassicas grow as rosettes prior to receiving the flowering stimulus (Dahanayake and Galwey, 1998). In brassicas, as with other species, the transition to maturity may be marked by various morphological criteria, such as production of minimum leaf number or

minimal stem diameter (Friend, 1985). Visser (1964) also demonstrated the importance of size in attaining the mature condition. Using 8 to 12 year-old apple and pear plants, he found an inverse correlation between plant size (stem diameter) and length of juvenile period. In herbaceous species, the number of nodes to first flower is often used as the measure of the length of the juvenile phase. Purvis (1934) first postulated this concept of a minimal leaf number. The requirements for a period of early vegetative growth before becoming responsive to low temperature was demonstrated in cabbage as early as 1918 by Gassner, as reported by Friend (1985). It is therefore well established that vernalization is most effective when imposed on plants after a juvenile stage and that cabbage cultivars differ in their sensitivity to chilling and subsequent flowering (Nieuwhof, 1969; Yamaguchi, 1983). Young plants with relatively few developed leaves and less than 5 or 6 mm stem diameter are considered to be at the juvenile growth stage and do not vernalize, however, plants developed beyond the juvenile stage are sensitive to vernalization. Franck (1976) suggested that transition from juvenile to mature morphological characters in several plants is correlated with an increase in the size of the shoot apical meristem. It is also known that the rate of apical development is controlled by the supply of nutrients. Allsop (1954) reported that reduction in the supply of nutrients to a certain level leads to a reversion of apical development with a gradual return to increasing juvenile condition and decrease in apex size, and this phenomenon has also been reported for cabbage (Gauss and Taylor, 1969). This suggests that nutrition plays an important role in the transition from juvenile stage of cabbage to enable it to respond to environmental signals inducing flowering.

1.7 MEASUREMENT OF HEAT TOLERANCE

The cultivation of temperate crops in the tropics is often hampered by heat stress (McWilliams, 1980). It is therefore necessary to screen for heat tolerance in temperate crops intended for cultivation in the tropics and in this study, attempts will be made to screen cabbage lines meant for the tropics. The rate of temperature change and the duration of high temperature all contribute to the intensity of heat stress. It is difficult to define adequately heat stress in plants because response to heat stress depends on thermal adaptation, duration of exposure and the stage of growth of the exposed tissue (McWilliams, 1980). In spite of these difficulties, several methods have been developed for measuring the heat tolerance of crop plants (Martineau *et al.,* 1979; Onwueme, 1979; Sullivan and Ross, 1979) as reported by Chen *et al*. (1982). Heat tolerant and susceptible genotypes have been identified, based on fruit set and yield under high temperature field conditions, in tomato (*Lycopersicon esculentum*) (Ahmadi and Stevens, 1979; Stevens and Rudich, 1978), beans (*Phaseolus vulgaris*) (Halterlein *et al.,* 1980), soyabean (*Glycine max*) (Martineau *et al.,* 1979) and potato (*Solanum tuberosum)* (Mendoza and Estrada, 1979). Conductivity and 2, 3, 5-triphenyl tetrazolium reduction tests have been successfully used in measuring plant heat tolerance (Chen *et al*., 1982; Levitt, 1980; Onwueme, 1979). Metabolic activities such as photosynthesis and respiration are more sensitive to heat stress in cool season species than warm season plants (Bjorkman *et al.,* 1980). Such important metabolic functions appears sensitive to heat stress in temperate species but a cell membrane system that remains functional during heat stress appears

central to adaptation of plants to high temperature (Raison *et al.*, 1980). Despite the fact that the physiological basis for the stability of membranes under heat stress is still not known, the loss of membrane integrity under high temperature may be attributed to ion leakage from plasma membranes, as a consequence of inhibition of membrane-bound enzymes responsible for maintaining chemical gradients (Reynolds *et al.*, 2001). Sullivan and Ross (1979) were able to develop a test that measures the amount of electrolyte leakage from leaf discs bathed in deionised water after exposure to a heat treatment. They interpreted the measurement as an indicator of cell membrane thermostability (CMT) in response to heat stress and have used the procedure to identify genetic variation in heat tolerance in grain sorghum (*Sorghum bicolour* [L.] Moench). The CMT test is based on the fact that the injury inflicted on leaf tissue under high temperatures weakens the cell membrane which leads to leakage of electrolytes out of the cell. If the leaf tissue is washed in de-ionized water, the amount of leakage can be determined with an electrical conductivity meter (Martineau *et al*., 1979) and can be compared among lines. They further reported that the results obtained with this technique correlate with observations on strains known to be tolerant from field experience, with observation of the heat stability of photosynthetic activity in isolated chloroplasts and with the test in which whole plants were exposed to elevated temperatures (Sullivan, 1972). Martineau *et al.* (1979) and Wallner *et al.* (1982) used the CMT to characterise soyabean (*Glysine max* [L.] Merr) and turf grass species respectively for heat tolerance.

Another method that has potential to be rapid and effective for screening existing germplasm for heat stress tolerance is the measurement of chlorophyll fluorescence (Sipos and Prange, 1986). The photosynthetic apparatus is known to be highly susceptible to heat (Alexandrov, 1964; Berry and Bjorkman, 1980; Smillie, 1979) and is damaged before visible symptoms of high temperature injury are manifested (Bjorkman, 1975). Santarius (1974) and Krause and Weis (1984) stated that one of the first components of photosynthetic apparatus to be damaged by heat is the thylakoid membrane leading to a change in chlorophyll fluorescence. The ability of the thylakoid membrane, which contains the carriers for the electron transport, photosystems I (PSI) and 2 (PSII), to resist heat damage varies amongst plant species (Smillie and Hetherington, 1983; Smillie and Nott, 1979) and even among lines of the same species (Hetherington *et al.*, 1983). By measuring chlorophyll fluorescence, which is the re-emitted energy absorbed by photosystem II (PS II) but not transferred to PS I with subsequent carbon dioxide reduction, the efficiency of electron transfer in the photosystems can be determined (Sipos and Prange, 1986). In other words, the amount of chlorophyll fluorescence indicates thylakoid membrane integrity and the relative efficiency of electron transport from PS II to PS I (Krause and Weis, 1984; Smillie, 1979). Thermal damage in the photosystems always leads to a marked increase in initial fluorescence (Fo), decrease in variable fluorescence (Fv) and the ratio of the Fv to maximum fluorescence (Fm) (Maxwell and Johnson, 2000; Hansatech, 1996; Havaux, 1995; Sipos and Prange, 1986). As well as being a possible reliable indicator of heat damage, chlorophyll fluorescence measurements can be done rapidly *in vivo* with

various commercial instruments, requiring only minutes to obtain a reading (Sipos and Prange, 1986).

Some types of cabbage, e.g. Chinese cabbage, cannot form compact heads under conditions of high temperature and high humidity in tropical zones (Ping, 1986). Therefore, solidity of cabbage head [mean weight/(0.523 x mean head width x mean head length)] was used as an index for heat tolerance (Ping, 1986). It will therefore be of interest, in this study, to compare some of the heat tolerant screening methods and recommend the best for future cabbage screening.

1.8 PRIMING OF SEEDS AND SEED GERMINATION

Priming may help seed production in the tropics by ensuring that seed plants grow uniformly and vigorously (Harris *et al.*, 2001). If emergence can be suppressed during priming, it may also enable vernalization to occur in seeds (seed vernalized types) for a long time without germination and the associated growth of fungi. Osmotic priming consists of the incubation of seeds for a specific period of time at a specific temperature in an osmoticum of -1.0 to -1.5 MPa, usually salt or polyethylene glycol (PEG, molecular weight 6000) dissolved in water (Karssen *et al.,* 1989). Priming is usually followed by re-drying of seeds to allow storage and handling. It involves exposing seeds to an external water potential low enough to restrict germination and yet permit pre-germinative physiological and biochemical activities (Bradford, 1986). Commonly used osmotic priming agents include

inorganic salts such as $CaCl_2$, K_2HPO_4, KNO_3 or Na_2SO_4 and organic agents such as PEG, mannitol and sorbitol (Pill *et al.*, 1991). Hydro (water) priming also involves soaking in water and drying back to storage moisture prior to sowing of the seeds.

There have been several reports of the benefit of priming to seed quality after sowing. Water priming of barley (*Hordeum vulgari* L.) for about 12 hours, and subsequent seed storage of up to 9 weeks, increased germination from 65% to 95% (Ajouri *et al*., 2005). Seed priming has increased the uniformity and early emergence of many crops, especially under conditions of environmental stress (Bradford, 1986; Khan *et al.*, 1978). It has successfully increased the germination percentage and rate for lettuce and celery seeds sown at high temperature (Guedes and Cantliffe, 1980; Brocklehurst and Dearman, 1983). For cabbage, Khan *et al.* (1978) showed that primed seeds kept at 15^{o}C gave accelerated emergence and increased plant fresh weight.

There are three stages in germination, namely (i) the imbibition stage (which involves rapid water uptake and softening of the seed coat), (ii) the lag stage (where the water potential of the seed is in balance with the environment and major metabolic activities prepare the seed for radicle elongation) and (iii) seedling growth stage (where cell elongation and division lead to radicle emergence) (Karssen *et al.*, 1989). Osmotic priming is reported to allow the first and second stages to proceed but prevent the last stage (radicle emergence)

(Karssen *et al.,* 1989). Several other reasons have been proposed to explain the observed stimulation in early and total germination. When seeds imbibe, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986). The author further noted that priming up to this point can have a positive effect, while extended priming duration will negatively affect germination. In addition, priming is reported to induce a range of biochemical changes in the seed that are required to start the germination process (breaking of dormancy, hydrolysis, mobilization of inhibitors, imbibition and enzyme activation) (Ajouri *et al.*, 2005) which is prerequisite for quick expansion of radicle cells. Hydro priming is also reported to cause the hydrolysis of abscisic acid (ABA) and the leaching of cytokines, coumarin and phenolic compounds (some are known to be germination inhibitors) from the seed to the aqueous solution (Hopkins, 1995). It will therefore be of interest to find out in this study, whether the benefits of priming (higher seed germination and vigour) could still be achieved when cabbage seeds are primed and vernalized at the same time for a longer duration of 8 weeks to pave the way for seed production of seed vernalized brassicas in the tropics. Recent studies showed that inclusion of certain growth regulators in priming solution even further improved germination of seeds and this has been confirmed in *Poa pratensis* L. (Tiryaki *et al.*, 2006), *Amaranthus cruentus* L. (Tiryaki *et al.*, 2005) and *Citrullis lanatus* (Thunb.) Mansf. (Korkmaz *et al.*, 2004). This new finding is likely to promote germination in some recalcitrant seeds.

1.9 SUMMARY OF THE THESIS STRUCTURE

The thesis is divided into two parts. The overall aim of experiments in the first part (Chapters 2-4) was to ascertain whether cabbage seed produced under high temperature is viable and to select suitable lines and methods for further investigation. Chapter 2 reports the evaluation of some morphological and flowering characteristics of ten cabbage lines for selecting suitable lines for the study of flowering and seed production in the tropics and ascertaining the viability and vigour of cabbage seed produced under high temperatures (20^{o}-30^{o}C). In Chapter 3, screening of some cabbage lines for heat tolerance using cell membrane thermostability and fluorescence tests is reported, as a complement of Chapter 2, for selecting heat tolerant lines for the study. Chapter 4 reports the effect of an osmotic priming pre-treatment (polyethylene glycol, PEG 6000) on the viability and vigour of cabbage seed at non-vernalization and vernalization temperatures. A method of applying a seed vernalization treatment that does not adversely affect the viability and vigour of the seed but arrests growth during seed vernalization was needed for future work.

The aim of the second part (chapters 5-6) was to determine whether there are alternatives for plant vernalization that can lead to less expensive cabbage seed production in the tropics. Chapter 5 examines whether cold treatment of imbibed seeds or *in vitro* plants and the application of gibberellic acid (GA_3) later to juvenile plants can induce flowering in cabbage raised from seeds and ratoons. Chapter 6 examines whether increasing the sucrose concentration to seedlings *in*

*vitr*o and subsequently increasing nitrogen supply to plants in soil can affect head parameters or greenness which may lead to flower induction in cabbage at relatively high temperature.

Finally, the appendices contain full published papers from this study. The Chapters that contain work included in these publications are Chapter 3 (Nyarko *et al*., 2006a, Appendix 1), Chapter 4 (Nyarko *et al*, 2006b, Appendix 2) and Chapter 5 (Nyarko *et al*., 2007, Appendix 3). The references and acknowledgements for all Chapters have been collated into the References and Acknowledgements sections.

PART ONE: SCREENING AND SELECTION OF LINES AND METHODS FOR FUTURE WORK AND THE FEASIBILITY OF PRODUCING CABBAGE SEED IN THE TROPICS

CHAPTER TWO: TOWARDS CABBAGE (*Brassica oleraceae* var. *Capitata* L) SEED PRODUCTION IN THE TROPICS.

2.1 INTRODUCTION

Studies into cabbage seed production in the tropics have not received the needed attention despite the economic benefits it may give to these countries as already indicated in Chapter 1. It is believed that, even if plants can be vernalized, flowering and seed development will be poor due to high temperatures (Wang *et al.*, 2000). The need to study the possibility of local seed production of some of the popular 'exotic' vegetables like cabbage in the tropics has therefore become imperative. The evaluation of some morphological and floral characteristics of ten cabbage lines is reported here with the aim of selecting suitable lines for the study of flowering and seed development in the tropics and to ascertain the viability and vigour of cabbage seed produced under high temperatures (20^{o}-30^{o}C).

2.2 MATERIALS AND METHODS

Seeds of ten cabbage lines with accession numbers 'HRI 011446, 'HRI 013011', 'HRI 002605', 'HRI 003202', 'HRI 003720', 'HRI 005237', 'HRI 006556,' HRI 009617', 'HRI 009837' and 'HRI 007827'(Table 2.1) were obtained from Warwick–Horticultural Research Institute, Wellesbourne, Warwickshire, UK.

Table 2.1: List of names and countries of collection of ten cabbage lines obtained from Warwick–Horticultural Research Institute, Wellesbourne, Warwickshire, UK.

Access. number	Species	Subtaxa	Accession Name	Common name	Country of collection
HRI 002605	oleracea	capitata	Extra Early Roundhead	Cabbage	Britain
HRI 003202	oleracea	capitata	Express	Early summer cabbage	Italy
HRI 003720	oleracea	capitata	Golden Acre Exra Early	White summer cabbage	Britain
HRI 005237	oleracea	capitata	Copenhagen	Summer cabbage	Italy
HRI 006556	oleracea	capitata	-	Heading cabbage	Egypt
HRI 007827	oleracea	capitata	Suttons Express	Cabbage	India
HRI 009617	oleracea	capitata	Cape Spitzkool	Cabbage	Zimbabwe
HRI 009837	oleracea	capitata	Sakata's Succession No 1	Cabbage	Japan
HRI 011446	oleracea	tronchuda	Covo Tronchuda	Cabbage	Zimbabwe
HRI 013011	oleracea	capitata	Copenhague 4	Cabbage	France

Accession Name = Line name

The seeds were sown at a depth of 2 mm in each 4 cm-square cell of a plastic modular tray filled with 46 g Levington F2S compost (Scotts Professional, Bramford, Ipswich, UK). When seedlings were 24 days old, they were transplanted into plastic pots of 23 cm top diameter containing 3 kg Levington M2 soil mix. The pH of the compost was 5.5. Five plants of each of the ten lines were kept in a controlled environment room (CER) held at 12 h photoperiod and 400 $\mu mol.m^{-2}s^{-1}$ photosynthetic photon flux and temperature of 20°C. Another group of five plants of each line were kept in a glasshouse receiving natural light with a blackout system to give a 12-h photoperiod and maintained between 20° to 30°C. The pots were arranged in a randomised complete block design in both controlled environments. Eight weeks after sowing, two plants of each line from both the CER and the glasshouse were transferred to another CER for vernalization at 4°C for 8 weeks, after which they were returned to their respective controlled environments. Parameters measured included the number of leaves, height of stem, canopy spread, length of the most recent fully expanded leaf at 40 days after sowing, days from sowing to flowering, number of nodes and leaves, stem girth (2 cm from the soil level) and height at flowering. Greenness was also measured fortnightly from two weeks after transplanting for 8 weeks using a SPAD meter (Minolta Camera Company, Osaka, Japan) on two most recently fully expanded leaves of each of the plants. Five readings were taken from each leaf and the average SPAD reading of all leaves was computed for each plant.

Due to the absence of pollination agents in the controlled environments, five of the lines that flowered were randomly selected, hand pollinated (selfed) and seeds

collected. Germination (%) and coefficient of velocity (CV) as described below were subsequently determined to assess the viability and vigour of seed produced under temperatures (20°-30°C) that were higher than are normal for cabbage seed production. Fifty seeds of each of the five lines were sown into 4 cm-square plastic modular trays filled with Levington F2S compost at a depth of 0.5 cm and kept at 20°C. The emergence of seedlings was recorded daily till day-10 after which the normal seedlings were recorded and the germination percentage determined as defined by International Seed Testing Association (ISTA, 1993)

Germination (%) = (Number of normal seedlings x 100)/ Total number of seed used for the test

The normal seedlings had well developed roots and shoots and excluded damaged seedlings (seedlings with any of the essential structures missing or badly and irreparably damaged), deformed seedlings (seedlings with weak development or in which essential structures are out of proportion), decayed seedlings (seedlings with any of their essential structures so diseased as a result of primary infection), and ungerminated seeds (which include hard seeds, fresh seeds and dead seeds) (ISTA, 1993)

The CV, a measure of vigour, was calculated as follows:

$$CV = \frac{\sum_i N_i}{\sum_i N_i D_i} \times 100$$

where Ni = number emerging in day i.

Di = days from sowing.

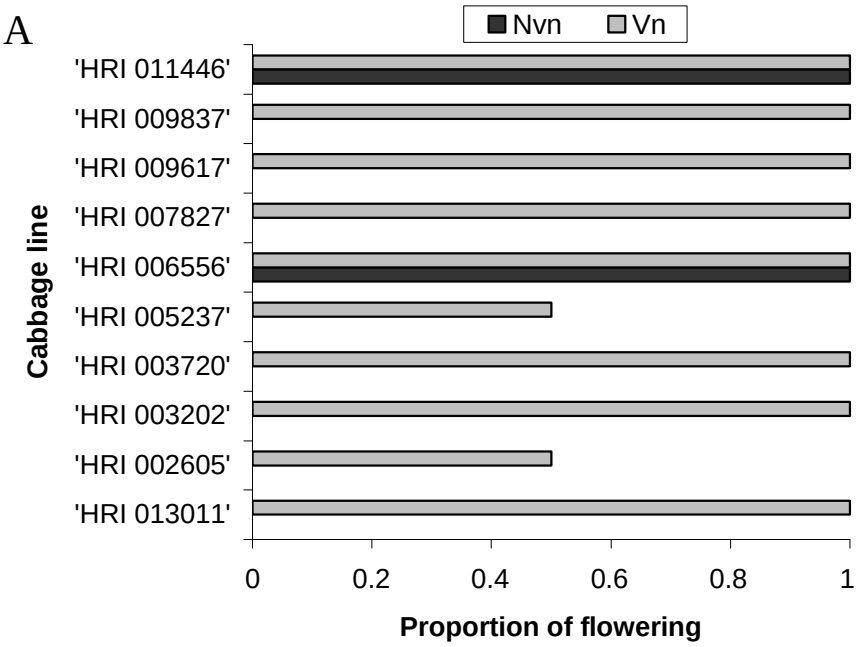
The CV gives an indication of the rapidity and uniformity of seedling growth. Higher CV means higher vigour (Scott *et al*., 1984; Kittock and Law, 1968).

Analysis of variance was used to determine the variation among cultivars for all parameters measured except proportions of flowering, head splitting, tip burn and sign of heading where logistic analysis (Binomial) was adopted using Genstat-release 7.2 statistical package. The CV was square root transformed to normalize the error distribution before the analysis was performed.

2.3 RESULTS

Two of the ten lines of cabbage (‘HRI 011446’ and ‘HRI 006556’) used for the study were able to flower without vernalization in the glasshouse (Figure 2.1A) while in the controlled environment room (CER) only one, HRI 006556 flowered without vernalization (Figure 2.1B).

A



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B

FIGURE 2.1: Flowering responses of ten lines of cabbage after plant vernalization (8 weeks). A, grown at 20º-30ºC (glasshouse). B, grown at 20ºC (controlled environment room, CER). (Vn = vernalized plants; Nvn = Non-vernalized plants)

One of the lines that flowered in the glasshouse without vernalization ('HRI 011446') did not form a head while the other ('HRI 006556') formed a loose head. There was an indication that all the lines of cabbage have a tendency to flower when vernalized at 4ºC and grown at the tropical temperatures (20º-30ºC), however, only four lines ('HRI 011446', 'HRI 006556', 'HRI 003202' and 'HRI 013011') had all the vernalized plants flowering both in the glasshouse and in the CER. Generally, more plants flowered in the glasshouse where the temperature varied from 20º-30ºC as compared to the CER where the conditions were relatively constant (20ºC).

The mode of flower stalk emergence after plant vernalization placed the cabbage lines into three categories (Figure 2.2): those that were able to flower without head formation (‘HRI 011446’ and ‘HRI 006556’), those that formed a loose or small head and unfolded later to allow flower stalks to elongate (‘HRI 009617’, ‘HRI 007827’, ‘HRI 003202’, ‘HRI 003720’ and ‘HRI 002605’) and those with dense heads where flower stalk elongation was only possible after the heads were cut (‘HRI 009837’, ‘HRI 013011’ and ‘HRI 005237’).

The lines ‘HRI 011446’ and ‘HRI 006556’ flowered significantly earlier than the others (Figure 2.3). Apart from those two lines (‘HRI 011446’ and ‘HRI 006556’), ‘HRI 009617’ flowered significantly earlier than the rest. Lines ‘HRI 003720’ and ‘HRI 013011’ were very late in flowering after plant vernalization.

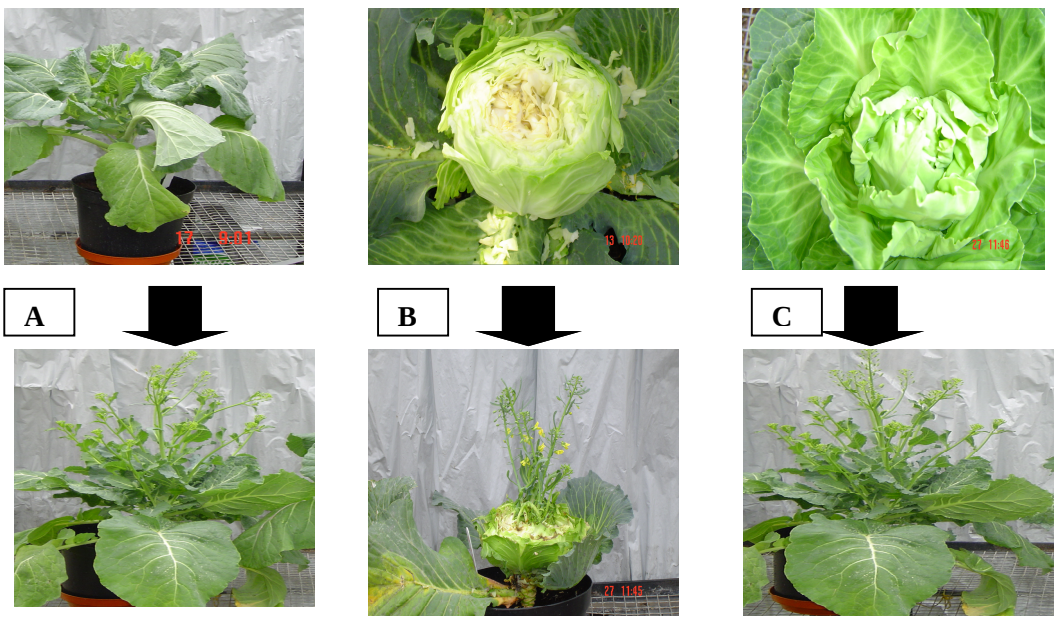

FIGURE 2.2: Modes of flower stalk emergence. Panel A, plants flowered without head formation. Panel B, plants formed dense heads and flower stalk elongation was only possible after the heads were cut. Panel C, plants formed a loose or small head and unfolded later to allow flower stalks to elongate.

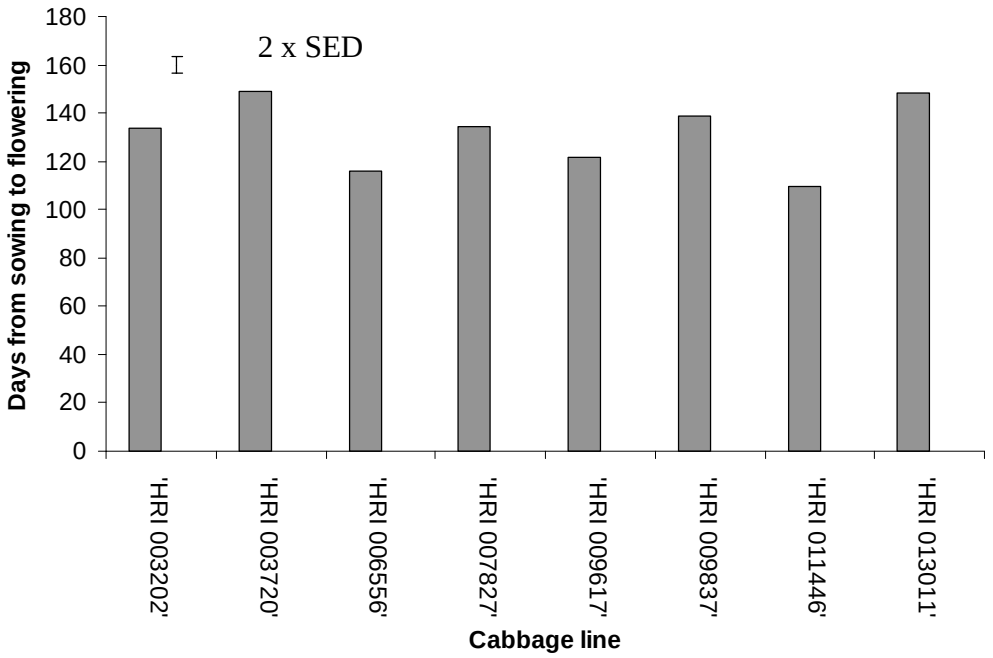

FIGURE 2.3: Days from sowing to flowering of eight lines of cabbage which had been vernalized and raised in the glasshouse (20^{o}-30^{o}C).

Lines ‘HRI 011446’ and ‘HRI 006556’ did not differ from each other in their height, girth, number of nodes and leaves at the time of flowering (Table 2.1).

Also, there were no significant differences between vernalized and non-vernalized plants in terms of these four morphological characteristics, however, non-vernalized plants appeared to have more leaves (P = 0.069), more nodes and were generally taller than the vernalized plants.

TABLE 2.2: Some morphological characteristics of vernalized (Vn) and non-vernalized (Nvn) cabbage lines ('HRI 011446' and 'HRI 006556') at the flowering stage.

Line	Mean height (cm)		Mean girth (cm)		Mean # of nodes		Mean # of leaves	
	Nvn	Vn	Nvn	Vn	Nvn	Vn	Nvn	Vn
'HRI 006556'	27.7	27.5	8.8	8.0	56.3	28.5	32.7	24.0
'HRI 011446'	63.3	33.0	8.0	8.0	36.3	20.5	25.7	13.5
F prob.	> 0.05		> 0.05		> 0.05		> 0.05	
SED	20.87		0.833		20.01		7.51	
df	6		6		6		6	

= number Nvn = non-vernalized Vn = vernalized

The ten lines varied significantly in terms of the number of leaves, height of the stem and the length of the most recently developed leaf (Table 2.2). Lines 'HRI 011446' and 'HRI 006556' were significantly taller than the rest. However, the lines did not differ (P > 0.05) in canopy shape index.

TABLE 2.3: Some horticultural characteristics of ten cabbage lines (non-vernalized plants) at 40 days after transplanting.

Line	Number of leaves		[1]Canopy shape index		Height of stem (cm)		[2]LMRL (cm)	
	GH	CER	GH	CER	GH	CER	GH	CER
'HRI 002605'	12.2	11.6	1.34	1.11	3.70	3.80	18.8	13.4
'HRI 003202'	12.2	12.6	1.07	1.08	3.00	3.80	17.2	14.3
'HRI 003720'	13.4	14.6	1.09	1.13	3.60	4.60	15.0	11.5
'HRI 005237'	13.0	12.4	1.18	1.04	4.40	4.70	16.8	14.5
'HRI 006556'	13.0	11.4	1.08	1.02	6.90	9.20	15.8	14.0
'HRI 007827'	14.2	12.2	1.10	1.41	5.50	4.30	17.2	11.5
'HRI 009617'	10.8	9.80	1.12	1.12	4.00	4.40	20.0	13.5
'HRI 009837'	14.0	12.2	1.15	1.12	4.00	6.30	18.2	14.5
'HRI 011446'	12.2	11.2	1.09	1.14	13.80	19.30	16.6	16.6
'HRI 013011'	14.4	14.0	1.11	1.04	4.40	4.30	19.4	15.7

F probability	<0.05	<0.01	>0.05	>0.05	<0.01	<0.01	<0.05	<0.01
Replication	5	5	5	5	5	5	5	5
df	36	36	36	36	36	36	36	36
SED	0.931	0.994	0.096	0.123	1.215	1.113	1.884	1.241

[1]Canopy shape index = canopy length/canopy breadth

GH = glasshouse CER = controlled environment room

[2]LMRL = Length of most recent developed leaf

df = degrees of freedom

SED = Standard error of the difference

There were significant differences between some of the lines of cabbage ($F_{(9, 36)} = 9.60$, $P < 0.001$) in terms of SPAD meter value (greenness) over all dates (Figure 2.4). Generally, there was an increase in greenness for the first 42 days after which there was a decline, with the exception of 'HRI 005237' and 'HRI 007827' that increased at 56 days after transplanting. Two lines ('HRI 011446' and 'HRI 006556') showed a consistent high level of greenness throughout the 56 days, while two other lines ('HRI 002605' and 'HRI 003720') were consistently less green as compared to the others.

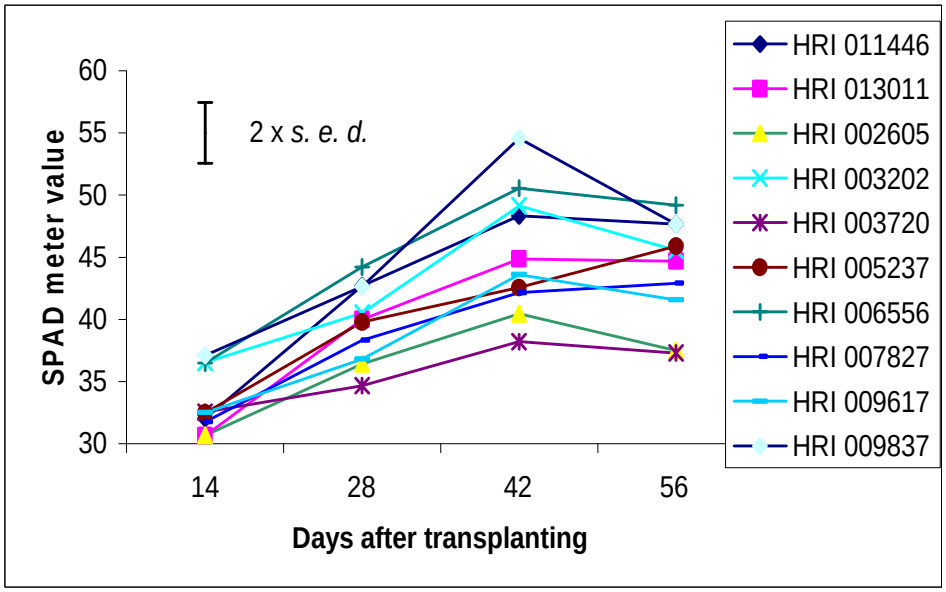

FIGURE 2.4: The relative SPAD meter value (greenness) of plants with time after transplanting.

The mean proportion of plants showing splitting, tip burn and sign of heading at 70, 60 and 40 days after transplanting (DAT) respectively showed significant differences between some of the lines (Table 2.3). The two lines, 'HRI 002605' and 'HRI 003720' were most susceptible to head splitting, especially the vernalized plants. Almost all the plants that had head splitting also had tip burn of the leaves. Plants kept in the CER showed less incidence of tip burn as compared to plants in the glasshouse and also the tall lines ('HRI 011446' and 'HRI 006556') had less incidence of the tip burn disorder.

Lines 'HRI 003720', 'HRI 005237' and 'HRI 013011' showed indications of head

formation at 40 d after transplanting, whereas lines ‘HRI 011446’, ‘HRI 009617’ and ‘HRI 006556’ did not show any sign of heading at the same period of time.

TABLE 2.4: Mean proportion plants showing splits (70 DAT), tip burn (60 DAT) and sign of heading (40 DAT) of ten lines of cabbage (DAT = days after transplanting)

Key: 0 = no splitting, tip burn or sign of heading 1 = all showing splitting, tip burn or sign of heading

Line	Splitting				Tip burn				Sign of heading			
	CER (20ºC)		Glasshouse (20º-25ºC)		CER (20ºC)		Glasshouse (20º-25ºC)		CER (20ºC)		Glasshouse (20º-25ºC)	
	Nvn	Vn	Nvn	Vn	Nvn	Vn	Nvn	Vn	Nvn	Vn	Nvn	Vn
'HRI 002605'	0.7	0.5	0.3	0.5	1.0	0.0	1.0	1.0	0.7	0.0	0.1	1.0
'HRI 003202'	0.0	0.0	0.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	0.7	0.5
'HRI 003720'	1.0	1.0	0.3	1.0	0.3	0.5	1.0	1.0	0.3	1.0	1.0	1.0
'HRI 005237'	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.5	1.0	0.5	1.0	1.0
'HRI 006556'	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	0.0	0.0	0.0
'HRI 007827'	0.0	0.5	0.0	0.0	0.0	0.0	1.0	0.0	0.7	0.0	1.0	1.0
'HRI 009617'	0.0	0.0	0.0	0.0	0.3	0.0	1.0	0.0	0.0	0.0	0.0	0.0
'HRI 009837'	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.3	0.0	0.7	0.5
'HRI 011446'	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
'HRI 013011'	0.0	0.0	0.0	0.0	0.3	0.0	1.0	0.0	0.7	1.0	1.0	0.0
Probability	*P* = 0.001		*P* = 0.001		*P* = 0.032		*P* < 0.01		*P* = 0.001		*P* < 0.001	
No of plants	3	2	3	2	3	2	3	2	3	2	3	2

CER = Controlled environment room

The germination (%) and the CV of seeds obtained from cabbage plants raised in the glasshouse (20°-30°C) were compared with the original seeds obtained from Warwick-HRI (Figure 2.5). With the exception of 'HRI 007827' where seeds produced at 20°-30°C gave an average germination percentage of 38, the rest gave germination percentages above 80. Again, the germination (%) of the seeds produced was not significantly different from that of the original seeds obtained from Warwick-HRI for all lines tested except for 'HRI 007827'.

The CV indicated that seeds produced in the glasshouse (20°-30°C) were slightly more vigorous than the original seeds, obtained from the Research Station, except for 'HRI 007827' where the original seeds performed better.

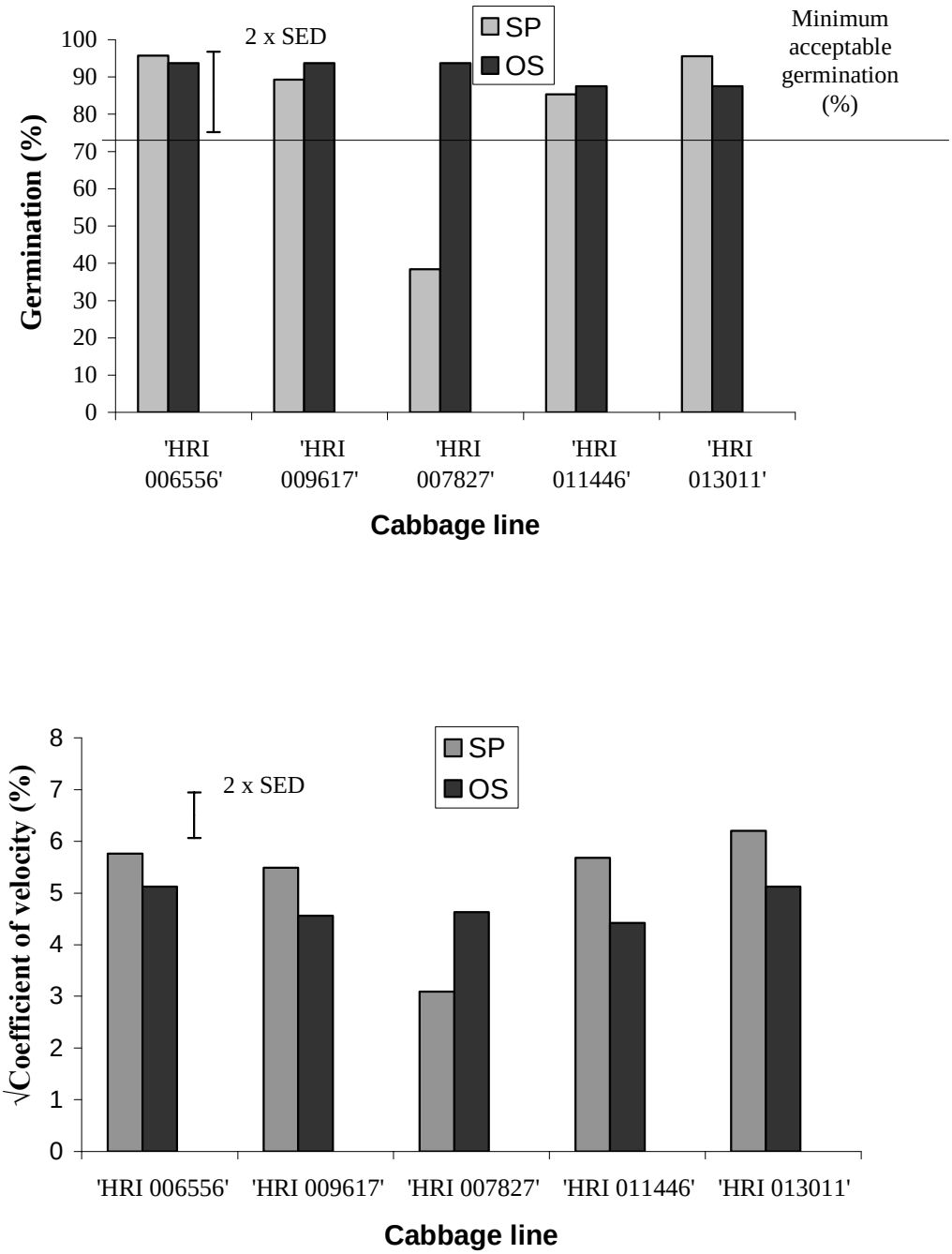

FIGURE 2.5: Germination percentages and √Coefficient of velocity (CV) of five lines of cabbage produced in the glasshouse (20º-30ºC). Number of seeds in each of the four replicates was 50. SP = seeds produced at 20º-30ºC; OS = original seeds obtained from Warwick-HRI.

2.4 DISCUSSION

The two lines 'HRI 011446' and 'HRI 006556' could flower without vernalization in the glasshouse probably because they undergo a vernalization-independent pathway (Dennis *et al.*, 1996). The non-vernalized plants of 'HRI 006556' were not able to flower in the CER (Figure 2.1b) and this may be due to the fact that they were not given enough time since the experiment was terminated earlier due to a disease outbreak.

In the glasshouse, where the temperature varied from 20^{o}-30^{o}C, more plants generally flowered as compared to the CER where conditions were relatively constant (20^{o}C). This gives an indication that, after vernalization, cabbage plants may flower better in the field where temperature varies, provided the temperature is within 20^{o}-30^{o}C. These results contradict the earlier view that temperatures between 15^{o} and 25^{o}C would cause the vernalization effect of low temperature in cabbage to be reversed (devernalization) (Heide, 1970, cited by Wiebe *et al*., 1992; Friend, 1985). The fact that some lines can flower at such high temperatures gives great hope for cabbage seed production in the tropics.

The different mode of flower stalk emergence between cabbage lines is relevant (Figure 2.2). It is very important that, in selecting cabbage lines for seed production in the tropics, efforts are made to avoid those that require head cutting or splitting before flower emergence because it is labour intensive. The two lines ('HRI 011446' and 'HRI 006556') that flowered significantly earlier than the

others (Figure 2.3) were the same that were able to flower without vernalization (Figure 2.1). This suggests that the earliness of flowering after plant vernalization may give an indication of lines that could most easily be induced to flower. If this assumption is correct, then it is expected that line 'HRI 009617' will also be relatively easy to induce to flower because, apart from the two lines, it flowered significantly earlier than the rest. However, lines 'HRI 003720' and 'HRI 013011' were very late in flowering after plant vernalization and consequently they may be difficult to induce to flower without vernalization.

During the eight week period of vernalization (at 4^{o}C), vernalized plants were expected to grow more slowly than the non-vernalized ones due to lower thermal time accumulated, and this may have accounted for the lower number of nodes and leaves of the vernalized plants at flowering stage (Table 2.1). Chilling during vernalization was found to suppress leaf initiation and leaf growth at the shoot apex, which consequently allowed increased availability of dry matter at the apical point (dome), thereby permitting its development to flower initiation (Fontes and Ozbun, 1972). It appeared that girth at flowering was more consistent, both between the lines and also between vernalized and non-vernalized plants, and thus it may serve as a good indicator of time of flowering. Ito and Saito (1961) used the girth of 15.7-18.0 mm (5-6 mm diameter) as an indicator of stages for vernalization for the cabbage cultivar, Yosin.

The differences in the number of leaves, height of stem and length of the most recent developed leaves (Table 2.2) showed how distinct the cabbage lines were. It appeared that stem height had a relationship with flowering. Lines 'HRI 011446' and 'HRI 006556', which were able to flower even without vernalization, were significantly taller than the rest, whereas vernalized plants of most of the short cultivars (e.g. 'HRI 002605' and 'HRI 003720') did not all flower in both controlled environment rooms (Figures 2.1a and 2.1b). The lack of differences in canopy shape index between lines and the fact that the value for each lines in both growth rooms was approximately one indicated that all the cultivars had a similar shape and the canopy length was approximately similar to the canopy breadth.

The two lines ('HRI 011446' and 'HRI 006556') which showed a consistent high level of greenness as compared to the others were the same which flowered without vernalization. In contrast, the two lines ('HRI 002605' and 'HRI 003720') that were consistently less green even had some of their vernalized plants not able to flower either in the CER or in the glasshouse. Greenness (SPAD meter value) is closely related to the nitrogen content. Correlations of R = 0.91 between the measured SPAD value of greenness and leaf N concentration of rice have been reported (Anderson *et al.*, 1993). Further, from data pooled from four lines of cabbage, there were positive relationships ($P < 0.001$; R = 0.90) between measured SPAD value of greenness and leaf total chlorophyll (Chapter 6). This gives an indication that the amount of nitrogen in the plant may be one of the important factors promoting flowering in cabbage. Earlier, Colder and Cooper (1961)

reported that cold requiring plants such as *Dactylis glomerata* lose their need for low temperature in the presence of high nitrogen levels. Shortage of nitrogen also delayed curd initiation and maturity in cauliflower (Parkinson, 1952 cited by Hand, 1988). It is probable that where nitrogen deficiency retards floral initiation it is due to a reduction of the level of metabolites at the stem apex. From these findings, the amounts of nitrogen in relation to flowering of cabbage need to be investigated.

Almost all the plants that had head splitting also had tip burn of the leaves and these phenomena may be physiologically related. Plants kept in the CER where conditions were almost constant (20^{o}C) showed less incidence of tip burn as compared to plants in the glasshouse where the temperature varied from 20^{o} to 30^{o}C. This agrees with the suggestion that high temperature may contribute to tip burn disorder. Nagata and Stratton (1994) used elevated temperatures of 28^{o}-37^{o}C for testing susceptibility to tip burn in new lettuce cultivars. Misaghi and Grogan (1979) also reported that symptoms of tip burn can be induced even in detached lettuce heads if temperatures of 25^{o}-28^{o}C are applied for a few days.

The sign of heading at 40 days after transplanting gives an indication of earliness of maturity since early maturing cabbages start forming a head at that stage. Lines 'HRI 003720', 'HRI 005237' and 'HRI 013011' can be considered as early maturing types. Lines 'HRI 011446', 'HRI 009617' and 'HRI 006556' did not show any sign of heading because 'HRI 011446' is not a head forming cabbage

and the other two are late maturing lines. The rest of the lines ('HRI 002605', 'HRI 003202', 'HRI 007827' and 'HRI 009837') can be regarded as intermediate between the early and late maturing lines.

Seventy five (75) is the minimum acceptable germination percentage of cabbage seeds in most countries (FAO, 1993; Douglas and Imman, 2002; Minnesota Seed Rules and Laws, 2003). The slight reduction in CV observed in seeds obtained from Warwick-HRI may be due to the long storage. The germination and the CV results suggest that some cabbage lines can produce viable and vigorous seeds in a tropical environment (20°-30°C), which is contrary to the view of Wang *et al*. (2000) who indicated that, even if the plants can be vernalized, flowering and seed development will be poor due to high temperature. The poor germination and CV of the seeds obtained from line 'HRI 007827' indicated that some lines of cabbage may not produce viable and vigorous seeds under such high temperatures and therefore selection of lines intended for growth in the tropic is very important.

2.5 CONCLUSION

Vernalization at 4°C promoted flowering in all lines. However lines 'HRI 011446', 'HRI 006556' and 'HRI 009617' flowered earlier than the other lines and seeds produced in tropical conditions (20°-30°C) were viable and vigorous with most lines having 80% germination or higher. The exception was 'HRI 007827' which performed poorly in terms of germination and CV. The lines varied significantly in terms of number of leaves, height of stem and length of leaves but canopy shape

index did not vary significantly and was approximately one. The taller lines of cabbage ('HRI 011446' and 'HRI 006556') flowered easily and consistently showed higher levels of greenness as compared to the shorter lines. There was a strong suspicion that earliness of flowering after plant vernalization may give an indication of the ease by which lines could be induced to flower without vernalization. Apart from the two lines, 'HRI 0011446' and 'HRI 006556', which flowered without vernalization, lines 'HRI 009617' and 'HRI 003202' could be used for future studies on flowering and seed development in the tropics because they are head forming types that flower early and easily after plant vernalization without head cutting or splitting. Unlike 'HRI 009617', 'HRI 003202' is susceptible to tipburn and is early maturing**.**

CHAPTER THREE: HEAT TOLERANCE IN TEN LINES OF CABBAGE AS DETERMINED BY CELL MEMBRANE THERMOSTABILITY AND CHLOROPHYLL FLUORESCENCE.

3.1 INTRODUCTION

Previous work (Chapter 2, Figure 2.5) revealed that cabbage line 'HRI 007827' performed poorly in terms of germination and vigour at 20°-30°C which suggests that not all cabbage lines can produce viable seeds in the tropics. Techniques for the rapid identification of heat tolerant lines for commercial production, potential seed production and breeding programmes in tropical areas are therefore required.

As described in Chapter 1, two tests that have gained popularity for rapid screening of agricultural crops for high temperature tolerance are cell membrane thermostability (CMT) and chlorophyll fluorescence (CF). However, it is not known whether the methods will be effective to assess cabbages for heat tolerance in the tropics and whether there is a relationship between the two methods. The aim of this study was the selection of heat tolerant cabbages for future study using CMT and CF tests and determination of the best method(s)/parameter(s) for future screening of temperature tolerance in cabbage. A further aim was to determine whether there is a relation between CMT and CF.

3.2 MATERIALS AND METHODS

Ten lines of cabbage considered in Chapter 2 were used for the study. Heads of cabbage plants, grown in the previous season, were removed to allow axillary buds to develop on the remaining stalk. A month later, 5 developed buds were cut from each of the ten lines and rooted in perlite-sphagnum moss mixture for three weeks, after which they were transferred into Levington F2S compost (Scotts Professional, Bramford, Ipswich, UK) in 4 cm-square modules in plastic modular trays and kept in a closed propagator in a controlled environment room at 20^{o}C with a 12 h photoperiod and 400 μmol m^{-2} s^{-1} photosynthetic photon flux. After three weeks, the plants were transferred into 15 cm top diameter plastic pots containing 0.85 kg Levington M2 soil mix (Scotts Professional, Bramford, Ipswich, UK) and maintained in a glasshouse at 20^{o}-30^{o}C. Seven weeks after rooting, the CMT and CF tests were performed as described below.

3.2.1 Cell membrane thermostability test

The procedure used was similar to that described by Chauhan and Senboku (1996) and Martineau *et al.* (1979). It was repeated on four plants from each of the ten lines of cabbage. Two young leaves (the uppermost fully expanded leaves) from each plant, each of about 100 cm^2 area, were used for the assay. The leaves were washed thoroughly first with tap water and then with distilled water to remove any soil particles. From each leaf sample, paired sets next to each other (control and treatment) of 10 leaf discs were cut, avoiding the midrib, with a 1 cm diameter cork borer and this was repeated for the other leaf. The ten control and ten

treatment leaf discs were then placed into two separate test tubes, and washed thoroughly with distilled water, including at least four changes of water. This procedure was necessary to remove exogenous electrolytes adhering to tissue surfaces and to remove endogenous electrolytes that were released from cut cells at the periphery of the discs. After the final wash, the tubes were drained of excess water, although sufficient water adhered to the discs and tube interior to maintain a high humidity. The treatment tubes were then covered with plastic wrap and incubated in a thermostatically controlled water bath for 15 min at 50^{o}C while the control tubes were maintained at 25^{o}C for the same period of time. After the elevated temperature treatment, the treatment tubes were quickly cooled to 25^{o}C and both the control and treatment tubes were filled with 15 ml of distilled water and incubated overnight for 18 h at 10^{o}C to allow the diffusion of electrolytes from the leaf discs. The tubes were then transferred into a water bath at 25^{o}C, the contents mixed thoroughly for 5 s on a vortex and the initial conductance reading was made with a conductivity meter (Jenway, UK). Upon completion of this measurement, the control and the treatment tubes were covered with plastic wrap and autoclaved at 121^{o}C for 15 min to kill the leaf tissue and release all the electrolytes. All the tubes were then cooled to 25^{o}C, the contents thoroughly mixed and the final conductance measurements were taken.

The relative injury (RI) induced, as a result of the temperature treatment, was calculated as follows:

$$RI\ (\%) = \{1 - ([1 - (S1/S2)] / [1 - (C1/C2)]\} \times 100$$

where, S and C refer to conductance values for treatment and control tubes respectively and 1 and 2 refer to the initial and final conductance readings respectively.

3.2.2 Chlorophyll fluorescence test

Fluorescence measurements, Fo and Fm, were taken from the most recent fully expanded leaf of each of the ten lines of cabbage using a portable Photosynthetic Yield Analyser (Mini Pam, Heinz Walz GmbH, Germany) after the plants had been dark-adapted using leaf-clips for 30 min. Measurements were taken from five plants of each line, which had been arranged in a completely randomized design. Measurements of Fo and Fm were made on two occasions, i.e. after the plants had been stressed at 30°-45°C for three hours (T2) achieved by closure of glasshouse ventilators during the hot summer and early the next morning when the plants were not under any stress and when the temperature was between 23°-26°C (T1). Values of Fv were calculated as a difference between Fm and Fo on each plant, and the ratio Fv/Fm was subsequently determined for each of the temperature regimes.

3.2.3 Statistical procedures

Analysis of variance was used to determine the cultivar variation for RI and the CF parameters, Fo, Fv and Fv/Fm, using the Genstat statistical package. The values for Fv and Fo were transformed (Log_{10}) to normalise the error distributions before the analysis.

3.3 RESULTS

The RI values did not show any differences between the lines (P = 0.113) (Figure 3.1) despite the fact that values ranged from 32.7 (‘HRI 005237’) to 68.0 (‘HRI 009617’). The variability of the RI within lines was so high (CV % = 28.3) that no differences between the lines in response to heat stress were detected.

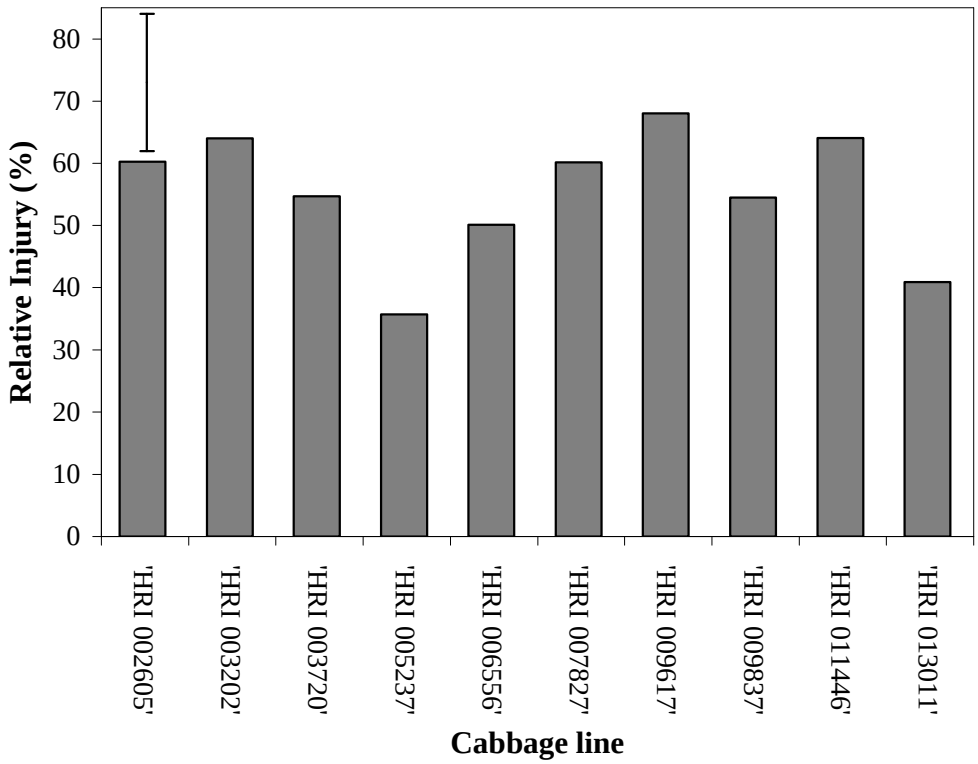

FIGURE 3.1: Mean values for Relative Injury (RI) of ten lines of cabbage raised at 20º-30ºC (n = 40; df = 30). Error bar represents two standard error of the difference.

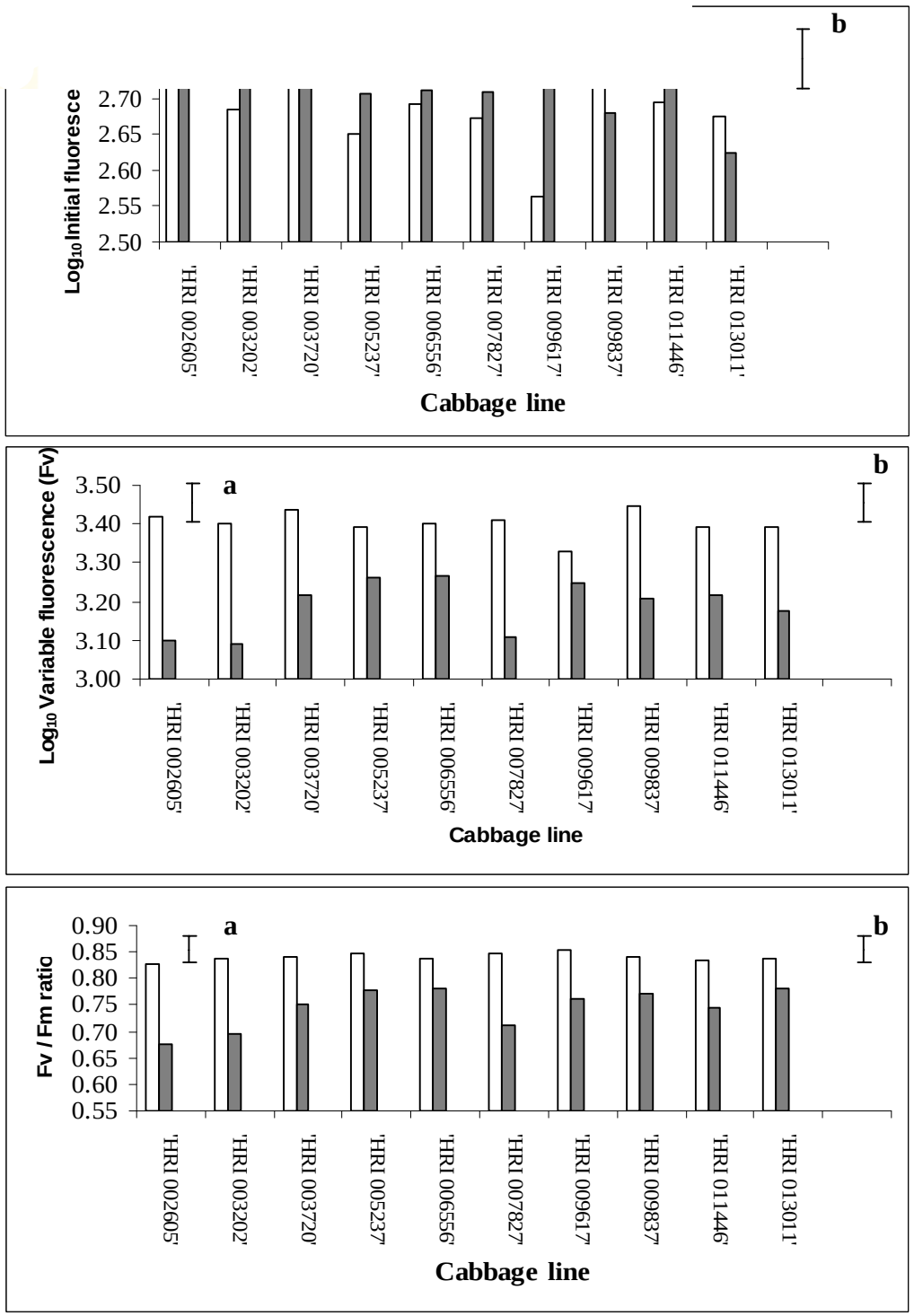

FIGURE 3.2: Comparison of variable fluorescence (Fv), minimum fluorescence (Fo) and Fv/Fm ratio measured at 23º-26ºC (T1, □) and 35º-40ºC (T2, ■) of ten lines of cabbage. Error bars represent two standard errors of the difference (*a* compares means between different lines and *b* compares T1 and T2 means for the same line) (n = 100; df within a line = 40 and between lines = 80).

There were differences ($P < 0.01$) between the stressed (T2) and non-stressed (T1) treatments overall for Fo, Fv and the Fv/Fm ratio and interactions between stress and line ($P = 0.019$; 0.012; 0.062 respectively) indicating that the response to stress differed among lines. This can be seen clearly in Figure 3.2 which shows the pattern of all three fluorescence parameters of the ten lines of cabbage at T1 and T2. Generally, at T2 the Fo values increased while that of Fv and Fv/Fm decreased relative to the values at T1. The Fv/Fm ratios at non-stressed conditions are relatively constant; ranging between 0.83 and 0.86 with an average of 0.84. The greatest decreases in Fv and the Fv/Fm ratio of the stressed plants were shown by 'HRI 002605', 'HRI 003202' and 'HRI 007827' indicating higher susceptibility to heat, whereas 'HRI 005237', 'HRI 009617' and 'HRI 006556' showed smaller decreases in the two fluorescence parameters. For two lines, 'HRI 009837' and 'HRI 013011', the Fo appeared to increase under non-stressed conditions.

There was no relationship ($P > 0.05$) between RI and the three fluorescence parameters at T1 (Figure 3.3), however, at T2 there were significant relationships ($P < 0.05$) between the RI and Fo and between RI and Fv/Fm and a suggestion of a relationship ($P = 0.092$) between RI and Fv. Generally, as RI increased, Fv and Fv/Fm decreased whereas Fo increased.

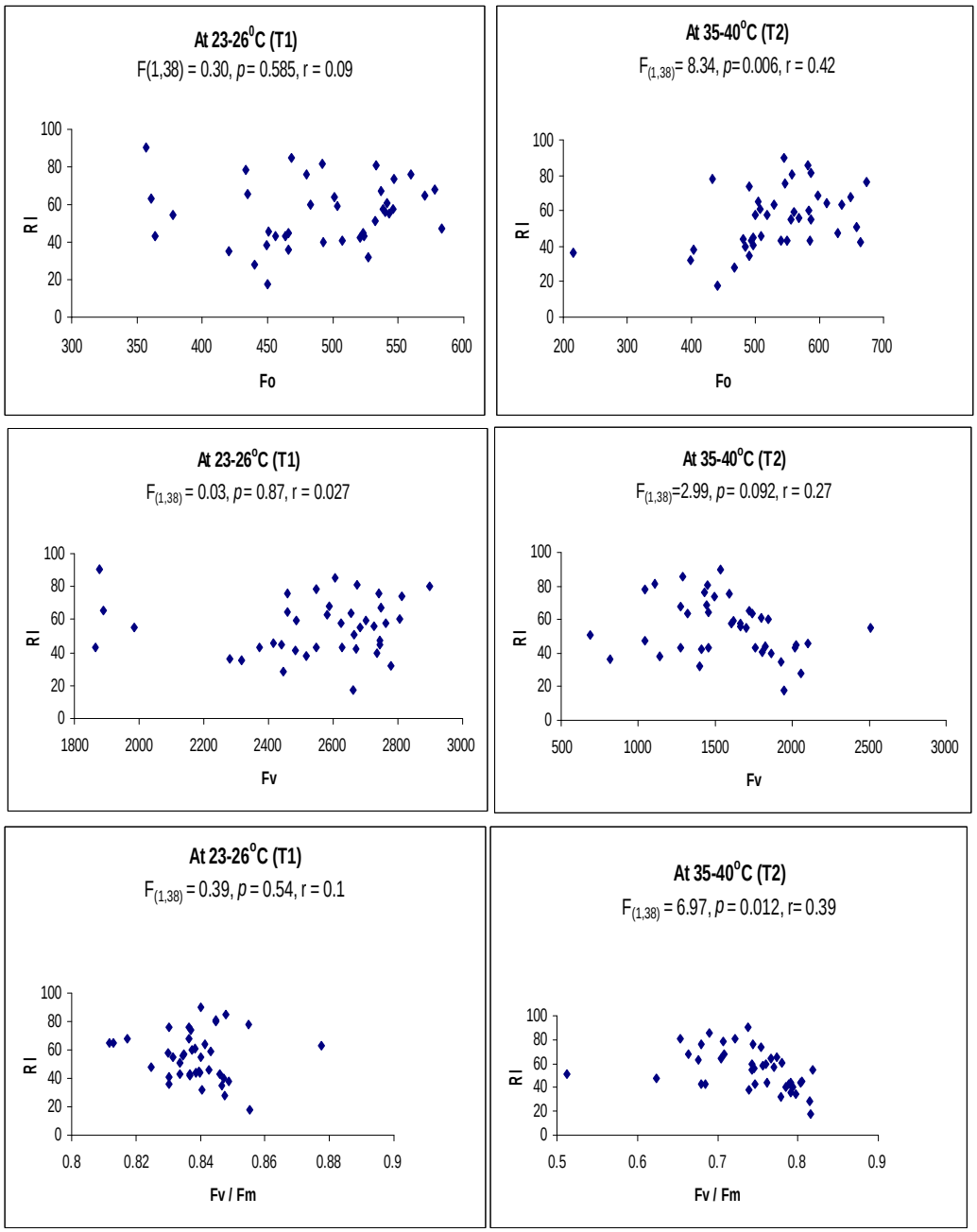

FIGURE 3.3: Relationship between Relative Injury (RI) and the fluorescence parameters (Fo, Fv, Fv/Fm)* taken at non-stress (23º-26ºC, T1) and stress (36º-40ºC, T2) temperature conditions.

*Fo = Initial fluorescence; Fv = Variable fluorescence; Fm = Maximum fluorescence

3.4. DISCUSSION

Only the most recent fully expanded leaves were used for the determination of RI because it has been reported that genotypic differences in membrane thermostability were greatest in newly developed leaves (Martineau *et al*., 1979). The scale of within-line variation observed in RI means that differences between lines would have to be larger than observed in this study (Figure 3.1) to be detected. Similarly, Martineau *et al.* (1979) also found that plant-plant variation was a significant source of variation in RI measurements in four soyabean (*Glycine max* L.) cultivars. It seems that RI would be a useful tool to screen cabbage lines for heat tolerance only in lines that exhibit extreme cases, as reported by Chauhan and Senboku (1996). They found differences in RI between the heat tolerant line 'Sousyu' and heat susceptible line 'YR Kishun'. It appeared that the ten lines in the present study do not exhibit extreme cases of heat tolerance and therefore the differences of the cell membrane thermostabilty between them may be too small to detect. Techniques to reduce variability within lines are therefore needed to utilize the CMT test in such cabbages. From the coefficient of variation and the standard error of the data, the minimum number of replications needed for differences to be detected between the cabbage lines at a significant level of 0.05, with a power of 0.90, using a two-sided test, was calculated to be 6. This information was not available prior to the experiment and it is expected that researchers who want to use the method in future screening of cabbage lines for heat tolerance adhere to the minimum replication (6) or by bulking samples from more plants.

The differences between stressed and non-stressed plants among lines and their interaction for the CF parameters Fv, Fo and the Fv/Fm ratio (Figure 3.2) showed how variable the cabbage lines were and the different values give an indication that there were some physiological differences. The Fo represents the level of fluorescence when primary quinine electron acceptors of PSII are maximally oxidized (PSII centres are open); the Fm represents the level of fluorescence when primary quinine electron acceptors of PSII are maximally reduced (PSII centres are closed), whereas the Fv demonstrates the ability of PSII to perform primary photochemistry (Baker and Rosenqvist, 2004). It is of interest to note that the Fv/Fm values at T1 gave almost a constant average value of 0.84 for all of the cabbage lines (Figure 3.2) while the ratio at T2 was lower than 0.84 for all the lines. These values agree with earlier literature (Bjorkman and Demming, 1987; Johnson *et al.*, 1993; Maxwell and Johnson, 2000) which indicated that optimum Fv/Fm values of around 0.83 were recorded for most plant species and that heat susceptible lines were much less than this average value. The dark adapted values of the ratio reflect the potential quantum efficiency of PSII and are used as an indicator of plant photosynthetic performance (Maxwell and Johnson, 2000). It also gives the efficiency with which light (low irradiance) is used (Chauhan and Senboku, 1996). Therefore, the extent of the decrease of the Fv/Fm ratio from 0.84 may be used to screen cabbages for heat tolerance. The general pattern of the CF parameters as shown in Figure 3.2 confirmed earlier reports (Baker and Rosenqvist, 2004; Maxwell and Johnson, 2000) that, under stress, the Fo increases while the Fv and Fv/Fm decrease, however, in the present study, Fo was not

consistent for all lines. The Fo for ‘HRI 009837’ and ‘HRI 013011’ appeared to decrease under stress (Figure 3.2). An increase in Fo may therefore not be a reliable indicator of heat tolerance for all lines of cabbage. Sipos and Prange (1986) also observed significant differences in Fv and Fv/Fm but not in Fo for ten lines of potato (*Solanum tuberosum* L.) of different heat tolerance. Differences in Fv and Fv/Fm between the lines at T2 revealed that these CF parameters are more sensitive indicators of heat tolerance in cabbage than RI and hence could be used for screening cabbages for growth at high temperatures. This supports earlier literature in which Bjorkman *et al*. (1980) reported that the thylakoid membrane (which contains PSII) is more sensitive to heat than the cell membrane. The large decrease in values for both Fv and Fv/Fm at T2 for lines ‘HRI 002605’, ‘HRI 003202’ and ‘HRI 007827’ and the lower decrease in lines ‘HRI 005237’, ‘HRI 009617’ and ‘HRI 006556’ from that obtained at T1 gives an indication of high susceptibility and high tolerance to heat respectively, and the latter lines may tolerate better the high temperature stress in the tropics. The remaining lines can be said to be intermediate between these two groups in their response to heat.

The fact that there were no relationships detected between RI and the CF parameters measured at T1 whereas, at T2, there were some significant relationships (Figure 3.3), suggests that, although there were differences between CF parameters measured at non-stress conditions (Figure 3.2), parameters measured at stress temperatures are more important in the determination of heat tolerance and that, at high temperatures, cell membranes and thylakoid membranes

(PSII) are damaged simultaneously. The results re-affirmed the fact that the thylakoid membrane (PSII) is more affected by heat as reflected by the decrease in fluorescence parameters (Fv and Fv/Fm) at T2. In general, the relationships at T2 revealed that, as RI increased, Fv and Fv/Fm decreased whereas Fo increased. This agrees with earlier work which showed that thermal damage to cell membranes is characterised by a marked increase in RI (Yeh and Hsu, 2004), whereas damage to thylakoid membranes (PSII) is characterized by a marked increase in Fo and decrease in Fv and the ratio of Fv to Fm (Hansatech, 1996; Sipos and Prange, 1986; Maxwell and Johnson, 2000).

3.5 CONCLUSION

Chlorophyll fluorescence parameters, especially Fv and Fv/Fm, were found to be more sensitive for screening cabbages for heat tolerance than RI, an index of CMT. Values from both Fv and Fv/Fm ratio of the stressed plants showed a marked decrease for lines 'HRI 002605', 'HRI 003202' and 'HRI 007827' indicating higher susceptibility to heat, whereas lines 'HRI 005237' and 'HRI 006556' showed indications of high tolerance to heat and may tolerate best the high temperature stress in the tropics. There were relationships ($P < 0.05$) between the RI and Fo and Fv/Fm only at stress conditions (35^{o}-40^{o}C), which suggests that parameters measured at stress temperatures may be more important in the determination of heat tolerance.

CHAPTER FOUR: PROMOTION OF VIGOUR IN CABBAGE SEED BY OSMOTIC PRIMING PRE-TREATMENT AT BOTH VERNALIZATION AND NON-VERNALIZATION TEMPERATURES

4.1 INTRODUCTION

In a preliminary study, it was found that exposure of cabbage seed to a prolonged period of cold treatment in a refrigerator, in combination with gibberellic acid treatment, appeared to induce flowering, although more work needed to be done to confirm this. However, an earlier attempt to vernalize cabbage seeds had resulted in weak stemmed seedlings, with fungal infections, as a consequence of the seeds having germinated while still in the fridge at the low temperatures used for vernalization (0^{o}-5^{o}C). To overcome this, the seeds needed to be primed during the vernalization period, but it was uncertain whether this treatment would adversely affect the viability and vigour of the seed. Experiments were carried out to determine the effect of osmotic priming pre-treatment (PEG 6000) on the viability and vigour of cabbage seed under non-vernalization and vernalization temperatures to address the following questions:

1. How does osmotic priming (PEG 6000) pre-treatment affect germination and vigour of cabbage seed compared to non-primed seed at 25^{o}C?
2. How does osmotic priming (PEG 6000) pre-treatment affect the germination and vigour of cabbage seeds kept for a prolonged period under vernalization temperature (8 weeks at 0^{o}-5^{o}C) compared to non-primed seeds, or to primed seeds kept at a higher (tropical) temperature (25^{o}C)?

4.2 MATERIALS AND METHODS

4.2.1 Effect of PEG 6000 on the viability and vigour of cabbage seed at non-vernalization temperatures

Seeds of ten lines of cabbage (Chapter 1) were imbibed in 5 cm Petri dishes lined with three filter papers. For each line, three replicate dishes, each containing 20 seeds were treated by moistening the filter paper with 2 ml 302.44 g l^{-l} PEG 6000 providing an osmotic pressure of 1,200 kPa. Three replicate control dishes were moistened with 2 ml distilled water. The dishes were kept in a controlled environment room for 2 weeks at 25^{o}C with an 8 h photoperiod and 400 μmols m^{-2} s^{-1} photosynthetic photon flux. The seeds were then washed in distilled water and sown at a depth of 0.5 cm in 46 g Levington F2S compost in each 4 cm-square module in plastic modular trays. The final proportion of seeds germinating and the coefficient of velocity (a measure of seed vigour) were determined as described in Chapter 2.

4.2.2 Effect of PEG 6000 on the viability and vigour of cabbage seed at the vernalization temperatures

Seeds of each of four lines of cabbage (including 'K.K. Cross', a line commonly grown in Ghana, West Africa) were heat-treated to protect against pathogens by placing them in a 50^{o}C water bath for 30 min, after which they were quickly cooled and imbibed in 302.44 g l^{-1} PEG 6000. Fifty seeds of each line were then placed in 9 cm Petri dishes (i.e. 50 seeds per dish) lined with three filter papers, moistened with 4 ml PEG solution. This was replicated three times.

The seeds were then exposed to vernalization temperatures (0°-5 °C) for 8 weeks in a refrigerator (referred to as 'Prim + Ver'). After the period of vernalization, the seeds were washed in sterile water and sown immediately in 4 cm-square plastic modular trays filled with Levington F2S compost. There were two controls. In the first control, seeds (referred to as 'Prim only') were primed as above and kept at 25°C for 11 d prior to sowing of the treated ('Prim + Ver') seeds. This was to ensure that both batches of seeds had received the same thermal time and were of similar physiological age. For the second control (referred to as 'No Prim + No Vern'), seeds were sown without priming or vernalization. All seeds were planted in similar containers at a depth of 0.5 cm. CV values and the final proportion of seeds germinating were determined as described Chapter 2.

4.2.3 Statistical procedures

A linear logistic regression analysis (binomial) and analysis of variance were used to analyse the proportion of seeds that germinated and their CV values, respectively, using the Genstat (Release 8.1) statistical package.

4.3 RESULTS

4.3.1 Effect of PEG 6000 on the viability and vigour of cabbage seed at non-vernalization temperatures

Priming did not have any significant effect on the proportion of seeds germinating ($P > 0.05$, Figure 4.1). For CV, there was no line x priming interaction, nor a significant main effect of line; therefore, only the main effect of priming is presented (Table 4.1). Priming with PEG 6000 did enhance the CV for all ten lines.

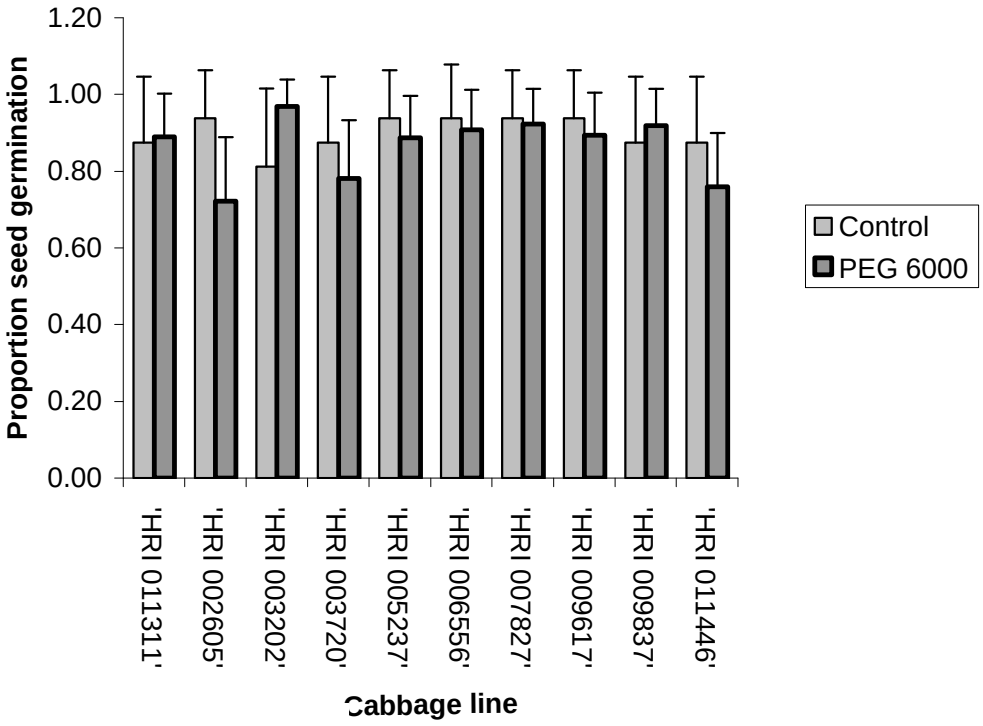

FIGURE 4.1: Lack of effect of priming on the proportion of germination of cabbage lines. Seeds were primed in PEG 6000 or kept in distilled water (control) at 25ºC with an 8 h photoperiod for 2 weeks. Error bars = Standard error of the mean proportion predicted from regression model.

TABLE 4.1: Enhancement of the average coefficient of velocity (vigour) of cabbage seeds (at 0^{o}-5 ^{o}C) by osmotic priming.

Treatment	**Coefficient of velocity (d^{-1})**
Priming (PEG 6000)	**34.4**
No priming (Control)	**23.0**
SED	**4.16**
Probability (*P*)	**0.014**

Seeds primed in PEG 6000 or distilled water (control) and kept at 25^{o}C with 8 h light and 16 h dark for two weeks. SED = standard error of the difference.

4.3.2 Effect of PEG 6000 on the viability and vigour of cabbage seeds at the vernalization temperatures

None of the treatments had any significant effect ($P > 0.05$) on the proportion of cabbage seeds that germinated (Figure 4.2). Thus there was no evidence that priming had any adverse effect on the percentage germination of cabbage seed kept at vernalization temperatures (0^{o}-5^{o}C) for 8 weeks.

There was neither a line x priming treatment interaction, nor a line main effect on the CV of cabbage seed kept under the two temperatures ($P > 0.5$). Priming treatment had a significant effect ($F_{(2, 24)} = 20.6$; $P < 0.001$); therefore only the main effect of treatment is presented (Table 4.2).

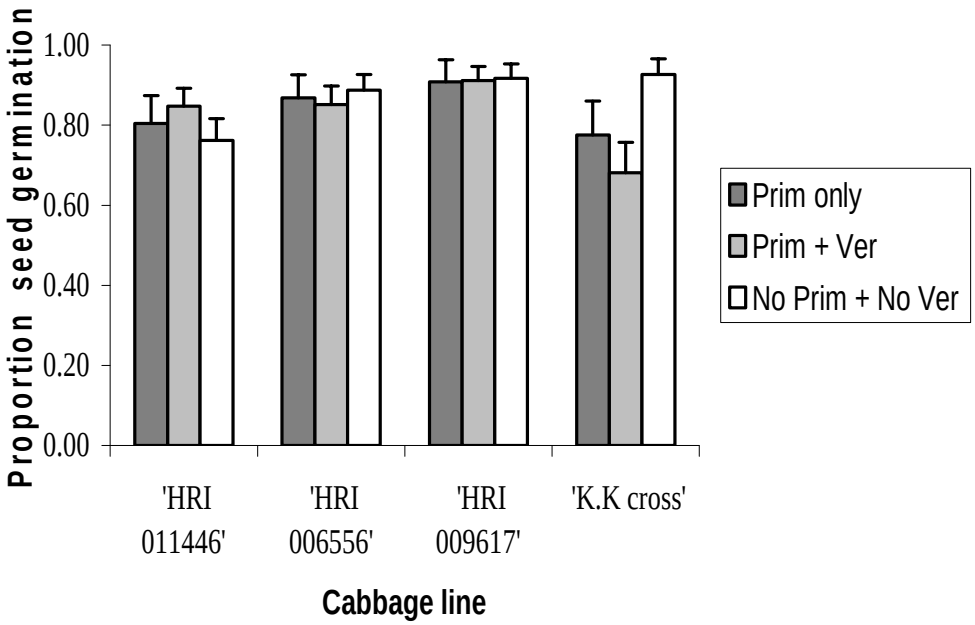

FIGURE 4.2: Lack of effect of priming on the proportion of seeds germinating in four lines of cabbage kept at two temperatures. 'Prim only' = seeds primed and kept at 25°C for 11 d; 'Prim + Ver' = seeds primed with PEG 6000 and exposed to vernalization temperature (0°-5°C) for 8 weeks; 'No Prim + No Ver' = seeds sown without priming or vernalization. Error bars = One standard error of the mean proportion predicted from regression model.

TABLE 4.2: Enhancement of the coefficient of velocity (vigour) of cabbage seeds (at 0°-5°C) by osmotic priming

Treatment	Coefficient of velocity
Prim only	**42.72**
Prim + Ver	**48.00**
No Prim + No Ver	**36.23**
SED*	**1.299**
Probability (*P*)	**<0.001**

'Prim only' = seeds primed and kept at 25°C for 11 d; 'Prim + Ver' = seeds primed and exposed to vernalization temperature (0°-5°C) for 8 weeks; 'No Prim + No Ver' = seeds sown without priming or vernalization. *SED, standard error of the difference.

The CVs for seeds that had received priming treatments were higher than the non-primed seeds ('No Prim + No Ver' control). Among the priming treatments, seeds which were primed and exposed to vernalization temperature (0°-5°C) for 8 weeks ('Prim + Ver') had a significantly higher CV than those that were primed and kept at 25°C for 11 d.

4.4 DISCUSSION

The fact that the proportion of germination of the primed and non-primed seeds was not statistically different between cabbage lines in the two experiments (Figure 4.1; Figure 4.2) indicated that priming with PEG 6000 had no adverse effect on the embryo either at the imbibition or the emergence stage. It has also been suggested that part of the improvement in germination performance resulting from priming may arise from repair, during priming, of deterioration sustained previously during maturation or storage (Dearman *et al.*, 1986). Primed seeds gave higher CV values in both experiments (Table 4.1; Table 4.2). The results obtained in the present study confirmed the results obtained by Khan *et al.* (1980) where primed cabbage seeds kept at 15^{o}C gave accelerated emergence and increased plant fresh weight.

The second experiment showed that cabbage seeds can be primed and vernalized at the same time, without any adverse effect on the viability. This means that seeds of seed vernalized-responsive plants could be vernalized for a long period of time (8 weeks) without growth and loss of viability. When seeds imbibe, the water content reaches a plateau and changes little until radicle emergence (Bradford, 1986). Priming up to this point can have a positive effect, while extended priming duration will negatively affect germination. This implies that, at 8 weeks, the primed cabbage seeds had still not gone beyond the point to affect adversely the viability. Similarly, osmotic priming is also reported to more-than-double germination rate, increase the uniformity of germination and raise the upper temperature limit for

germination of celery seeds (Brocklehurst and Dearman, 1983).

Seeds exposed to vernalization temperature (0°-5°C) for 8 weeks ('Prim + Ver') had a higher CV than those which were primed for 11 d at 25°C (Table 4.2). This may be due to the fact that 8 weeks was enough time for priming to have an effect on seed coat membrane integrity. Upon wetting, those membrane constituents associated with dormancy, such as C_7, C_8 and C_9 lipid acids, change their position in the membrane and do not revert to the initial position (Bewley and Black, 1982; Ajouri *et al*., 2005), thus weakening the membrane to allow the rapid elongation of the radicle. It has also been reported that, during priming, water imbibition and some major metabolic events prepare the seeds for radicle emergence, but radicle elongation is prevented (Karssen *et al.*, 1989). Moreover, slow germinating seeds catch up with faster ones during priming. This ensures uniform and rapid emergence of seedlings which may have accounted for the higher CV after priming in both experiments.

Priming is reported to induce a range of biochemical changes in the seed that are required to start the germination process (breaking of dormancy, hydrolysis and the mobilisation of inhibitors, imbibition and enzyme activation) (Ajouri *et al.*, 2005) which are prerequisite for rapid expansion of the radicle cells. Further, it seems that endosperm weakening associated with priming contributed to the higher CV observed in the present studies as reported for tomato (Haigh, 1988).

During priming of tomato seeds, the breakdown of protein bodies was more extensive in endosperm cells at the micropylar region than was observed prior to germination in non-primed seeds (Haigh, 1988). Therefore, there is ample evidence that priming of cabbage seeds, prior to vernalization, inhibits radicle elongation during vernalization and improves the rate of germination after sowing.

4.5 CONCLUSION

Priming of cabbage seed with PEG 6000 had no adverse effect on the proportion of seeds that germinated, and promoted the CV (vigour) both for seeds kept under vernalization and non-vernalization temperatures. In future work, cabbage seeds can therefore be primed and kept at vernalization temperature (0^{o}-5^{o}C) for up to 8 weeks, to promote vigour.

PART TWO: ALTERNATIVES FOR PLANT VERNALIZATION THAT CAN LEAD TO LESS EXPENSIVE CABBAGE SEED PRODUCTION IN THE TROPICS

CHAPTER FIVE: INDUCTION AND GENERATION OF FLOWERING IN CABBAGE PLANTS BY SEED AND *IN VITRO* VERNALIZATION, GIBBERELLIC ACID TREATMENT AND RATOONING

5.1 INTRODUCTION

Previous work (Chapter 2) showed that cabbage can produce viable and vigorous seeds at tropical temperatures (20°-25°C) provided that the plant had previously been vernalized by exposure to cold (0°–10°C) for 6-8 weeks. This finding is encouraging for cabbage seed production in cooler parts of the tropics [e.g. Abetifi (in Ghana) and Kabete (in Kenya)] where the daily minimum and maximum temperatures are 19°-28°C and 13°-23°C respectively in the rainy seasons. However, the need to vernalize plants poses a major limitation to seed production in the tropics and therefore finding less-expensive alternatives to plant vernalization has become important for cabbage seed production in the tropics.

In the last chapter, it was established that seeds of some cold-requiring plants like cabbage can easily be primed and exposed to vernalization temperature (0°-5°C) for 8 weeks without affecting viability. However, reports (Chapter 1, section 1.4.1) that cabbage needs seven-to-nine leaves, or a stem diameter of 6 mm before it becomes sensitive to low temperatures (i.e. plant-vernalization responsive type) suggest that cold treatment alone will not cause vernalization of cabbage seeds. Some researchers have also used *in vitro* techniques to achieve flowering in some plants (Tang *et al.*, 1983; Naik and Latha, 1996; Yoshikawa and Furuya, 1983;

Virupakshi *et al*., 2002). It has been further reported that, in some species, excised apices grown *in vitro* are sensitive to some flower promoting factors such as photoperiodic treatments (Francis, 1987) and it is likely that cabbage can be sensitive to cold. Examining cold treatment of seeds or *in vitro* plants, combined with other flower-inducing factors, may be more effective. Gibberellic acid (GA_3) promotes flowering in many plants (Hanks, 1985; Halevy, 1990; Kurtar and Ayan, 2005). Combining GA_3 with cold-treated seeds or *in vitro* plants may prove beneficial for flowering in cabbage, and allow seed production in the tropics.

Two separate experiments were carried out. The aims of the first were to assess whether cold treatment of imbibed cabbage seeds and the application of gibberellic acid (GA_3) to juvenile plants and ratoons could induce flowering at a relatively high temperature (20^o-25^oC), and whether more flowering plants could be generated from flower-induced plants by vegetative means. The aim of the second experiment was to determine whether cabbage plants raised from seeds and cold-treated *in vitro* plants could flower with or without GA_3 application at relatively high temperatures (20^oC) as an alternative to the normal green plant vernalization.

5.2 MATERIALS AND METHODS

5.2.1 Seed vernalization and GA_3 on flower induction

Seeds of each of ten lines of cabbage (Chapter 1) were primed with PEG 6000 as described in Chapter 4. For each line, five seeds were placed in each of three replicate 5 cm Petri dishes lined with three filter papers for the priming. The seeds were exposed to vernalization temperatures (2.0°-4.5°C) for 8 weeks using a separate refrigerator for each replicate. Control seeds were primed for 11 d immediately prior to planting, to ensure that the vernalized and control seeds had been primed for the same thermal time (°Cd).

At planting, the seeds were washed in sterile water and sown immediately in 4 cm-square plastic modular trays filled with Levington F2S compost (Scotts Professional, Bramford, Ipswich, UK). After 3 weeks, seedlings were transplanted into 23 cm plastic pots filled with Levington M2 compost and kept in a glasshouse receiving natural light, with supplementary lighting at 150 µmol m^{-2} s^{-1} photosynthetic photon flux density, to give a 12 h photoperiod and maintained between 20° - 25°C. Plants were arranged in a randomised complete block design with three replications. In each block, a plant from the vernalized-seed and control treatments of each line was sprayed until run off with a GA_3 solution, and another with distilled water, using a hand sprayer. Two drops of Tween 80 (wetting agent) were added l^{-1} to each spray. The GA_3 solutions were prepared at 250 mg l^{-1} by dissolving 250 mg of GA_3 in 10 ml ethanol and making up to 1 l with distilled water. GA_3 applications started 45 d after transplanting, and seven applications

were made at 1-week intervals.

Parameters measured included days to flowering-after-sowing (DFS), stem height 90 d after sowing (measured from soil level to the apical bud or the base of the head), number of leaves, girth (circumference of the stem at 5 cm from soil level) and stem height at flowering (measured from soil level to the last node before flower appearance), and percentage germination of seed produced from every plant that flowered, determined in accordance with the International Seed Testing Association Regulations (ISTA, 1993).

As a further experiment, the heads of control or seed-vernalized plants of eight lines were removed carefully, leaving the large basal leaves intact, to allow ratoons (axillary buds developing on the stem of mother plant) to form. Two cabbage lines that had flowered in the previous experiment, even without cold treatment, were excluded. After 2 weeks, the axillary buds that developed were thinned to two, then, after a further 1-week, only the largest bud on each plant was allowed to grow. Four weeks after removing the heads, GA_3 was applied to the ratoon, as described earlier. The number of leaves, stem girth and stem height at flowering were recorded for each plant.

For the single line that was induced to flower, axillary buds developed on both flowering stalk (FS) and the main stalk (MS) after flowering. Four of these buds were cut from the FS, and four from the MS and rooted in a 50:50 (v/v) mixture of

sphagnum moss peat and perlite for 21 d, then transplanted into 4 cm-square plastic modular trays filled with 46 g Levington F2S compost (Scotts Professional). Fourteen days after transplanting, plants were transferred to 15 cm-diameter pots containing Levington M3 compost and kept in a growth-room at 20^{o}C with a 12 h photoperiod at a photosynthetic photon flux density of 335 µmol m^{-2} s^{-1}. The time of flower appearance was recorded and, when the cuttings that developed from the FS reached the flowering stage, the stem height, length and width of the longest leaf and stem girth were measured on plants raised from both FS and MS buds.

5.2.2 *In vitro* vernalization and GA_3 on flower induction

A similar experiment, except it attempted to vernalize *in vitro* plants rather than seeds, was also conducted. Based on the findings from chlorophyll fluorescence parameters (chapter 3) and the morphological and flowering studies (chapter 2) the following lines were chosen for the work reported in this second experiment due to the reasons listed below;

'HRI 009617' - Flower stalk appears without artificial intervention
- Forms dense head
- Flower early after plant vernalization
- Matures late (takes > 10 weeks after transplanting)
- Moderately resistant to heat

'HRI 006556' - Flower even without plant vernalization

- Flower stalk appears without artificial intervention
- Forms loose head
- Flower early after plant vernalization
- Resistant to heat

‘HRI 003202’ - Flower stalk appears without artificial intervention

- Forms dense head
- Flower early after plant vernalization
- Matures early (takes < 10 weeks after transplanting)
- Susceptible to heat

A fourth line, ‘K.K’ Cross was later added because it is the commonest line grown in some West African countries like Ghana. It forms a very dense head, is resistant to heat and has almost the same maturity time as ‘HRI 009617’.

Seeds of the four selected cabbage lines were separately dipped in 2 ml of 5 % Parozone bleach for 5 minutes. The seeds were then washed five times in 4 ml of purified water and cultured in 100 ml screw-capped glass jar (Beatson Clark and Co. Ltd, Rotherham, UK) containing 15 ml of heat sterilized agar medium (Murashige and Skoog, 1962) with 3 % sucrose (weight / volume). The sucrose was added to the agar before autoclaving. The cultured plants were kept at 25^{o}C for 56 d after which they were cold treated (6^{o}-13 ^{o}C) for another 56 d while in culture. The control plants were kept at 25^{o}C for 67 days. Plants generated were transplanted into 4 cm-square plastic modular trays (filled with 46 g Levington

F2S compost) for 10 d after which they were again planted into plastic pots of 15 cm top diameter containing 0.58 kg Levington M2 soil mix. Ammonium nitrate (1.4 g N / pot) was applied as a top dressing on two occasions to all plants, i.e. one month after transfer of plants to Levington M2 compost in 15 cm pots and one month later (a total of 2.8 g N / pot). The GA_3 application started 24 d after planting out from the glass jars and there were seven applications at one-week intervals. The pots were arranged in a randomised complete block design with three replications. In each block, an *in vitro* vernalized plant and control plant of each line was sprayed with GA_3 solution which were prepared and applied as described above. Parameters measured included stem girth and leaf number just after 56 d cold treatment. Leaf number, length of the longest leaf, stem girth and height were also measured at 21 d (before GA_3 application), 60 d (i.e. 30 d after first GA_3 was applied) after transplanting (DAT) and at flowering. Also recorded were days to flowering-after-transplanting (DFAT) of all plants that flowered.

Analysis of variance (Genstat Release 8.1) was used to analyse the data.

5.3 RESULTS

5.3.1 Seed vernalization and GA_3 treatment on flower induction

Among the ten cabbage lines tested, only two ('HRI 011446' and 'HRI 006556') were able to flower in the glasshouse without prior vernalization. The effects of GA_3 and seed vernalization on the DFS of these two cabbage lines are presented in Table 5.1.

TABLE 5.1: Main effects of gibberellic acid (GA_3) and seed vernalization on the average number of days to flowering-after-sowing (DFS) for cabbage lines 'HRI 011446' and 'HRI 006556'.

Treatment	DFS	SED*	Probability (*P*)
GA_3	98		
		5.81	0.004
No GA_3 (control)	118		
Seed vernalization	102		
		5.81	0.03
No seed vernalization (control)	115		

*SED, standard error of the difference (n = 24; df = 14)

There was no interaction between treatments, and no main effect of line ($P > 0.05$), therefore only the main effects of GA_3 and seed vernalization are presented. GA_3 reduced the average DFS ($P = 0.004$) of the two lines. Exposing the seed to cold (2^o-4.5^oC) for 8 weeks also reduced the average DFS ($P = 0.03$) of the two lines. For the other eight cabbage lines, seed vernalization alone did not induce flowering, and had no effect on the stem height ($P > 0.05$; Figure 5.1A). GA_3 alone increased the stem height of almost all cabbage lines, but did not induce flowering (Figure 5.1B; Figure 5.2). There was no significant increase ($P > 0.05$) in stem height due to GA_3 in the two lines that flowered without vernalization ('HRI 011446' and 'HRI 006556') when compared with their controls (Fig. 5.2).

Seed vernalization, followed by GA_3 treatment, induced flowering in cabbage line 'HRI 009617' (Figure 5.1C), on average, 153 d after sowing, when the average number of leaves, stem height and girth were 47, 97 cm and 5.3 cm, respectively. Similarly, the GA_3 x seed vernalization combination only induced flowering in ratoons of line 'HRI 009617' (Figure 5.1D), on average, 85 d after heading-back, when the average number of leaves, stem height and girth were 38, 64 cm and 5.8 cm, respectively. However, neither treatment (GA_3 or seed vernalization), applied without the other, was able to induce flowering in any line.

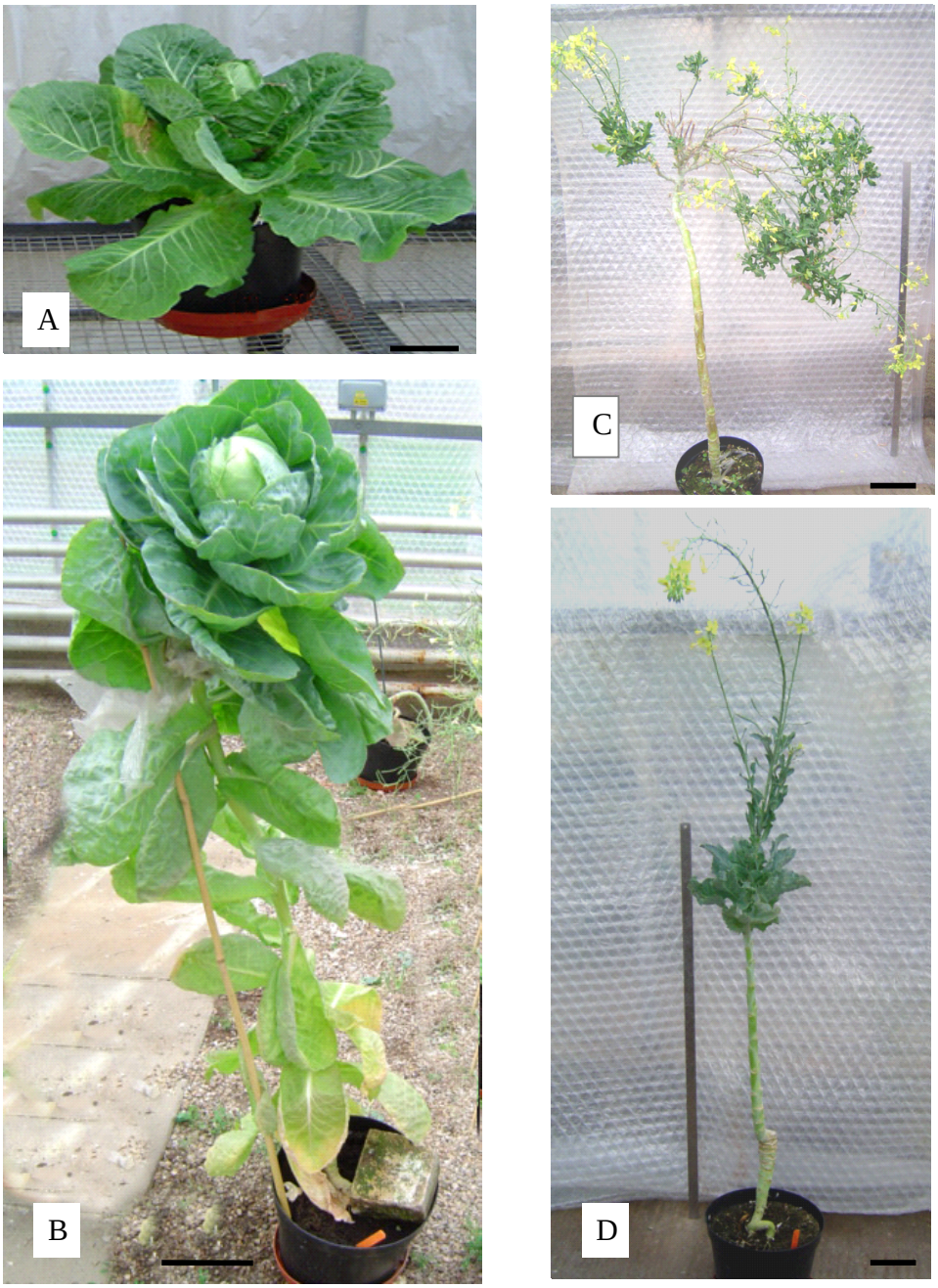

FIGURE 5.1: Responses of cabbage to GA_3 and seed vernalization**.** Panel A, normal plant growth following seed vernalization alone, or control treatment. Panel B, increased plant height following seven successive GA_3 treatments. Panel C, flowering in cabbage line 'HRI 009617' following seed vernalization and GA_3 treatment. Panel D, seed vernalization and GA_3 treatment-induced flowering in ratoons of cabbage line 'HRI 009617' (Bars = 10 cm).

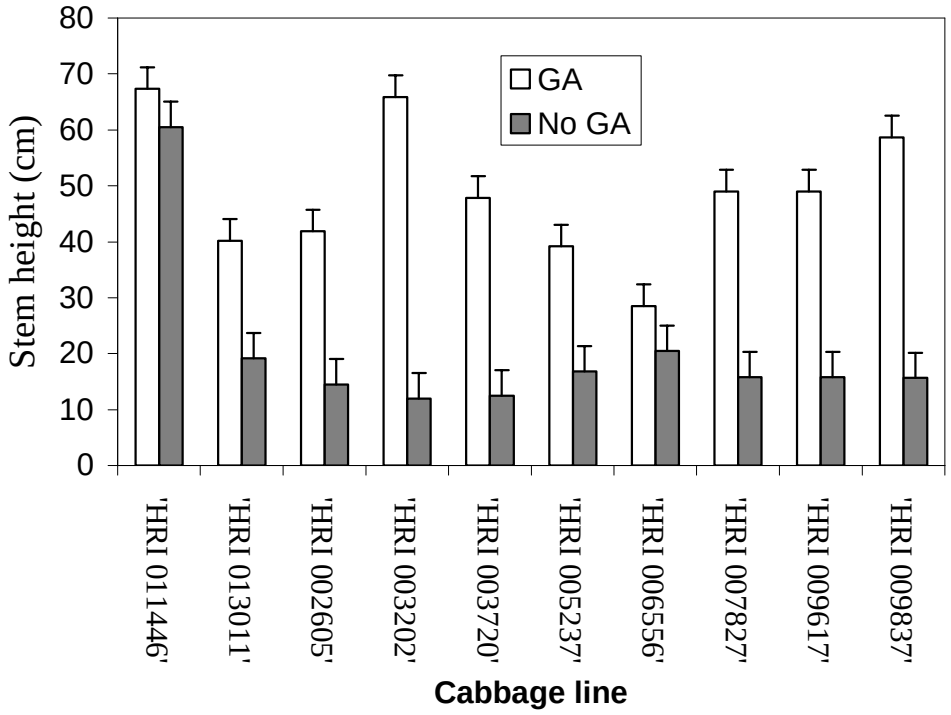

FIGURE 5.2: Effect of gibberellic acid (GA_3) on stem height of ten lines of cabbage 90 days after sowing. Error bars show standard errors of the mean. (n = 120, df = 78)

Although there were slight differences ($P < 0.05$) in the germination (%) of the three lines that flowered and produced seeds under the various treatments, seed produced from all lines gave ≥ 89 % germination (Figure 5.3).

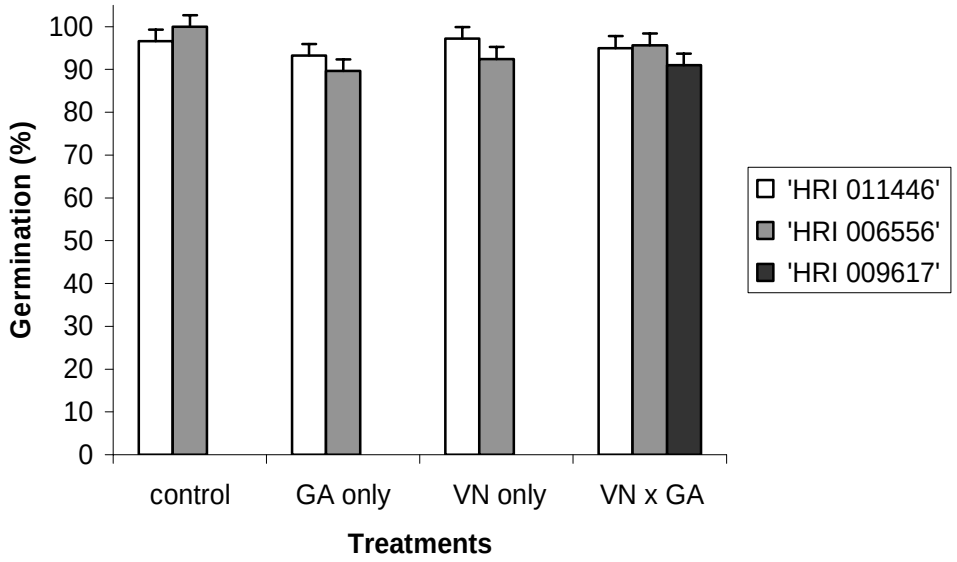

FIGURE 5.3: Germination (%) of three lines of cabbage that flowered and produced seeds under the various treatments**.** GA = seeds from GA_3 treated plants; VN = seeds from seed vernalized plants; VN x GA = seeds from plants that received both treatments; control = seeds from plants that received neither of the treatments. Error bar shows standard error of the difference**.**

In cabbage line 'HRI 009617', approximately 48 d after rooting, most cuttings taken from the flowering stalks (FS) flowered at 20^{o}C, whereas those taken from the main stalk (MS) failed to flower. The morphology of the two sets of plants developed from the FS and the MS was very different (Table 5.2). Although both sets of plants had similar girth and length of the longest leaf, the offshoots developed from FS had significantly longer stems and more, thinner leaves (P < 0.001).

TABLE 5.2: Morphological differences in cuttings developed from the flowering stalk (FS) or main stem (MS) of cabbage line ‘HRI 009617’ 48 d after rooting.

Part of plant	Stem height (cm)	Number of leaves	Width of longest leaf (cm)	Length of longest leaf (cm)	Stem girth (cm)
FS	16.00	12	3.67	13.25	2.75
MS	8.25	8	13.00	13.85	2.38
SED*	1.031	0.629	1.353	1.554	0.228
Probability (*P*)	< 0.001	< 0.001	< 0.001	0.724	0.228

*SED, standard error of the difference (n = 8; df = 6)

5.3.2 *In vitro* vernalization and GA_3 treatment on flower induction

Data taken immediately after the 56 d cold treatment showed that line and cold treatments significantly affected leaf number independently**.** Line 'HRI 003202' produced more leaves ($P < 0.001$) than the other three lines (Table 5.3), which did not differ in the number of leaves produced within the period. Plants which were given cold treatment also produced more leaves ($P = 0.027$) than the control plants (Table 5.3). The four lines did not show any differences in stem girth ($P > 0.05$) after the cold treatment.

At 21 DAT, 'HRI 003202' had the smallest stem girth, shortest leaves and stem and highest leaf number (Table 5.4). The length of the longest leaf and stem girth were not different for the other three lines, however all lines varied significantly from each other in terms of stem height; line 'HRI 006556' was the tallest. Plants exposed to the eight weeks cold treatment had significantly taller stems ($P = 0.010$) compared with the controls (data not shown).

Similar to observations at 21 DAT, plants which were exposed to eight weeks cold treatment grew taller than the untreated plants at 60 DAT. GA_3 generally increased the stem height, leaf number and length of the longest leaf of all cabbage lines at 20^{o}C (Table 5.5).

TABLE 5.3: Line and cold treatment main effects on leaf number after 58 days exposure to cold (6º-13ºC)

Line	Leaf number	Treatment	Leaf number
'HRI 006556'	2.67	No cold treatment	2.70
'HRI 009617'	2.71	Cold treatment	2.84
'HRI 003202'	3.03		
'K.K. Cross'	2.67		
SED	0.089	SED	0.063
df	24	df	24
Probability	<0.001	Probability	0.027

Data were square root transformed and transformed data was presented.

SED = standard errors of the differences of means.

TABLE 5.4: Line main effects on leaf and stem parameters at 21 d after transplanting

Line	Leaf number	Length of longest leaf ($cm^{1/2}$)	Stem girth ($cm^{1/2}$)	Stem height ($cm^{1/2}$)
'HRI 006556'	3.17	2.08	1.28	2.90
'HRI 009617'	3.07	2.24	1.34	2.35
'HRI 003202'	3.41	1.85	1.01	1.95
'K.K. cross'	3.28	2.25	1.30	2.44
SED	0.080	0.157	0.10	0.159
df	14	14	14	14
Probability	0.004	0.074	0.021	<0.001

Data were square root transformed and the transformed data is presented.

SED = standard errors of the differences of means.

TABLE 5.5: The effects cold treatment and GA_3 treatments on stem girth, stem height, leaf number and length of longest leaf of four lines of cabbage at 60 d after transplanting.

Line	Stem girth ($cm^{1/2}$)				Stem height ($cm^{1/2}$)				Leaf number				Length of longest leaf ($cm^{1/2}$)			
	No cold		Cold		No cold		Cold		No cold		Cold		No cold		Cold	
	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA	GA	NGA
HRI 003202	2.0	2.0	2.0	1.8	4.8	2.5	4.9	2.6	5.0	4.2	5.0	4.6	5.2	5.3	5.1	4.9
HRI 006556	2.1	2.0	1.9	1.9	5.3	3.7	5.3	4.9	4.8	4.6	5.5	4.7	5.8	5.7	6.0	5.6
HRI 009617	2.0	2.0	2.1	1.9	5.0	2.6	5.6	3.3	4.6	4.2	4.6	3.8	5.4	5.3	5.6	5.4
K.K. cross	2.2	2.0	2.0	2.0	4.1	2.9	4.7	3.2	5.3	5.1	4.8	4.8	5.5	5.2	5.5	5.3
SED	0.065				0.421				0.240				0.225			
df	30				30				30				30			
Replication	3				3				3				3			

Data were square root transformed before analysis and the transformed data is presented.

Among the four cabbage lines tested, only one ('HRI 006556') was able to flower in the growth room without prior vernalization. The combination of GA_3 and cold treatment affected leaf number at flowering and number of days to flowering of line 'HRI 006556' (Figure 5.4). GA_3 did not have any effect ($P > 0.05$) on cold treated plants but significantly reduced both the leaf number at flowering and DFAT of plants that did not receive the cold treatment (control). The stem height at flowering of 'HRI 006556' was not affected by any of the treatments (Table 5.6). Cold treated plants had narrower stem girth at flowering as compared to the controls.

TABLE 5.6: GA_3 and cold treatments main effects on length of longest leaf, stem girth and stem height (stem ht) of 'HRI 006556' taken at the day of flowering.

Treatment	Longest leaf ($cm^{1/2}$)	Stem ht ($cm^{1/2}$)	Treatment	Stem girth ($cm^{1/2}$)	Stem ht ($cm^{1/2}$)
No GA_3	29.83	31.0	No cold treatment	5.17	36.8
GA_3 treatment	34.17	40.5	Cold treatment	3.92	34.7
SED	1.351	6.02	SED	0.180	6.02
df	6	6	df	6	6
Probability	0.018	0.166	Probability	0.003	0.731

Data were square root transformed and the transformed data is presented.
SED = standard errors of the differences of means.

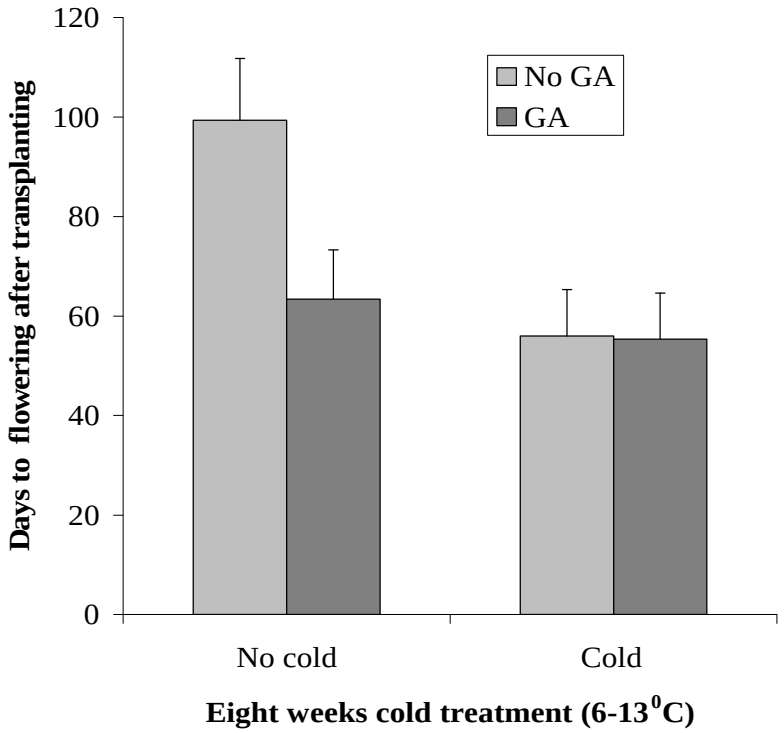

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FIGURE 5.4: The combined effects of GA_3 and cold treatment on the number of leaves at flowering and number of days to flowering after transplanting of 'HRI 006556'**.** Error bars = one standard error of the mean predicted from regression model.

For the other three cabbage lines, neither GA_3 nor cold treatments, applied without the other, was able to induce flowering in any of the lines**.** However, one of the three replicates of line 'HRI 007617' flowered when a combination of cold and GA_3 treatments was applied to plants, and flowering occurred at 148 d after sowing, when the number of leaves, stem height and girth were 48, 96 cm and 2.6 cm respectively.

5.4 DISCUSSION

The fact that GA_3 caused early flowering in the two cabbage lines ('HRI 011446' and 'HRI 006556') in the first experiment (Table 5.1) and 'HRI 006556' in the second experiment (Figure 5.4) is relevant. It is known (Chapter 2) that these two lines can flower without vernalization and therefore are thought to undergo a vernalization-independent pathway (Dennis *et al.*, 1996). Dennis *et al*. (1996) hypothesised that GA_3 influenced the vernalization-dependent pathway; but were not certain whether it also influenced the vernalization-independent pathway. This finding shows that GA_3 also has some influence on the latter pathway. Unlike the first experiment where GA_3 and seed vernalization caused early flowering independently for the two lines (Table 5.1), it was also revealed (Figure 5.4) that GA_3 caused early flowering only in plants that have not received the *in vitro* cold treatments and the combined effect was similar to when the factors are applied without the other. The implication is that GA_3 can be substituted for *in vitro*-cold in that line to promote early flowering and that it is not necessary to use both factors to achieve the same result. The different temperature ranges and plant materials (seeds or *in vitro* plants) for the two experiments may account for the slight differences in GA_3 effects.

Although it has been reported that cabbage needs to complete a juvenile stage before it becomes sensitive to low temperatures (i.e. plant vernalization-responsive type; Ito *et al.*, 1966; Friend, 1985; Lin *et al*., 2005), it now seems likely that juvenility differs between cabbage lines, and imbibed seeds of some lines may be sensitive to vernalization. The reduction of DFS by seed vernalization (Table 5.1) supports Nakamura and Hattori (1961), reported by Wien and Wurr (1997), who challenged the existence of a juvenile phase in some lines of cabbage.

It was not surprising that GA_3 increased the stem height of almost all the cabbage lines used in the two experiments. Gibberellins (e.g. GA_3) are known to increase stem height of many crops (Suge and Rappaport, 1968; Behringer *et al*., 1990). The biophysical mechanism triggered by GA_3 to cause stem elongation in plants is still a contentious issue. For example, Cleland *et al.* (1968) proposed that GA_3 increases the elongation rate in plants by exerting greater osmotic pressure on the growing cells, which generate greater turgor pressure and subsequently drive cell wall extension at a greater rate. This conclusion was contradicted by Behringer *et al*. (1990) who suggested that GA_3 enhances stem elongation by both decreasing the wall yield threshold and increasing the wall yield co-efficient. The stem heights of the two lines ('HRI 011446' and 'HRI 006556') after GA_3 treatment did not differ from their controls ($P > 0.05$; Figure 5.2), probably because these lines undergo the vernalization independent pathway, and both the GA_3-treated and control plants bolted. A similar result was obtained for the later experiment (Table 5.6) where neither *in vitro* cold nor GA_3 affected stem height at flowering of one of these lines. Temperate vegetables such as cabbage often bolt (i.e. elongate

their stems before flowering; Roberts *et al*., 1997) and normally attain a certain height before flower initiation.

The induction of flowering by the combined effect of GA_3 and seed vernalization (Figure 5.1C) indicates that at least one cabbage line (plant vernalization type) was sensitive to seed vernalization, but the effect on its own was not sufficient to induce flowering; the supplementary effect of GA_3 was needed for the induction of flowering. Again, the results reaffirmed the finding (Table 5.1) that seed vernalization may affect the flowering of some cabbage lines. This is contrary to the views expressed by Lin *et al.* (2005) and Ito *et al.* (1966) that cabbage has to reach a certain developmental stage before it can be sensitive to cold treatment. The earlier results were confirmed when GA_3 and seed vernalization, in combination, again induced flowering in ratoons of line 'HRI 009617' (Figure 5.1D). This result is important because it implies that farmers in the tropics could obtain both a head and seeds from the same plant of this cabbage line. If adopted, this technique is likely to increase the revenue of cabbage farmers.

It was expected that the combined effect of *in vitro* vernalization and GA_3 would cause flower induction in 'HRI 009617' as happened in the earlier experiment when the cold (0^o-4.5^oC) was applied to primed seeds. One out of the three replicates of that line was able to flower in this case when the combined treatments were applied, giving an indication that the others might have sensed the cold but not enough to cause flower induction. Unlike the seed-vernalized experiments where a cold temperature range of 0^o-4.5^oC was applied, for the *in vitro*-plants a temperature of 6^o-13^oC was applied because that was the minimum temperature

range that could be obtained in the growth room during the experimental period (hot summer). The temperature range for vernalization in many Brassicas does not usually exceed 10^{o}C (Nieuwhof, 1969; Yamaguchi, 1983; Dixon, 2006). Hence, the combined effect of the factors may not have induced all the plants to flower probably because the cold temperatures applied were not optimal for vernalization. The fact that one plant of the line 'HRI 009617' flowered in response to the combined effects of *in vitro* vernalization and GA_3 may be due to chance but it may also be a confirmation of the earlier result that the same line flowered after vernalization as seed and that not all cabbage lines need to reach a certain developmental stage and size before they are sensitive to flower induction factors. Further work is needed, however, perhaps with lower temperatures and longer vernalization durations, to confirm this.

A previous experiment (Chapter 2) showed that the other eight lines were able to flower when eight week old plants in pots were exposed to cold (0^{o}-4^{o}C) for 8 weeks. That result affirmed the earlier observations that some cabbage lines need between seven and nine leaves, or a stem diameter of 6 mm (stem girth = 1.9 cm) before they become sensitive to low temperatures (i.e. plant-vernalisation responsive type; Friend, 1985; Lin *et al*., 2005). In the second experiment, the minimum leaf number (> 7 leaves) required for the plants to sense cold was attained after growing the plant *in vitro* for 8 weeks, however, the plants could only attain a stem girth of 0.4-0.5 cm instead of the required 1.9 cm, due to growth restriction in the glass jar. It is, therefore, likely that most of the plants could not sense the cold to allow for the subsequent induction of flowering, because they had not reached the critical stem girth. Attainment of threshold size was reported to be

particularly important in biennials such as cabbage since it is related to the amount of resources accumulated (Bernier and Perilleux, 2005). It may therefore be useful, in future experiments, to ensure that the critical girth of 1.9 cm be attained before cold treatment for vernalization is imposed. The stem girth at flowering of the cold treated plants was smaller than that of the control (Table 5.6) probably because the former flowered earlier. The control plants therefore had more time to grow and increased in stem girth before flowering.

Line 'HRI 003202' is the shortest and the earliest maturing of the four cabbage lines used in the second experiment. This fact may not only have accounted for the ability of that line to produce the most leaves within the 8 weeks cold treatment period but also the smallest stem girth, shortest leaves and stem, and most leaves 21 d after transplanting. The cold treated plants were kept at 6^{o}-13^{o}C for 56 d while control plants were kept for 11 d at 25^{o}C. Therefore, the cold treated plants had higher thermal time (°Cd) than the control which may partially explain why they were able to develop more leaves (after 8 weeks, Table 5.3) and produce taller plants at 21 d after transplanting than the controls.

The percentage germination for all lines that flowered and produced seeds was ≥75%, which is the minimum acceptable germination percentage for cabbage seed in most countries (FAO, 1993; Douglas and Imman, 2002; Minnesota Seed Rules and Laws, 2003). The implication is that seed vernalization and gibberellic acid treatments, applied alone or in combination, did not have any adverse effect on the seeds subsequently produced. Therefore these treatments could be adopted for cabbage seed production.

The morphological differences (Table 5.2) clearly show that plants developed from FS retained the reproductive stage, while the others remained vegetative. Morphological changes occur in some *Brassica* species when they reach the adult stage (Thomas, 1980; Wurr *et al.*, 1993). Wurr *et al.* (1993) observed, in cauliflower, that the apical meristem changes to a generative state when vernalizing temperature is applied. The fact that flowering plants can be generated from cuttings developed from FS indicated that the induced state caused by the combined effect of GA_3 and seed vernalization can be inherited through mitotic division**.** It seems that the induction of flowering by GA x seed vernalization interaction has similar characteristics to the thermo-induced state resulting from plant vernalization, a state which Lang (1965) has suggested can also be inherited through successive mitotic divisions but it is not passed through meiosis.

Cuttings from the flowering stalk may have generated flowering plants because they had already developed flower primordia on the mother plants. This was confirmed by the dissection of apices under a compound microscope. Line 'HRI 009617' required an average of 153 d to flower after sowing whereas offshoots developed from FS took only 48 d after rooting. The finding that flowering plants can be generated easily and quickly from FS, at a relatively high temperature (20^{o}C), is significant. It means that only a few plants need to be induced to flower, and many flowering plants could be generated quickly for seed production in some of the cooler areas in the tropics, where the average temperature is around 20^{o}C. This will facilitate less-expensive seed production, as the use of GA_3 will only be needed for the initial induction of flowers. Future research will therefore be

geared towards finding ways that will allow more lines of cabbage to respond to flower induction at tropical temperatures.

5.5 Conclusion

The interaction between seed vernalization and GA_3 can induce flowering in at least one line of cabbage developed from seeds or ratoons, while, the combined effect of *in vitro* cold treatment and GA_3 induced flowering in one of the three replicates of line 'HRI 009617'. For genotype(s) which flower even without cold treatment, GA_3 and seed vernalization cause early flowering independently, however GA_3 alone did not have any effect ($P > 0.05$) on cold-treated *in vitro* plants but can be substituted for *in vitro* vernalization to cause early flowering of non-treated (control) plants. The use of GA_3 and seed vernalization in cabbage did not have any adverse effect on the viability of seeds produced. Potential flowering plants can easily be generated from flowering stalks through vegetative propagation methods.

CHAPTER SIX: SUCROSE AND NITROGEN EFFECTS ON GREENNESS, HEAD PARAMETERS AND FLOWERING OF FOUR LINES OF CABBAGE

6.1 INTRODUCTION

Sucrose and nitrogen (as discussed in Chapter 1) were strongly suspected to influence flowering in Brassicas. It was also observed in a previous experiment (Chapter 2, Nyarko *et al*., 2006a;) that two lines of cabbage ('HRI 0011446' and 'HRI 006556'), which flowered without vernalization, showed a consistently high level of greenness as compared to the other lines throughout the eight week study period. Greenness (amount of chlorophyll in a plant) was closely related to the nitrogen content of the leaves (Anderson *et al.*, 1993). The aim of this experiment was to determine whether increasing the sucrose concentration to seedlings *in vitr*o and subsequently increasing N supply to plants in soil could affect greenness or head parameters which might lead to flower induction in cabbage. A further aim was to find out whether there was a consistent relationship between SPAD meter values for greenness and total chlorophyll of the four cabbage lines under study.

6.2 METHODS:

6.2.1 Sucrose and nitrogen effect

Seeds of the four selected cabbage lines (Chapter 5) were surface sterilized and raised *in vitro* as described in Chapter 5 (Section 5.2.2.). The agar medium contained 0 or 3 % sucrose (weight / volume). When seedlings were 21 days old,

they were transplanted into plastic pots of 23 cm diameter containing 3 kg Levington M2 soil mix. Then, 3.5 g N (ammonium nitrate) per pot was applied as a top dressing on two occasions, i.e. 30 days and 60 days after transfer of plants to the 23 cm pots (a total of 7 g N / pot). The control had no N top dressing. The experimental design was 4 x 2 x 2 factorial in a randomized complete block with four replications. In addition to the N treatment, all the plants had basal nutrients from Sangral compound fertilizer (1:1:3). Fifty ml of a stock solution (100 g l^{-1}) were mixed with 5 litres of water and applied to 5 plants. There were weekly applications for 12 weeks starting from 6 weeks after transplanting.

The following assessments were made:

Parameter	Explanation
Greenness	Measured weekly for 6 weeks with SPAD meter as described previously (Chapter 2, Section 2.2).
Head length	Length of cut head longitudinal from base to tip
Head width	Width of cut head about mid portion
Head shape index	Head length / head width
Solidity	Head weight / (0.523 x horizontal width x head length)
Days at flowering	Days from transplanting to flower appearance
Stem height at flowering	From soil level to last node before flower appearance

6.2.2 Relationship between SPAD values and total chlorophyll

At 28 days after sowing, two leaves (of distinct different visual level of greenness) from the control and N treated plants respectively of each line of cabbage were selected and three plants per line were used. For each leaf, SPAD meter values for greenness were measured using a Minolta SPAD meter avoiding the mid rib. Leaf discs were cut (with 1 cm diameter cork borer) from the exact points where the measurement of greenness was taken. Each leaf disc was ground separately with a pestle and mortar in 1 ml of 80 % acetone (volume by volume) which was then made up to 4 ml of 80 % acetone in a Falcon tube. The tube and the contents were centrifuged at 3000g for 5 minutes. Absorbance was then measured at 645 and 663 nm using a Sp6-500 ultra-violet spectrometer (Pye Unicam, UK) with 80 % acetone as a blank. The total chlorophyll was determined using the following formula developed by Arnon (1946):

Total chlorophyll = $(8.02 \times A_{663}) + (20.21 \times A_{645})$

where A_{663} and A_{645} are absorbance at 663 nm and 645 nm respectively.

Analysis of variance was used to determine the variation among cultivars for all parameters measured except proportions of head splitting where logistic analysis (Binomial) was adopted using Genstat-Release 8.1 statistical package. The data for the head parameters were square root transformed to normalize the error distribution before the analysis was performed. For the SPAD meter value for greenness where measurements were taken over time on the same plants, repeated measures analysis was done.

6.3 RESULTS

6.3.1 Sucrose and nitrogen effect

Among the four lines, only one, 'HRI 006556', flowered. For this line, there was a significant interaction between the nitrogen and sucrose treatments (P = 0.02) on the days to flowering after transplanting (DFAT). (Table 6.1). Application of 7 g N reduced DFAT (P = 0.02) of the plants raised from medium with elevated (3 %) sucrose but not when no sucrose was added. None of the treatments or their interactions had effects (P > 0.05) on the girth and the number of leaves at flowering of the line 'HRI 006556', therefore that data is not presented. Sucrose and N increased the stem height at flowering (P < 0.05) of 'HRI 006556' independently; there was no interaction (P > 0.05) (Table 6.2).

TABLE 6.1: Effect of additional nitrogen and sucrose application on days to flowering after transplanting of line 'HRI 006556'.

Nitrogen	Days to flowering after transplanting	
	No sucrose	3 % sucrose
No additional N	12 (140)	13 (174)
7 g additional N	13 (157)	11 (118)
SED	0.735	
Sucrose x N interaction probability (P)	0.020	

Data were transformed (square root). Original scale data are in brackets.

SED = Standard error of the difference

TABLE 6.2: Main effects of additional nitrogen and sucrose on the average stem height at flowering for cabbage line 'HRI 006556'.

Treatment	Stem height (cm)	SED	Probability (*P*)
No additional N	5.55 (31)		
		0.418	0.032
7 g additional N	6.63 (44)		
No sucrose	5.59 (31)		
		0.418	0.043
3 % sucrose	6.59 (43)		

Data were transformed (square root). Original scale data are in brackets.
SED = Standard error of the difference.

The application of 7 g N as a top dressing significantly increased the proportion of splitting (P = 0.004) in the three lines of cabbage that did not flower (Figure 6.1). Line 'HRI 003202' appeared to be more resistant to head splitting than the other lines, with no splitting in the absence of N and the smallest proportion of splitting when N was applied.

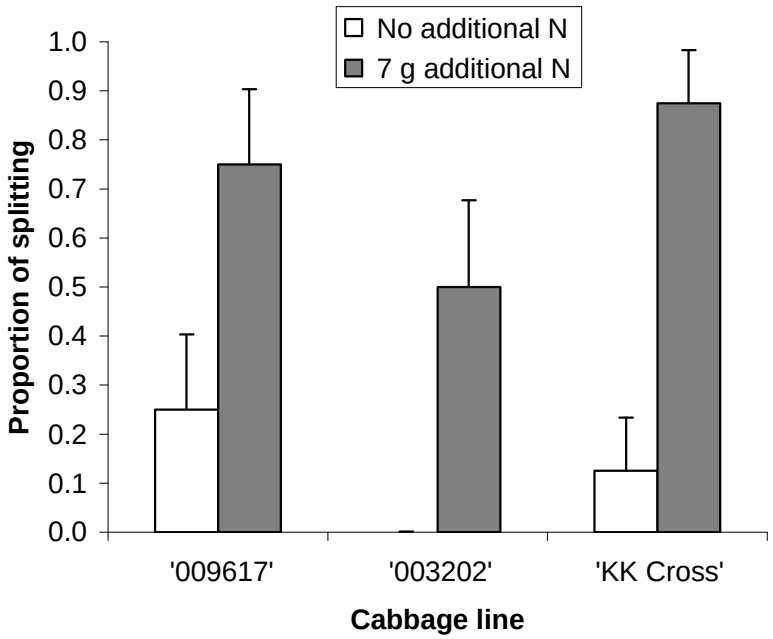

FIGURE 6.1: The proportion of splitting in three lines of cabbage in response to 7 g N supply. Bars show standard error of the mean proportion of splitting predicted from the regression model (n = 48, df = 36).

Most of the head parameters measured were influenced by the line or addition of 7 g N independently (Table 6.3). Head width, length, shape and weight varied depending on the line, however, head length and weight also decreased (P = 0.008 and P = 0.015 respectively) with addition of 7 g N supply. 'K.K. Cross' seemed to be the best line in terms of head parameter; it had the highest head weight, width and was more rounded.

TABLE 6.3: Line and nitrogen main effects on head parameters (Head length: length of cut head longitudinal from base to tip; Head width: width of cut head about mid portion; Head shape index: head length/head width).

Line	Head width ($cm^{1/2}$)	Head length ($cm^{1/2}$)	Head shape index	Head weght ($g^{1/2}$)
HRI 009617	3.409	3.966	1.168	24.33
HRI 003202	3.123	3.594	1.153	18.28
KK Cross	3.648	3.675	1.008	25.78
SED	0.0899	0.0754	0.0278	1.418
df	33	33	33	33
Probability (*P*)	<0.001	<0.001	<0.001	<0.001

Nitrogen	Head length ($cm^{1/2}$)	Head weight ($g^{1/2}$)
7 g additional N	3.666	21.31
No additional N	3.824	24.28
SED	0.1066	1.582
df	33	33
Probability (*P*)	0.008	0.015

Data were transformed (square root) and transformed data presented.

SED = Standard error of the difference; df = Degrees of freedom

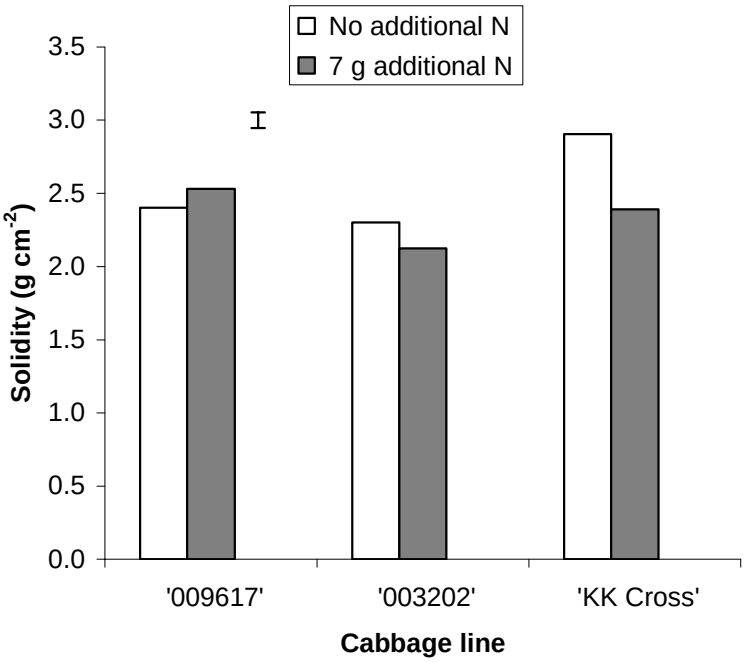

FIGURE 6.2: Head solidity in three lines of cabbage in response to 7 g additional N supply. Bars show standard error of the difference (n = 48, df = 33).

All the three and two way interactions (except that of the line x N) and the sucrose main effect were not significant ($P > 0.05$) as far as head solidity was concerned. The combined effect of line x N was therefore presented (Figure 6.2). There were no differences between the plants that were supplied with N compared with the control for all lines except K.K. Cross where additional supply of 7 g N reduced the solidity significantly.

Most of the combined effects of the three factors for SPAD meter value for greenness were not significant. The exceptions were line x date and nitrogen x date interactions (Figures 6.3a and 6.3b respectively). K.K. Cross consistently showed the lowest level of greenness from 21 d onwards while the rest did not

show differences over almost all dates. The additional 7 g N supply to the cabbage plants led to higher level of greenness compared to the control over all dates except 14 and 28 DAT where the differences were not significant (Figure 6.3b). There was a decline in greenness from week 4 onwards for the control while plants supplied with additional N continuously increased in greenness throughout the 6 week period. Sucrose increased the SPAD meter value for greenness independently (Figure 6.3c).

Error! Not a valid link. FIGURE 6.3a: Average SPAD meter values of four lines of cabbage over time (bar a represents one standard error of the difference for comparing means between lines, df = 225; bar b compares different dates for the same line, df = 113)

Error! Not a valid link.

FIGURE 6.3b: Average SPAD meter values of additional N application over time (bar a represents one standard error of the difference for comparing means between N levels, df = 225; bar b compares different dates at the same level of N, df = 40).

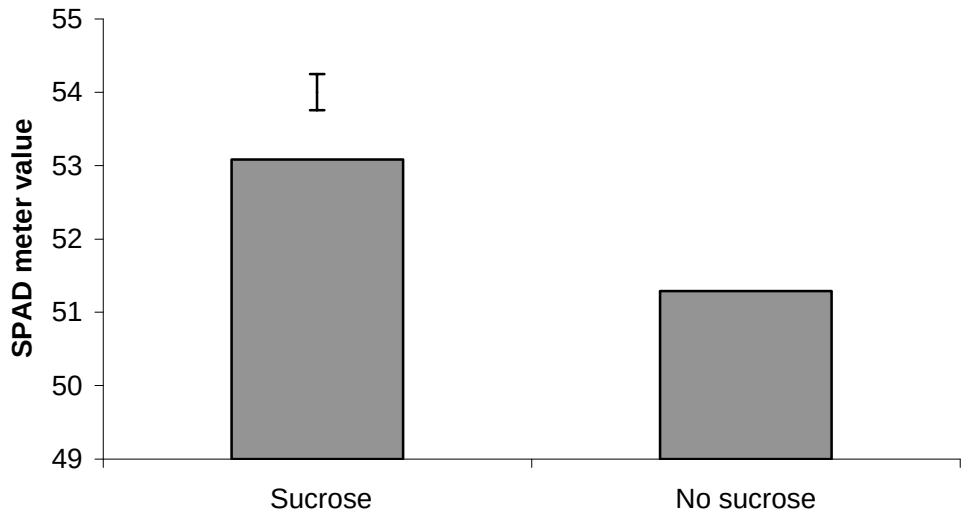

FIGURE 6.3c: Average SPAD meter values of sucrose and control treated plants (bar represents one standard error of the difference; df = 225).

6.3.2 Relationship between SPAD meter values and total chlorophyll

There were significant positive relationships ($P < 0.001$; $R^2 = 0.8216$) between SPAD meter value for greenness and the total chlorophyll for data pooled from all the four cabbage lines (Figure 6.4), however, the slopes and intercepts varied between lines ($P < 0.001$, Figure 6.5). Hence the total chlorophyll associated with a SPAD meter value of 60, for example, ranges from 1.6-2.8 mg m^{-2} depending on the line.

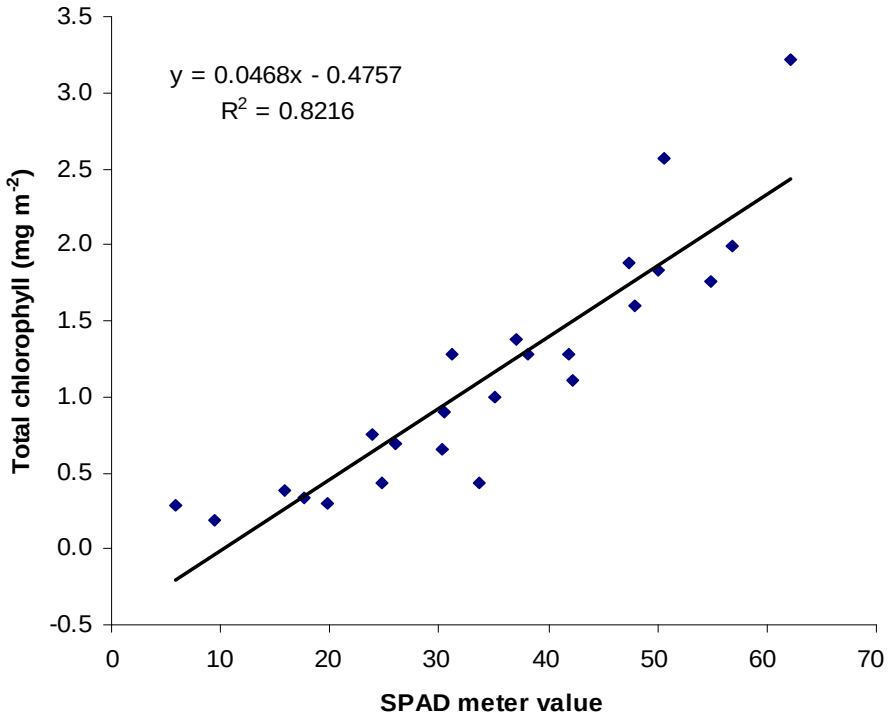

FIGURE 6.4: Linear regression for SPAD value for greenness and total leaf chlorophyll from four lines of cabbage.

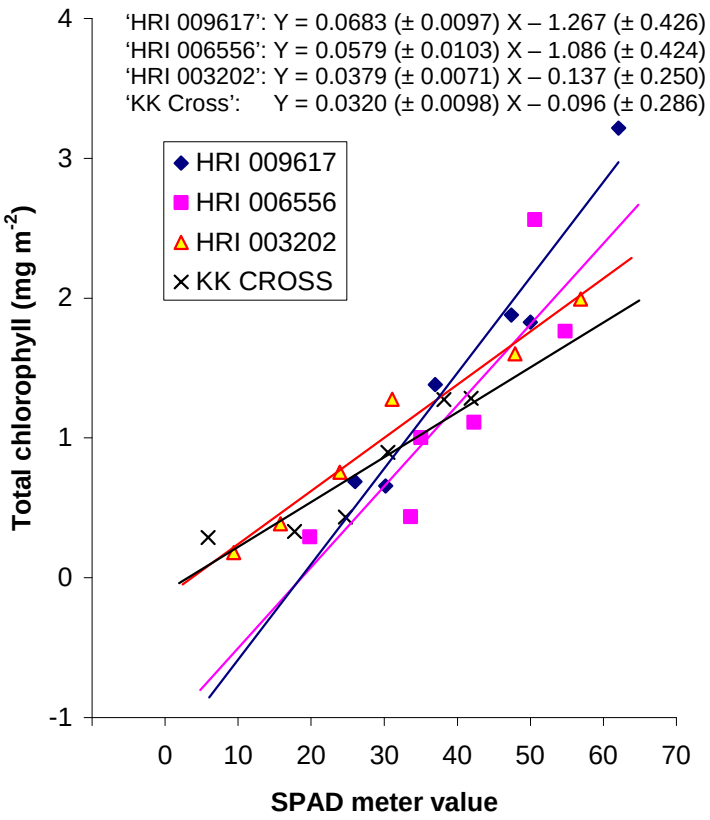

FIGURE 6.5: Linear regression for SPAD value for greenness and total leaf chlorophyll of four lines of cabbage.

Figures in brackets represent standard errors of the slope and intercept respectively.

6.4 DISCUSSION

6.4.1 Sucrose and nitrogen effects

The plants supplied with either sucrose or N grew more vigorously and appeared greener than the control. Hence, it was not unexpected that treated plants of line 'HRI 006556' took more time for vegetative growth before flower initiation and that may have accounted for the slight delay in flowering of either N or sucrose treated plants (Table 6.1). The reduction in number of days to flowering after transplanting (DAT) of this line by the combined effect of sucrose and additional N as compared to when applied on their own is relevant. This line can flower without vernalization and is believed to undergo a vernalization-independent pathway which utilizes sucrose at the apical point to stimulate the plant to flower (Dennis *et al.*, 1996). N is also known to promote flowering in some plants (Parkinson, 1952 cited by Hand, 1988; Colder and Cooper, 1961). In winter rye, for example, Gott *et al.* (1955) showed that N has little or no effect at any stage of flowering in fully vernalized plants, but in unvernalized material a low N level may slightly retard progress to flowering. It was therefore not unexpected that the combined effect of the two factors promoted early flowering of the line as compared with when the treatments were applied independently. The results gave an indication that neither N nor sucrose alone was involved in the flowering of that line. It is probable that sucrose and other N related metabolites are transported to the stem apex to stimulate flowering. This assertion agrees with the recent findings of Bernier and Perilleux (2005), who identified sucrose and reduced N compounds as part of substances involved in the flowering of *Sinapis alba.*

Supply of 3 % sucrose and 7 g N increased stem height independently (Table 6.2) without affecting the number of leaves at flowering. Plants supplied with either additional N or sucrose were probably able to photosynthesize better than the control and accumulated more carbohydrate into the cells. This may have led to more rapid cell division and cell expansion resulting in increased stem height. Wien and Wurr (1997) indicated that under conditions marginal for induction of flowers in cabbage, stem elongation appears to aid flowering as well. Therefore, the increase in stem due to the application of 3 % sucrose or 7 g N may have aided flowering in 'HRI 006556' to some extent.

Additional supply of 7 g N caused head splitting of all the three lines that did not flower (Figure 6.1). This implied that N led to rapid and continuous growth of the plants after heading. It is known that under favourable growing conditions, the inner leaves can exert sufficient pressure at maturity to cause head splitting (Dickson and Wallace, 1986). Although, head splitting is not a very good trait when a marketable head is required, it can be a good physiological phenomenon when it comes to seed production because it facilitates flower stalk emergence in vernalized plants, especially for lines that have a very dense head and require head splitting prior to flower emergence (Chapter 2, Nyarko *et al*., 2006a). In addition, the N reduced the solidity of 'K.K. Cross' (Figure 6.2). Unlike the other two lines ('HRI 009617' and 'HRI 003202') which formed loose or small heads and unfolded later to allow flower stalks to elongate (Nyarko *et al*., 2006a), 'K.K. Cross' formed a very dense head and reduction in head solidity (head density) caused by additional N supply would be expected to facilitate easier flower stalk

emergence. Supplying additional 7 g N may therefore be a good practice in seed production of 'K.K. Cross'.

It was observed that when the control plants were forming heads, the N treated plants were still forming wrapper leaves. Therefore, it likely that photosynthate was diverted for wrapper leaf production instead of head formation for plants that were given additional 7 g N which resulted in the reduction of head length and weight. The complex structure of chlorophyll is made up of the four basic elements C, H, O and N while that of sucrose is made up C, H and O (Streitweiser and Heathcock, 1981). It is therefore likely that both the additional supply of 7 g N and 3 % sucrose helped in the formation of more chlorophyll that resulted in the increased greenness (Figures 6.3b and 6.3c).

6.4.2 Relationship between SPAD meter values and total chlorophyll

Comparison between the regressions of the four lines gave an indication that, in order to predict the amount of chlorophyll from SPAD readings, it is necessary to develop a regression equation for each line of cabbage. Other workers have earlier reported that SPAD meter value for greenness may depend on the cultivar (Westerveld *et al.*, 2004; Soval-Villa *et al.*, 2002; Abdelhamid *et al.*, 2003). SPAD meter readings are based on the ability of leaf chlorophyll to absorb light of specific spectral band, i.e. red band (around 650 nm) where absorption by chlorophyll is high and in the infra red (around 940 nm) where absorption is low (Minolta, 1989; Peng *et al.*, 1993). This has made other workers suspect that differences in leaf thickness may account for the variability of SPAD meter readings between genotypes (Campbell *et al.*, 1990; Peng *et al.*, 1993) and this

assertion was confirmed when SPAD values adjusted for specific leaf weight (an index of leaf thickness) improved the prediction of rice leaf N from SPAD meter readings (Peng *et al.,* 1993). It is also known that SPAD meter readings vary greatly depending on growth stage (Campbell *et al.*, 1990; Peng *et al.,* 1993). Thus, the differences in growth stages of the four lines may also partly explain the different regression models for each line. 'HRI 003202' is early maturing, 'HRI 009617' and 'K.K. Cross' are late maturing and 'HRI 006556' is a non-heading line. This means that, at the time of measurements, the cabbage lines may have been at different growth stages which can lead to differences in SPAD readings and the corresponding leaf chlorophyll.

6.5 CONCLUSION

Sucrose and N increased the stem height at flowering ($P < 0.05$) of 'HRI 006556' independently without affecting the leaf number. The combined effect of 3 % sucrose and additional 7 g N promoted early flowering of 'HRI 006556' as compared to when sucrose was applied in isolation. Additional supply of 7 g N caused head splitting of three lines that did not flower and reduced the solidity of 'K.K. Cross'. SPAD meter value for greenness was affected by the combined effects of line x date and additional N x date with additional N supply increasing greenness consistently over almost all dates. *In vitro* sucrose (3 %) supplied to cabbage increased greenness independently. There were significant positive relationships between SPAD meter value for greenness and the total chlorophyll for data pooled from all the four cabbage lines, however, there were line

differences in the relationship that demanded the development of a regression equation for each line of cabbage for effective prediction of total chlorophyll.

CHAPTER SEVEN: GENERAL DISCUSSION AND CONCLUSIONS

The aim of this chapter is to discuss the thesis as a whole so as to draw conclusions based on all chapters. Therefore, in this section, a brief recapitulation of the study will be given, the benefits of the findings to the overall goal of the research and the possible future research in cabbage seed production in the tropics will be highlighted, and finally the general conclusions will be given.

7.1 RECAPITULATION OF THE STUDY

Part one (Chapters 2-4) evaluated some morphological and flowering characteristics of cabbage lines and ascertained the viability of cabbage seed produced under high temperature 20^{o}-30^{o}C (Chapter 2), evaluated two methods for screening lines of cabbage for heat tolerance and used the better method to select lines for future work (Chapter 3). The effects on the vigour of cabbage seed of osmotic priming with PEG 6000 at both vernalization and non-vernalization temperatures were determined in Chapter 4 to confirm the suitability of its application in subsequent experiments.

Part two (Chapters 5-6) determined whether cold treatment of imbibed seeds or *in vitro* plants, combined with the application of gibberellic acid (GA_3) to green plants, can induce flowering in cabbage at tropical temperatures (Chapter 5) and finally examined whether increasing the sucrose concentration to seedlings *in vitr*o and subsequently increasing nitrogen supply to plants in soil can affect head parameters, greenness and flowering in cabbage at 20^{o}C (Chapter 6).

7.2 THE FINDINGS OF THE STUDY AND THE OVERALL GOAL OF THE RESEARCH

The finding that cabbage can produce viable and vigorous seeds at temperature range between 20°-30°C (Chapter 2) which is typical of the cooler parts of the tropics was the base for subsequent experiments. The findings identified the absence of cold (0°-10°C) for vernalization of cabbage plants as the major hindrance to cabbage seed production in the cooler part of the tropics, such as Abetifi (in Ghana) and Kabete (in Kenya) where the daily average temperatures are less than 25°C in the rainy seasons. From these results, well established seed companies could obtain growth rooms where plants in pots could be vernalized before transplanting to the field for seed production. Many small scale seed producers in developing countries cannot afford such growth rooms individually, therefore it will be recommended for producers in a particular area to form a cooperative group to be able to acquire such facilities.

The author, who is from a tropical country, was familiar with cabbage cultivation only up to the heading stage. Therefore, the morphological and flowering studies in Chapter 2 were not only useful for studying the complete life cycle of the plant in tropical conditions but also served as an independent assessment of actual performance against which other subsequent tests were compared.

There are many cabbage lines whose heat tolerance is unknown. Warwick-Horticulture Research Institute (Wellesbourne, Warwickshire, UK) alone have 655 different genotypes in their possession. Chlorophyll fluorescence measurements

(Chapter 3) could therefore be used for rapid identification of heat tolerant lines for commercial production, potential seed production and breeding programmes in tropical areas.

The finding that priming of cabbage seed with PEG 6000 had no adverse effect on the vigour at both vernalization and non-vernalization temperatures (Chapter 4), would have a wider application for some *Brassica* species that are sensitive to seed vernalization. *Brassica* with A genome (n = 10) and B genome (n = 8) have been classified as seed vernalization type and weak seed vernalization type respectively (Kagawa, 1971) as reported by Inouye and Kuo (1981). Such *Brassica* can be primed and vernalized for relatively longer periods up to 8 weeks in a refrigerator and later grown in the tropical environment for seed production. In this study, the cabbage seeds were primed and vernalized at the same time in the subsequent experiment (Chapter 5) as a consequence of the usefulness of the practice revealed in the previous chapter (Chapter 4). Priming prevented seed germination and growth during vernalization in the refrigerator (dark) and the subsequent etiolation of seedlings, but rather promoted seed vigour.

For line 'HRI 009617', which was induced to flower by the combined effects of seed vernalization and GA_3 treatments (Chapter 5), and any other lines that were later found to respond to the treatments, their seed production could easily be achieved in the tropics as the problem of green plant vernalization could be overcome by vernalizing the seeds in a refrigerator. More importantly, the benefits of ratoons could be realized for such lines. Plants raised from vernalized seed could be grown for cabbage heads. After harvesting the heads, ratoons could

be raised and GA_3 applied later as described in Chapter 5. In this way, both heads and seeds could be obtained from each plant which may increase the revenue of the seed producer. Earliness in seed maturity could also be achieved from ratoon plants as compared to those obtained from seed since plants from ratoons took fewer days to flower. The major hindrance that is likely to affect the commercial seed production from ratoons will be the cost of GA_3 as it has to be applied thoroughly to each plant once a week for about seven weeks. Thus, cost analysis should be carried out to ascertain the profitability before widespread adoption.

For lines that do not respond to seed vernalization-GA_3 treatment, some findings in Chapters 1 and 5 could currently be combined to achieve reasonable results in their seed production in the tropics. In Chapter 1, it was established that cabbage can produce vigorous seeds in the cooler part of the tropics provided the green plants are vernalized whereas, in Chapter 5, it was found that more flowering plants could be generated from cuttings of the reproductive stalk without vernalization. With this information, it is suggested that a few controlled environment rooms could be built for vernalization of cabbage plants in pots in order to supply seed producers with some vernalized plants. From these, more flowering plants could be generated from cuttings of the flowering stalks for seed production. This option will be cheaper than the earlier one suggested because only a few vernalized plants (one or two plants) will be needed initially by a seed producer for the generation of more flowering plants. In this study, it was established that flowering plants can be generated from flowering stalks at 20^{o}C but the seed yield per hectare was not evaluated. There is therefore the need to

evaluate seed yield of plants generated from flowering stalks as compared to what would be obtained from the mother plants before widespread application.

In vitro supply of 3 % sucrose and application of 7 g N increased stem height and promoted early flowering in line 'HRI 006556'. The treatments also caused head splitting and increased greenness in most lines (Chapter 6). It has been reported that, when conditions are not optimal for flower production in cabbage, any factor that increases stem height appeared to promote flowering (Wien and Wurr, 1997). It was also found in a previous experiment (Chapter 2) that two lines which flowered easily without vernalization were consistently greener than those that were difficult to flower. Further, 7 g N reduced the solidity (head density) of 'K.K. Cross' (Chapter 6), a phenomenon that is likely to facilitate the easy emergence of flowering stalks from densely headed cabbage lines. These findings showed that despite the fact that sucrose and N could not directly induce flowering in almost all lines, the application of these treatments may facilitate flower formation and flower stalk emergence. Therefore, it will be appropriate to do more investigations to confirm the possibility of incorporating sucrose and N treatments to cabbage seed production in the tropics.

It has been the desire of the author to get cabbage flower induction at temperatures around 15^{o}-25^{o}C. These temperature regimes are normally found in the higher altitudes in the tropics and some of these areas can be reserved solely for seed production of *Brassica* species like cabbage. Farmers in the tropics normally grow cabbage for marketable heads at temperature range of about 28^{o}-38^{o}C. Therefore, getting cabbage to flower and produce seeds only at temperatures below 25^{o}C will

be beneficial because it will ensure that farmers using seeds grown in the high altitudes for production of cabbage heads will not have the problem of the plants developing flowering stalks prematurely (bolting). Bolting at low temperatures is one of the major problems of cabbage production in temperate countries (Roberts *et al.*, 1997) as it reduces the market value of heads. Thus, efforts should be made to ensure that cabbage do not bolt in areas where marketable heads are produced in the tropics.

7.3 RECENT SCIENTIFIC KNOWLEDGE AND FUTURE RESEARCH

Two of the ten lines of cabbage ('HRI 0011446' and 'HRI 006556') used for the study were able to flower with or without green plant vernalization (Chapter 2). The line 'HRI 0011446' is a non-heading type and both the vernalized and the non-vernalized plants of that line went straight to flowering without forming heads. Interestingly, while the vernalized plants of 'HRI 006556' flowered without head formation, the non-vernalized plants formed a large loose head at relatively high temperatures (20^{o}-30^{o}C) before they eventually flowered. It seems these lines (especially 'HRI 006556') could be used to introduce flowering trait into some of the head cabbage lines that are difficult to flower at the expected temperature regimes (15^{o}-25^{o}C). It is hoped that cabbage breeders will take this up in future research.

The induction of flowering in line 'HRI 009617' by the combined effect of seed vernalization and GA_3 (Chapter 5) and *in vitro* vernalization and GA_3 raised some issues that could be considered for future work. It seems that the treatments may

induce flowering in some lines of cabbage if the factors are varied. The duration of the vernalization period, for example, is known to vary between lines (Bernier *et al.*, 1981) and it has also been reported that when long vernalization duration was applied to cabbage plants, high temperatures at the end of the cold period were not effective in causing devernalization (Heide, 1970 as cited in Wien and Wurr, 1997). Further, recent study in *Arabidopsis* revealed that plants that had been cold-treated as seeds for less than 14 d did not bolt, whereas those that had been cold-treated for 28 d bolted easily at 23^{o}C (Sheldon *et al*., 2006; Sung and Amasino, 2004). It is also known (Ratcliffe *et al*., 2003) that the vernalization pathway involves two genes, *VERNALIZATION-INSENSITIVE 3 (VIN3)* and *MAD AFFECTING FLOWERING 2 (MAF2)*, which ensures that cold period of insufficient duration will not cause flowering. Thus, extending the vernalization period from 8 weeks to say 12 weeks may cause some of the other lines that were unable to respond to flower induction in the previous experiment to do so. This could easily be achieved if the seeds are primed to prevent germination and possible fungal infection to allow a longer period for vernalization. It will therefore be advisable to consider varying the factors, especially the duration for the seed vernalization, in future research.

Apart from sucrose and gibberellins, other long distance floral signals identified recently include reduced N-compounds and cytokinins that are translocated in the phloem sap from leaves to shoot apical meristems to induce flowering (Corbesier and Coupland, 2006). There is also evidence that many genes interact in various ways to either promote or repress flowering (Corbesier and Coupland, 2005; Bernier and Perilleux, 2005). The movement of the floral signals and the activities

of floral genes are known to be triggered by environmental factors which were attributed mainly to winter cold, photoperiod or both. It therefore seems that flowering of cabbage in the tropics would be best handled from both physiological and genetic approaches, since the identification of floral control genes of a particular species and the knowledge of the right environmental factors to initiate the genes' actions require both disciplines. In the last couple of years, more complex pathways have been proposed for *Arabidopsis* because of the increasing interest of other secondary environmental factors, such as irradiance, ambient temperature, light quality, water availability and mineral availability, which may substitute for vernalization. Obviously, as these environmental factors affect each other, Bernier and Perilleux (2005), in their recent review on flowering control, proposed a number of cross-talks between pathways involving various environmental factors and hormones (mainly gibberellins). Thus, easy induction of flowering in cabbage is likely to be achieved with a more comprehensive approach involving other environmental factors formerly considered as secondary, and understanding their relationships with floral genes and floral signals. The knowledge of these relationships is well advanced in *Arabidopsis* but it has not been applied in cabbage to determine whether there are similarities or *vice versa*. There were indications from the present work that GA_3, sucrose, nitrogen, ambient temperature, seed and plant vernalization can affect the flowering of some lines of cabbage. It will be of interest, in future research, to understand how these factors affect floral genes and signals in cabbage. It will also be of interest to repeat some of the experiments in field conditions in tropical countries for confirmation before widespread application.

7.4 CONCLUSIONS

- Viable cabbage seeds can be produced in a tropical temperature regime (20^{o}-25^{o}C) provided the plants are vernalized (0^{o}-10^{o}C), thus suggesting that seed production may be possible in the cooler parts of the tropics.

- Chlorophyll florescence parameters (Fv and Fv/Fm ratio) could be used to select cabbage lines (meant for the tropics) for heat tolerance. The cell membrane thermostability test may be useful provided the replication is increased to about 6 or by bulking samples from more plants.

- At least one line of cabbage can be induced to flower by seed vernalization and gibberellic acid (GA_3) treatments from plants raised from both ratoons and seeds.

- Cabbage seeds can be primed with PEG 6000 and vernalized for a longer period of time up to 8 weeks to prevent growth and to enhance vigour. This is a pre-requisite to flowering and seed development in the tropics for lines that can be induced to flower by the combined effects of seed vernalization and GA_3 treatment.

- More flowering plants can be generated from cuttings of the flowering stalks at 20^{o}C without further vernalization.

- Both GA_3 and seed vernalization promote early flowering independently in lines 'HRI 006556' and 'HRI 0011446'. Again, GA_3 treatment could be substituted for *in vitro* cold treatment to promote early flowering in line 'HRI 006556'.

- Therefore, there is hope for cabbage seed production in the cooler parts of the tropics.

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APPENDIX 1: TOWARDS CABBAGE (*Brassica oleraceae* var. *capitata* L) SEED PRODUCTION IN THE TROPICS

APPENDIX 2: PROMOTION OF VIGOUR IN CABBAGE SEED BY OSMOTIC PRIMING PRE-TREATMENT AT BOTH VERNALIZATION AND NON-VERNALIZATION TEMPERATURES

APPENDIX 3: INDUCTION AND GENERATION OF FLOWERING IN CABBAGE PLANTS BY SEED VERNALIZATION, GIBBERELLIC ACID TREATMENT AND RATOONING