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Manipulation of flowering for seed production of shallot (*Allium cepa* L. var. *ascalonicum* Backer)

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Beeinflussung des Blühverhaltens zur Saatgutproduktion von Schalotte (*Allium cepa* L. var. *ascalonicum* Backer)

Zusammenfassung

Die Schallote ist die wichtigste Untergruppe der Aggregatum Gruppe und die einzige, welche kommerziell genutzt wird. Die Vermehrung erfolgt meist vegetativ durch Zwiebeln, was einen großen Bedarf an Mutterzwiebeln (1,2 t/ha) voraussetzt. Diese besitzen jedoch schlechte Lagereigenschaften und können über Generationen hinweg Krankheiten beherbergen, weswegen sie als Pflanzmaterial nicht bevorzugt werden. Eine Alternative, welche in den letzten Jahren zunehmend diskutiert wird, stellt die Vermehrung durch Samen dar. Die Produktion von Samen setzt die Anlage einer großen Anzahl an Blütenknospen voraus. Diese resultiert einerseits aus für die Vernalisation optimalen Umweltbedingungen und andererseits aus der Wahl des richtigen Pflanzenmaterials. Ziel dieser Untersuchung war es daher, die optimale Temperatur und Photoperiode in Bezug auf unterschiedliche Pflanzenstadien zu finden. Es wurde die Schossneigung intakter Zwiebeln sowie wachsender Pflanzengeprüft, wobei das Pflanzmaterial sowohl aus Saatgut als auch aus Zwiebeln gezogen wurde. Ferner sollte ein für die Vernalisation optimales Entwicklungsstadium bestimmt werden.

Der Einfluss von Temperatur, Photoperiode und die Dauer dieser Stimuli auf das vegetative Wachstum, die Infloreszenzbildung und das Schossen der Schallote wurden anhand der vier Sorten ´Ambition F1´, ´Matador F1´, ´Bonila F1´ und ´Creation F1´ untersucht. Aus Samen gezogene Pflanzen wurden unter den Faktorkombinationen von 4, 8, 12 und 18 °C sowie einer Photoperiode von 12 und 16 Stunden für 30, 60 und 90 Tage kultiviert. Mit dem Ansteigen der Temperatur wurde insbesondere bei der 16 Stunden Lichtbehandlung ein verstärktes Schossen beobachtet. Pflanzen, welche über 90 Tage bei 18 °C kultiviert wurden, produzierten noch vor Ende der Behandlung reife Zwiebeln. Obwohl bei den Sorten ´Bonila F1´ und ´Creation F1´ bei 4, 8, und 12 °C Infloreszenzen angelegt wurden, waren diese nicht in der Lage sich weiter zu entwickeln. Die höchste Temperatur (18 °C) führte bei keiner Sorte zum Schossen oder zur Anlage von Blüten. Während die Sorte ´Ambition F1´ bei 8 °C und 16 Stunden Licht nach 60 Tagen 60 % Schosseranteil aufwies, benötigte ´Matador F1´ zusätzliche 30 Tage bei gleicher Behandlung, um dne gleiche Anteil Schosser zu erreichen.

Ein weiterer Versuch wurde zur Untersuchung der relativen Leistung von drei unterschiedlichen Pflanzenmaterialtypen aus Samen regenerierte Pflanzen (PS), Pflanzen aus

Zwiebeln (PB) sowie intakten Zwiebeln (B) angelegt. Das Pflanzenmaterial aller drei Varianten wurde 60 Tage bei 8 und 12 °C kultiviert. Der Anteil schossender Pflanzen bei PS und PB war signifikant höher als bei B. PS hatte verglichen mit PB und B kräftigere Blütenstände mit mehr Blüten pro Dolde. PB, PS und B produzierten durchschnittlich 8, 7 und 4,5 Blütenstände pro Pflanze. PB hatte somit im Vergleich zu PS und B 90 % bzw. 70 % mehr Einzelblüten pro Pflanze.

Aufgrund des geringen Anteils schossender Pflanzen (ca. 65 %) in dem vorangegangenen Experiment, folgte ein Versuch, der klären sollte, ob sich der Schosseranteil durch eine Verlängerung der Vernalisationsperiode erhöhen lässt. Zwiebeln der Sorten ´Ambition F1´, ´Matador F1´ sowie sieben weitere Schalottensorten, welche vegetativ vermehrt worden waren, wurden für 30, 60 und 90 Tage bei 8 °C kultiviert. Keine der vegetativ vermehrten Schalottensorten blühte. Im Gegensatz zu dem vorangegangenen Experiment wurden bei ´Ambition F1´ und ´Matador F1´ trotz verlängerter Vernalisationsdauer keine Zunahme des Schosseranteils erzielt.

Um das optimale Entwicklungsstadium zu bestimmen, bei welchem die Schalotte für ein zufriedenstellendes Schossen vernalisiert werden kann, wurde ein weiterer Versuch durchgeführt. Die Langtagssorte ´Matador F1´ sowie die beiden Kurztagssorten ´Tropix F1´ und ´Rox F1´ wurden bei 18 °C und einer Photoperiode von 16 bzw. 12 Stunden für jeweils 120, 90, 60, 30 und 0 Tagen auf der Basis gequollener Samen (IS) kultiviert. Im Anschluss wurden die Keimlinge und die IS in Klimakammern bei 8 °C für 60 Tage vernalisiert. Ein vollständiges Schossen der behandelten Pflanzen wurde bei ´Tropix F1´ mit Erreichen des 6-Blatt-Stadiums (nach 90 Tagen), bei ´Rox F1´ etwa beim 17-Blatt-Stadium (120 Tage) sowie bei ´Matador F1´ etwa beim 12-Blatt-Stadium erreicht.

Im 6-Blatt-Stadium von ´Rox F1´ und im 7-Blatt-Stadium von ´Matador F1´ schossten hingegen nach 90 Tagen Kulturdauer lediglich 75 bzw. 60 % der Pflanzen. Abgesehen vom hohen Schosseranteil besaßen Pflanzen, welche eine ausreichende Größe erreichten, auch einen hohen Anteil an Blütentrieben. Ferner vervollständigte ein Großteil dieser Pflanzen das Schossen kurz nach Beendigung der Vernalisationsphase.
Mittels der in den Experimenten gewonnen Daten wurde versucht, den Schosseranteil anhand einer logistischen Regression vorherzusagen.

Aus den Ergebnissen lässt sich folgern, dass für die Vernalisation wachsender Pflanzen, welche entweder aus Zwiebeln oder aus Samen gezogen werden, bei 8 oder 12°C eine Dauer von 60 Tagen ausreichend ist, um ein vollständiges Schossen zu erzielen. Bei den aus Samen gezogenen Pflanzen muss die Vernalisation nach Abschluss der Jugendphase erfolgen, welche je nach Sorte unterschiedlich lang dauert. Ist keine Saatgut, sondern eine Zwiebelproduktion beabsichtigt, sollten die Pflanzen nach Abschluss ihrer Jugendphase diesenBedingungen nicht mehr ausgesetzt werden.

Schlüsselworte: Schalotte, Vernalisation, Temperatur, Photoperiode, Schossen, Kohlenhydrate

Manipulation of flowering for seed production of shallot (*Allium cepa* L. var. *ascalonicum* Backer)

Abstract

Shallot (Alliaceae: *Allium cepa* var. *ascalonicum* Backer*)* is the most important subgroup of the Aggregatum group and the only one grown commercially. However, it is mainly propagated vegetatively by bulbs which requires a large quantity (1.2 ton ha^{-1}) of mother bulbs. Bulbs have poor keeping quality and also harbor diseases through generations. Thus, they are less preferable as planting material. Alternatively, reproduction through seeds was believed to overcome the aforementioned problems and enables genetic improvement of the crop through hybridization. Production of seeds requires high bolting resulting from optimum environmental and plant variables suitable for vernalization. The objectives of the present study were, therefore, to find out the optimum temperature and photoperiod, to compare the bolting capacities of intact bulbs and growing plants regenerated either from seeds or bulbs, and to identify the right age of the plants for vernalization treatment that gives high bolting.

The influence of temperature and photoperiod, and the duration of these stimuli on vegetative growth, inflorescence initiation and bolting of shallot were studied using four shallot varieties: Ambition F1, Matador F1, Bonila F1 and Creation F1. Plants raised from seeds were treated at factorial combinations of 4, 8, 12 and 18^{o}C under 12 and 16 h photoperiods for 30, 60 and 90 days. An increase in bulbing was observed with increase in temperature especially under 16 h photoperiod; plants treated at 18^{o}C for 90 days produced mature bulbs before the end of the treatment. Initiation of inflorescences was observed in plants of Bonila F1 at 8 and 12^{o}C and of Creation F1 at 8^{o}C, but they did not develop further. The highest temperature (18^{o}C) did not cause initiation of inflorescences in any of the varieties. The proportion of bolting obtained in Ambition F1 treated under 8^{o}C and 16 h for 60 days was more than 0.6, but Matador F1 required an additional 30 days of the same treatment to attain a similar magnitude of bolting.

An attempt was also made to investigate the relative performance of three different types of shallot planting materials: plants regenerated from seeds (PS), plants regenerated from bulbs (PB) and intact bulbs (B). The planting materials were treated at 8 and 12^{o}C for 60 days. The proportion of bolting plants of PS and PB was found to be significantly higher than of B. PS

had vigorous inflorescences that contained more florets per umbel compared to PB and B. On the average, PB, PS and B produced 6, 3.7 and 2.3 inflorescences per plant, respectively. Hence, PB had 90 and 75% more florets per plant than PS and B, respectively

Due to the low proportion of bolting (about 0.64) obtained form vernalization of bulbs in the previous experiment, a follow up experiment was initiated to increase botling by extending the duration of vernalization. Bulbs of Ambition F1, Matador F1 and of seven other vegetatively propagated shallot varieties were treated at 8^{o}C for a duration of 30, 60 and 90 days. However, none of the vegetatively propagated shallot varieties could flower. No increment in bolting was obtained, compared to the previous experiment, in Ambition F1 and Matador F1 despite increased duration of vernalization.

A further study was conducted to identify the optimal stage of development at which shallot plants can be vernalized to achieve satisfactory bolting. One longday (LD) variety, Matador F1 and two shortday (SD) varieties, Tropix and Rox F1, were grown in two separate greenhouses under 18^{o}C and 16 and 12 h photoperiods, respectively for a period of 120, 90, 60, 30 and 0 (imbibed seeds; IS) days. The seedlings and the IS were then vernalized in growth chambers at 8^{o}C for 60 days. Complete bolting was obtained in plants that were treated after they had attained about six-leaf stage (after 90 d) in Tropix, 17 leaf-stage (120 d) in Rox F1 and 12 leaf-stage in Matador F1. However, at six leaf-stage in Rox F1 and seven leaf stage in Matador F1 (after 90 d of growth), the proportion of bolted plants was only 0.75 and 0.60, respectively. Apart from the high proportion of bolting, plants that attained sufficient size also had a high proportion of floral shoots and bolted shortly after the end of vernalization. In all the experiments, an attempt was also made to predict the proportion of bolting using logistic regression.

In general, we can conclude that vernalization of growing plants regenerated either from bulbs or seeds at 8 or 12^{o}C for 60 days is sufficient to attain complete bolting in the varieties tested. In the case of plants regenerated from seeds, however, the plants must be treated at their post juvenile stage depending on the varieties. Whereas plants meant for bulb production should not be exposed to these conditions at their adult stage.

Key words: shallot, vernalization, temperature, photoperiod, bolting, carbohydrate

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List of abbreviations

Abbreviation	Description	Unit
AIC	Akaike information criterion	-
B	Intact bulbs	-
BR	Bulbing ratio	-
cv(s)	Cultivar (s)	-
D	Duration	days
d	Days	days
DAV	Days after vernalization	days
DFMB	Days to 50% of maximum bolting	days
DW	Dry weight	mg, g
IS	Imbibed seeds	-
LD	Longday	h
LN	Longnight	h
P	Photoperiod	h
PB	Plants regenerated from bulbs	-
PPFD	Photosynthetic photon flux density	$\mu mol\ m^{-2}s^{-1}$
PS	Plants regenerated from seeds	-
SD	Shortday	h
T	Temperature	^{o}C
TNB	Total number of buds	-
TSCHO	Total soluble carbohydrates	mg, g/100g DW
V (var)	Variety	-

Chapter 1

General introduction

Shallot (Alliaceae: *Allium cepa* var. *ascalonicum* Backer*)* is the most important subgroup of the Aggregatum group and the only one grown commercially (Rabinowitch, 1990). It is cultivated in southeast Asia and some east African countries (Currah and Proctor, 1990). It is also grown in the United States (Jones and Mann, 1963), in some European countries (Messiaen *et al*., 1993 cited by Krontal *et al*., 1998) and in South America (eg. Argentina; Krontal *et al*., 1998) because of its unique flavour.

Shallot is mainly propagated vegetatively by bulbs. The use of bulbs as sole planting material has several problems. A large quantity (1.2 ton ha^{-1}) of mother bulbs is used as planting material (Jackson *et al*., 1985) which is expensive, bulky to transport and needs well conditioned storage. Moreover, bulbs have poor keeping quality and also carry fungal diseases such as *Fusarium* spp. (Mengistu and Seid, 1990) and latent viruses (Proctor, 1987) from generation to generation. Thus, bulbs, are invariably less preferable as planting material.

Currah and Proctor (1990) reported that multiplication of shallot from true seeds has promising benefits in terms of size and productivity of plants and may solve some of the aforementioned problems. In addition to ease of propagation, it enables genetic improvement of the crop through hybridization. Many of these plants were reported to be more vigorous than their parents, possibly because they were free from viruses. However, bolting does not occur readily in many shallot varieties and needs pre-treatment of plants or bulbs. An attempt to produce shallot through seeds, therefore, requires an investigation of flowering behaviour.

Flowering

Flowering is an essential developmental process in the production of crops for food, feed and aesthetic values. Much effort is currently being put into regulating the time of flowering either to abbreviate or extend the vegetative phase, or to conveniently induce or repress flowering (van Nocker, 2001). When the desirable part of a crop plant consists of vegetative structures as in beet (*Beta vulgaris* L.), sugarcane (*Saccharum officinarum* L.), lettuce (*Lactuca sativa* L.), etc., flowering is to be avoided because it consumes energy that should preferentially be diverted to the desired vegetative organs (Kinet, 1993).

Flowering is a multistage (multi-sequential) process composed of sequences of events temporally and spatially ordered; no single initial event can set in motion all the subsequent events (Bernier *et al*., 1981). These sequential events have their own specific requirements and are affected differently by environmental variables such as temperature, photoperiod, light intensity and quality, etc. (Kinet and Sachs, 1984). Other species are less sensitive to these variables and appear to flower in response to internal (plant) variables such as plant size or number of vegetative nodes (Levy and Dean, 1998).

Genetic and physiological studies in *Arabidopsis* suggest that there are at least four pathways corresponding to these variables that promote flowering: vernalization pathway, photoperiod pathway, GA-dependent pathway (Wilson *et al*., 1992; Blazquez *et al*., 1998) and an autonomous or constitutive pathway (Koornneef *et al*., 1998; Simpson *et al*., 1999). The pathways are governed by a sequential action of two groups of genes: floral meristem identity genes (switch the fate of the meristem from vegetative to floral), and organ identity genes (direct the formation of the various flower parts; Levy and Dean, 1998).

One of the characteristics of mutants in the vernalization and autonomous pathways is that they exhibit a significant vernalization response, i.e., the late–flowering phenotype can be fully 'rescued' by a long-term cold treatment given to an imbibed seed or to a young plant. In contrast, cold is largely ineffective to accelerate flowering of the photoperiodic pathway mutants (Martinez-Zapater *et al*., 1994).

Temperature (vernalization)

Vernalization is the acquisition or acceleration of the ability to flower by chilling treatment (Chouard, 1960 cited by Bernier *et al*., 1981). It triggers (induces) vegetative meristems that had been producing vegetative structures such as leaves to switch to producing flowers (Evans, 1971). Vernalization requirement is a need for fulfilment of a low temperature period, in sensitive genotypes, in order to avoid delays in development to reach floral initiation (Appedino *et al*., 2003). It plays an important role in the control of heading time and adaptation to low winter temperatures (Kato and Yamashita, 1991 cited by Kato *et al*., 1997). It also prevents flowering from occurring before the end of the frosty period; it synchronizes flowering to the spring season (Levy and Dean, 1998).

Several researchers reported that growing leaves or mitotically active tissues (Wellensiek 1962 and 1964 cited by Bernier *et al*., 1981), apexes of intact plants (Lang, 1965), and fully expanded leaves and even some cells other than the apex (but not all cells; Metzeger, 1988) can be vernalized. The vernalized state, however, is not passed to the next generation; a progeny derived from vernalized plant must be re-exposed to cold to be vernalized.

In cold-requiring (vernalization responsive) plants, as in photoperiodic plants, there are species with an obligate (qualitative) and species with a facultative (quantitative) cold requirement (Bernier *et al*., 1981). As a rule, plants with a facultative requirement can be vernalized as imbibed seeds whereas those with an obligate requirement cannot and must reach a certain size for attainment of responsiveness to cold. Handerson *et al*. (2003) indicated that vernalization has a pronounced quantitative response; increasing exposure to low temperature leads to progressively accelerated flowering time. However, the response is saturable, reaching a point at which further exposure to cold does not lead to additional acceleration of flowering. The effectiveness of vernalization mainly depends on the magnitude and duration of temperature and its interaction with other variables that are known to affect flowering (Niu *et al*., 2002).

Genetic analysis of late and early flowering *Arabidopsis* ecotypes identified two major loci determining flowering time: the FRIGIDA (FRI) and the flowering locus C (FLC; Lee *et al*., 1993; Koornneef *et al*., 1998). Dominant alleles of these genes act synergistically and cause late flowering. The degree of lateness of non-cold treated plants is proportional to FLC copy number and presumably gene expression levels. An increase in FLC copy number can convert Arabidopsis from winter flowering into a biennial (Michaels and Amasino, 2000). Regardless of the FLC copy number and the consequent delay in flowering, vernalization can fully suppress the late-flowering effects of FLC; down regulates FLC transcripts. The extent of down regulation is proportional to the duration of the cold treatment and hence to flowering (Sheldon *et al.*, 2000). The down regulation of FLC activity persists throughout the development of the vernalized plant (eg. vernalization at seed germination and flowering occurring quite later) that change in gene expression being transmitted through many mitotic divisions. The high levels of FLC expression is reset in the progeny of a vernalized plant (Sheldon *et al.*, 2000).

On the other hand, it is hypothesized that vernalization activates flowering by demethylation of genes that are essential in the process of flowering (Finnegan *et al*., 1998 and Sheldon *et al*., 1999 cited by Sheldon *et al*., 2000). Growth at low temperature may disrupt maintenance methylation, the process by which patterns of DNA methylation are transmitted to newly synthesized DNA in dividing cells (Bird, 1978), perhaps through decreasing the activity of DNA methyltransferase. As a result methylation at sites in 'vernalization genes' would be diluted by successive cycles of DNA replication, accounting for the observed correlation between the duration of the cold treatment and the extent to which flowering is promoted (Napp-Zinn, 1957 cited by Sheldon *et al*., 2000).

Vernalization responsive genes are known in winter wheat (*Triticum aestivum* Vrc79; Zhao, 1999) and in a late-flowering ecotype of *A. thaliana* EARLI1 (Wilkosz and Schlappi, 2000) while a repressor of flowering and a controller of axiliary meristem was reported by Jenson *et al*. (2001). All together, more than 80 genes are known to be involved in the flowering process directly or indirectly.

As indicated above flowering is a multi-sequential process and plants usually integrate multiple cues during the decision to flower. In wheat and rape seed, for example, vernalization is often an obligate requirement but it is insufficient to induce flowering alone. Exposure to a subsequent longday photoperiod is required to induce the floral switch (Bernier *et al*., 1981).

Light duration (photoperiod) and quality

Photoperiod, the daily duration of light, is one of the environmental variables that influence flowering of plants (Bernier *et al*., 1981). Plants are classified as longday (LD) or shortday (SD) based on whether flowering is promoted when daylength is increased or decreased, respectively, but not on particular day lengths at which the plants flower. But in onion (*Allium cepa* L.) the classification refers to bulbing response rather than to flowering (Brewster, 1990). Qualitative (absolute) SD or LD plants have an obligate requirement for SD or LD without which flowering does not appear. These plants are characterized by an abrupt change in behaviour over a narrow range of daylengths and consequently have a sharp 'critical daylength'. On the other hand, quantitative (facultative) SD or LD plants produce flowers under any daylength but they do flower earlier in SD or in LD, respectively. Such plants may or may not have a clear cut critical daylength. The requirement of plants for photoperiod can

be a sequential LD and SD or *vice versa,* a repeated or a brief exposure to SD or LD, or a brief light interruption of long nights. On the other hand, *Pharabitis* spp., *Sinapis* spp. (Bernier *et al*., 1981) and onions (Rabinowitch, 1990) can flower in complete darkness. *Arabidopsis* spp. can also flower in complete darkness if the aerial portion of the plant is supplied with sucrose or glucose (Araki and Komeda, 1993).

Photoperiod is perceived in the leaves or cotyledons in photoperiodic plants, where a flower-inducing signal is produced and transmitted to the apex (O'Neil and Zhang, 1998). In contrast, *in vitro* cultures of root explants of *Cychorium intybus* and internode sections of *Plumbago indica* respond to photoinduction in the complete absence of leaves (Nitsch and Nitsch, 1967).

O'Neil and Zhang (1993) reported that the transition of the meristem from vegetative to reproductive growth is controlled by the cyclic alternation of light and darkness in photoperiodic plant, *Pharabitis nil*. The rhythmic changes in the red (R): far red (FR) light spectrum for photoperiodic induction of flowering is also a feature in LD plants and is controlled by phytochrome (Thomas, 1993). Guo *et al*. (1998) reported that the transition to flowering in *A. thaliana* is regulated by the antagonistic actions of R/FR light receptors (phytochrome-B, PHYTB) and blue/ultraviolet-A light receptors (cryptochrome-1, CRY1 and cryptochrome-2, CRY2). CRY2 normally acts not as a direct positive regulator under blue light, but as a negative regulator of the repression of flowering imposed by PHYB (Guo *et al*., 1998). FR, blue and R lights were all effective in promoting flowering when they are supplied in between dark periods although R light was the least effective (Goto *et al*., 1991; Hollyday *et al*., 1994; Carre, 1998). Daylength extensions with FR light or light rich in FR spectra (eg. incandescent light) are also very effective in promoting flowering (Goto *et al*., 1991; Bagnall *et al*., 1995). The effect of light on floral induction, however, may be permitted or denied at certain times of the day by the action of the circadian-clock (Brewster, 1982; Carre, 1998). In general, interactions of genes involved in the developmental control of floral initiation, regulation of circadian-clock and the signal transduction of photoreceptors control flowering in response to photoperiod (Thomas and Vince-Prue, 1997)

Wallace *et al*. (1993) reported that flowering response to photoperiod was controlled by a single gene in *Phaseolus vulgaris*; photoperiod sensitivity being dominant over insensitivity. They suggested that the photoperiod gene controls flowering due to its control over sink

activity (photosynthate partitioning) between reproductive and vegetative growth, presumably through changes in hormonal balance. Nevertheless, Koornneef *et al*. (1997) reported that the different types of phyotochrome that control photomorphogenesis are encoded by at least 4 to 5 different genes.

The requirement for photoperiod can be modified or completely replaced by a low temperature treatment (Barnier *et al*., 1981).

Others

In addition to temperature and photoperiod, endogenous plant factors such as age (Bernier *et al*., 1981; Meier-Dinkel and Kleinschmidt, 1990; Huang *et al.,* 1996; Garcia *et al*., 2000), type of planting material (Dowker, 1989; Krontal *et al*., 1998), contents of carbohydrates (Bodson and Outlaw, 1985; Araki and Komeda, 1993; Corbesier *et al*., 1998), gibberellins (Rood *et al*., 1989; Halvey, 1990; Kulikowska *et al*., 2000 ; Meier *et al*., 2001), cytokinins (Kinet, 1993), etc. at the time of vernalization determine the fate of the plants to flower or not.

Devernalization

Flowering in many vernalization and photoperiod responsive species is irreversibly induced before the appearance of the first flower, but in some species such as *A. cepa* there is no post inductive phase and flowering can be reversed by transfer to high temperature especially under long photoperiods even after floral buds are well developed (Rabinowitch, 1990; Summerfield *et al*., 1991) and under SD in some genotypes of *Arabidopsis* spp. (Okamuro *et al*., 1996). Some genotypes of garden pea (*Pisum sativum*) grown under non-inductive photoperiods may abort inflorescence initials and even undergo a period of vegetative reversion before flowering again at a higher node (Waller *et al*., 1997).

Flowering being a process influenced by multitude of factors, it is imperative to investigate and optimise those factors which can easily be utilized by horticultural crop growers. The objectives of the present study were, therefore, to find out the optimum temperature and photoperiod (Chapter 2), to compare the bolting capacities of intact bulbs and growing plants regenerated either from seeds or bulbs (Chapter 3), and to identify the right age of seedlings for vernalization treatment (Chapter 4) that will result in high bolting.

Chapter 2

The influence of temperature and photoperiod on growth, inflorescence initiation and bolting of shallot (*Allium cepa* L. var. *ascalonicum* Backer)

Abstract

Due to handling and disease problems associated with reproduction of shallot through bulbs and the need for improvement through hybridization, reproduction of shallot through seeds was sought as indispensable. However, bolting (flowering) does not readily occur in most varieties without pre-treatment of bulbs or growing plants. In order to achieve high bolting, the influence of two major environmental stimuli that induce bolting, temperature and photoperiod, and the duration of these stimuli on vegetative growth, inflorescence initiation and bolting of shallot was studied using four varieties: Ambition F1, Matador F1, Bonila F1 and Creation F1. Plants raised from seeds were treated at factorial combinations of 4, 8, 12 and 18^{o}C under 12 and 16 h photoperiods for 30, 60 and 90 days. An attempt was also made to predict the probability of bolting using logistic regression. Bulbing increased with increase in temperature especially under 16 h photoperiod; plants treated at 18^{o}C for 90 days produced mature bulbs before the end of the treatment. Although initiation of inflorescences was observed in Bonila F1 at 8 and 12^{o}C and in Creation F1 at 8^{o}C, no bolting was observed under any of the treatments in these genotypes. The highest temperature (18^{o}C) did not cause any initiation and hence bolting of inflorescences regardless of the photoperiods in any of the varieties. The second highest temperature (12^{o}C) also failed to induce bolting when applied only for 30 days in Matador F1, but the proportion of bolting was 0.09 and 0.46 in Ambition F1 under 12 and 16 h, respectively. Extension of the duration of treatment at 4 and 8^{o}C resulted in more bolting under both photoperiods; but it decreased bolting at 12^{o}C when the treatment was extended to 90 days. Therefore, vernalization at 8 and 12^{o}C under 16 h photoperiod for 60 days was found to be more effective at least in Ambition F1 and Matador F1.

Introduction

Shallot (Alliaceae: *Allium cepa* var. *ascalonicum* Backer*)* is the most important subgroup of the Aggregatum group and the only one grown commercially (Rabinowitch, 1990). However, it is mainly propagated vegetatively by bulbs which requires large quantity (1.2 ton ha^{-1}) of mother bulbs (Jackson *et al.*, 1985). Moreover, bulbs have poor keeping quality and also carry fungal diseases such as *Fusarium* spp. (Mengistu and Seid, 1990) and latent viruses (Proctor, 1987) from generation to generation. Thus, bulbs are invariably less preferable as planting material.

Alternatively, shallot can be reproduced by seeds with less problems. In addition to ease of propagation and handling, reproduction of shallot through seeds enables genetic improvement of the crop through hybridization and consequently plants with improved bulb size and quality will be obtained (Currah and Proctor, 1990). However, bolting (flowering), a prerequisite for reproduction through seeds, does not occur readily unless bulbs or growing plants are treated with temperature and photoperiod suitable to initiate the process. Some reports (Sinnadurai and Amuti, 1971; Tabor, 1996; Krontal *et al*., 2000) indicated that treating shallot bulbs or young plants with temperature ranging from 5-15^{o}C for a period of 70-90 days could initiate flowering depending on cultivar. Similarly, research results in other crops such as onion (*Allium cepa* var. *cepa* L.; Brewster, 1983; 1985), leek (*Allium porrum* L.; Wiebe, 1994), ornamental onion (*Allium cowanii* Lindl; Kodairal *et al*., 2000), annual bluegrass (*Poa annua* var. *reptans* L.; Johnson and White, 1997), and *Achillea filipendula* 'Parker' (Zoberi *et al*., 2003) show high and/or early bolting (flowering) within the above temperature range. In addition, high bolting percentage was obtained under longdays (16 h) in European longday onion cvs (Brewster, 1983; 1985) and under shortdays (10 h) in shortday shallot cvs (Krontal *et al*., 2000). Several researchers also attempted to quantify the flowering process in onion (Brewster, 1987), in quinoa (*Chenopodium quinoa* Willd.) and in cauliflower (Wurr *et al*., 1994).

Studies so far undertaken to investigate the bolting response of shallot to temperature and photoperiod stimuli are very few and less comprehensive. The relatively extensive findings in onion cannot be fully adopted because of the differences in the two crops (Krontal *et al*., 1998). Therefore, the objectives of the present study were to find out suitable vernalization

temperature and photoperiod that will result in high bolting, to investigate the interaction between vegetative and reproductive development of the plants in response to these stimuli, and to assess genotypic differences and interactions with these stimuli .

Materials and methods

Planting material, greenhouse and growth chamber management

Seeds of four shallot varieties: Ambition F1, Bonila F1, Matador F1 (Bejo Zaden, b.v. Warmenhuizen, Holland) and Creation F1 (Bruno Nebelung GmbH, Everswinkel, Kreis Warendorf, Germany), that were commercially grown in Europe and believed to have better homogeneity, were sown on Potgrond P peat (Klassmann, Geeste Gross Hesepe, Germany) on 25 January 2002 and grown in the greenhouse of the Institute of Vegetable and Fruit Sciences, University of Hannover, Germany. The greenhouse temperature was kept at 18/16^{o}C (day/night) and vented at 20^{o}C. Supplemental light (85 μmol $m^{-2}s^{-1}$ PPFD) was provided by SON-lamps (400 W) from 5:30 to 8:30 and from 15:30 to 19:00. The seedlings were transplanted to 12 cm pots on 5 March 2002 and were raised in the greenhouse maintained at the same temperature until they were transferred to growth chambers for treatment. During the period, the seedlings were supplemented with light from the SON-lamps between 6:00 to 18:00. PPFD measured at pot level by a LI-188B photometer (LI-COR Inc., USA) ranged from 183 μmol m^{-2} s^{-1} on cloudy days to 615 μmol $m^{-2}s^{-1}$ on sunny days. The seedlings also received weekly fertigation of 10 ppm Flory® 2 (Euflor GmbH, München, Germany) containing 15, 7, 22, and 6% N, P_2O_5, K_2O and MgO, respectively and micronutrients. Table 2.1 shows the conditions of the seedlings when transferred to the chambers.

The growth chambers were set at factorial combinations of four levels of temperature (4, 8, 12 and 18^{o}C) and two levels of photoperiod (12 and 16 h) each containing a factorial combinations of the three levels of the treatment durations (30, 60 and 90 days) and the four varieties. Twenty selected plants were randomly assigned and transferred to each experimental unit on 14 April 2002. The plants were supplied with constant irradiance (266±26 μmol $m^{-2}s^{-1}$ PPFD from fluorescent and incandescent lamps) and CO_2 (320 ppm). At the end of each treatment duration, three plants were sampled for vegetative growth and dissection data. The remaining plants were transferred into the greenhouse. In the greenhouse, the plants were subjected to natural daylight during the day but covered with double-faced

(black-white) polyethylene sheet between 19:00 and 7:00 to maintain 12 h photoperiod in order to discourage bulbing. The temperature and the radiation of the greenhouse during the growth period were as shown in Annex 2.1.

After destructive sampling at the end of each treatment duration, the portion of the plant comprising the base-plate and about 2 to 3 cm of the sheath was sub-sampled and preserved in 70% alcohol and 1% glycerol solution until dissection, for a maximum of four weeks. While dissecting, leaf sheaths and leaf initials were carefully removed using forceps and scalpels following the dichotomous division of the lateral shoots.

Data collection

The number of leaves (including leaf initials) and buds on each shoot was recorded. Developmental stages of the buds were identified under binocular (10-63x) based on the stages established by Brewster (1983) for onion. In order to avoid ambiguity in identification, only those buds at stage-4 and above were considered as floral. Number of shoots, bulb (sheath base) and sheath-neck diameters, and dry weights of leaves and leaf sheaths which also included bulbs when set, were measured from three plants harvested at the beginning of the treatment and at the end of each treatment duration. Bulbing ratio was taken as the ratio between maximum sheath (bulb) diameter and minimum sheath (neck) diameter. Leaf area was measured with LI-3100 leaf area meter (LI-COR Inc., USA). Bolting, the emergence of inflorescences about 1 cm or higher, was counted twice weekly.

Statistical analysis

The treatment effects were tested against plant-to-plant variation using SAS statistical software (SAS Institute Inc., 1999). Only significant main effects or interactions ($P \leq 0.05$) were considered for presentation.

Binomial data, bolting (1) and non-bolting (0), with their respective frequencies were used as inputs to predict the probability of bolting using PROC LOGISTIC procedure of the same software. Variety was considered as a classification variable in the model. The best-fit model was selected by backward elimination option based on the -2log likelihood ratio (-2Log L) or deviance and the Akaike Information Criterion (AIC). The AIC judges a model by how close its fitted values tend to be to the true values, in terms of a certain expected value (Agresti, 1996).

Log likelihood ratio = 2∑(observed)/ log (observed /fitted)

AIC = -2(maximized log likelihood - number of parameters in the model).

Deviance = -2(maximized log likelihood ratio for the fitted model - maximized log likelihood ratio for the saturated model); the saturated model is the one that contains all the possible parameters.

Moreover, type III analysis of effects was used to eliminate non-significant terms from the models ($p \leq 0.05$). Finally, correlation between the observed proportions and model-predicted probabilities was considered to detect the improvement of the successive models. Due to failure of plants treated at 18^{o}C to bolt, the prediction of bolting did not include data from 18^{o}C treatment.

Table 2.1 Vegetative characteristics of seedlings of the four shallot varieties at the beginning of the treatment*

Variety	Creation F1	Matador F1	Bonila F1	Ambition F1
No. of leaves and leaf-initials	15.50±3.50	13.00±2.08	17.20±2.28	12.66±1.89
No. of buds	3.75±0.96	2.67±1.55	4.20±1.30	3.06±0.58
Leaf DW (g)	1.23±0.35	1.60±0.33	1.07±0.22	1.64±0.40
Shoot DW (g)	0.47±0.13	0.66±0.41	0.31±0.07	0.47±0.29
Total shoot DW (g)	1.69±0.43	2.24±0.80	1.30±0.22	1.75±0.85
Sheath-neck diameter (cm)	0.99±0.12	0.93±0.12	1.11±0.85	1.01±0.13
Sheath base diameter (cm)	1.22±0.19	1.10±0.14	1.21±0.16	1.23±0.17
No. of shoots	1.12±0.32	1.08±0.28	1.10±0.30	1.13±0.33
Bulbing ratio	1.23±0.11	1.18±0.11	1.18±0.15	1.21±0.10

*Average of 5 plants ± standard deviation, DW = dry weight

Results

Dissection of plants

Regarding the vegetative data, treatment effects at the end of the longest duration of treatment (90 days) are discussed hereafter unless specified. Significant differences were observed among the levels of temperature and among the varieties in the total number of buds (TNB) (Figures 2.1a and b), number of leaves and leaf initials (Figure 2.1c), and number of floral

buds initiated (Figure 2.1d). The initiated TNB significantly increased with increase in temperature hence they were significantly lower at 4^{o}C than at 12 and 18^{o}C (Figure 2.1a). The TNB at 12 and 18^{o}C did not differ from each other. The difference among the temperatures in the number of leaves and leaf initials (Figure 2.1c) was also significant. It increased linearly with increase in temperature. Differences among the varieties in the number of buds, and the number of leaves and leaf initials were also observed (Figures 2.1 b and c). Bonila-F1 and Creation-F1 initiated significantly higher total number of buds, and leaves and leaf initials than Ambition F1 and Matador F1.

No visible floral bud of the specified stages was observed after 30 days of treatment (data not shown). Thus the comparison of inflorescences initiated among the temperature treatments and the varieties was limited to those initiated after 90 days of treatment. The highest number of floral buds was observed at 8^{o}C followed by 12^{o}C in both durations (Figure 2.1d). The numbers of inflorescences at 4^{o}C were significantly lower than what was observed at 8 and 12^{o}C. Despite the high TNB produced by Bonila F1 and Creation F1, fewer floral buds were induced compared to the other varieties (Figures 2.1b and d). A considerable increase in the number of floral buds was observed as the duration of treatment was extended from 60 to 90 days (data not shown).

As more shoots became visible, the TNB obtained by dissection increased considerably. Regression analysis of the two parameters showed strong relations ($r^2 \geq 0.86$) in all the cultivars (Figures 2.2 a to d). In the later growth stages, the buds were grown-up and considered as lateral shoots and followed a similar trend as TNB, except in Ambition F1, but with no significant difference between 4 and 8^{o}C (Figure 2.3f).

Each floral bud observed was accompanied by a vegetative bud (Plate 2.1). The development of the growing point (meristem) into floral bud did not terminate the further initiation of leaves and buds. Instead, vegetative buds emerged axiliary to the floral buds and continued the initiation of new leaves and buds until the plant attained its physiological maturity.

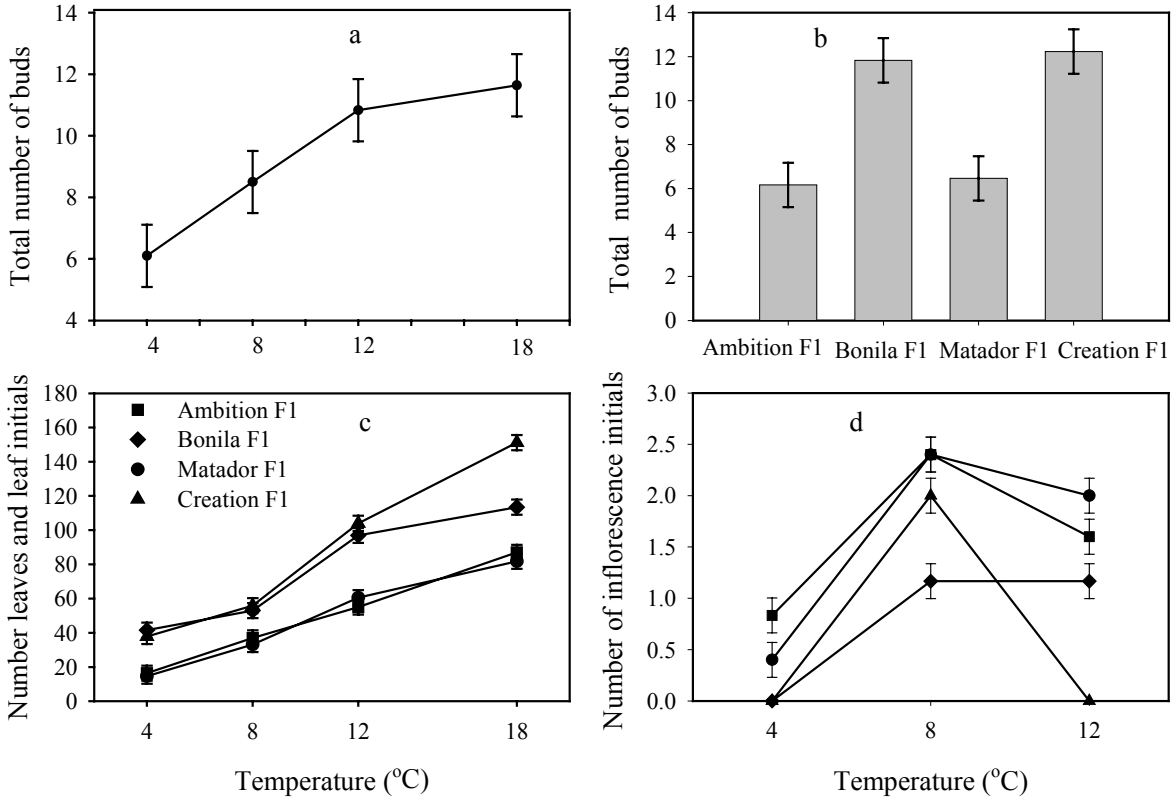

Fig. 2.1 Vegetative parameters and inflorescence initials of shallot as observed by dissection of plants treated for 90 days: a) total number of buds at the four temperatures (means across varieties and photoperiods, n=24), b) total number of buds of the four varieties (means across temperatures and photoperiods, n=24), c) number of leaves and leaf initials of the four varieties at the four temperatures (means across photoperiods n=6), and d) number of inflorescence initials of the four varieties at the three inductive temperatures (means across photoperiods n=6). Vertical bars represent ± two times standard error.

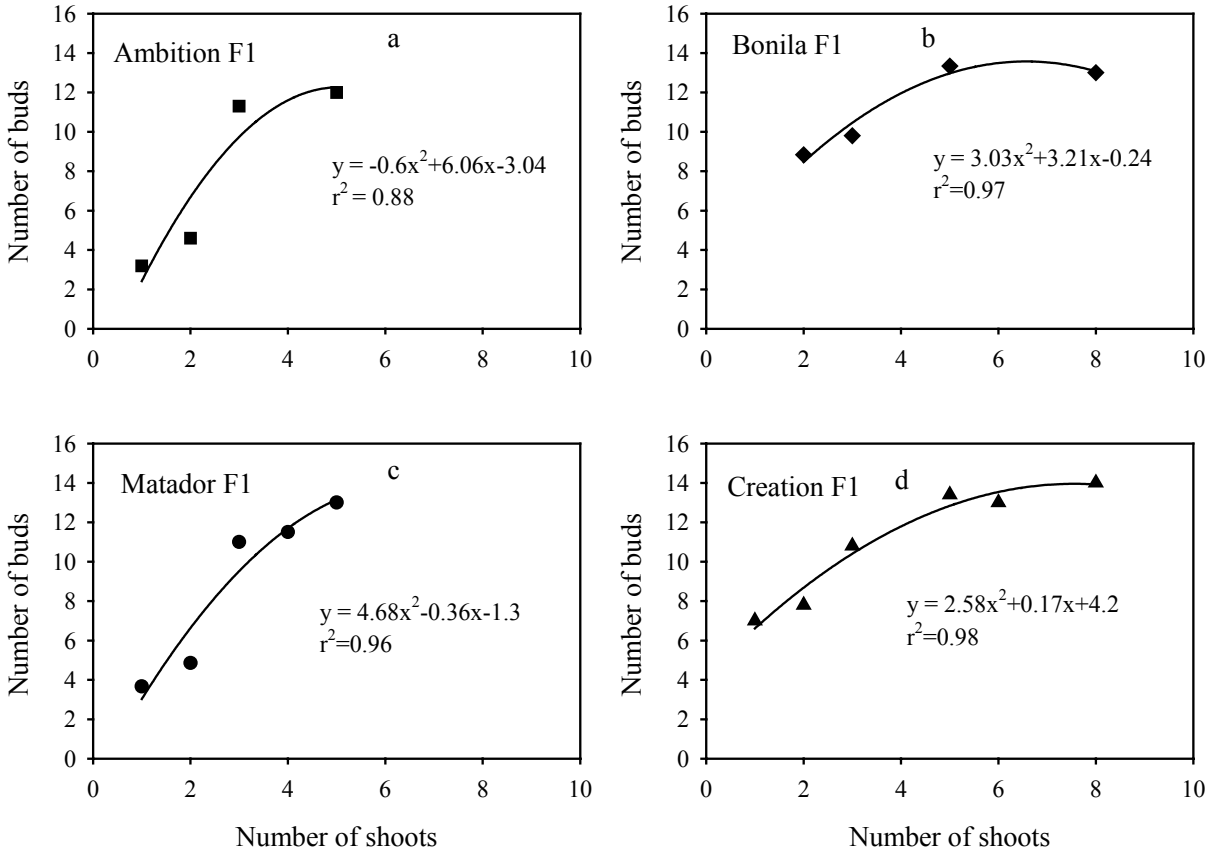

Fig. 2.2 Regression between the number of visible shoots and number of buds obtained by dissection in four shallot varieties (n varies from 1 to 9).

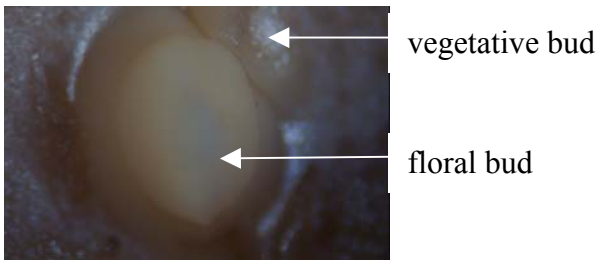

Plate 2.1 Shallot floral bud and the competing vegetative bud

Vegetative parameters

Significant interactions were observed between temperature and photoperiod in bulb diameter, bulbing ratio, leaf area, and sheath dry weight per plant (Figures 2.3a to c and e)) . Bulb development as indicated by bulb diameter (Figure 2.3a) and bulbing ratio (Figure 2.3b)

increased with increase in temperature in plants treated under 16 h photoperiod. They were very low and did not differ among the temperatures under 12 h photoperiod. Leaf area (Figure 2.3c) increased with increase in temperature in both 12 and 16 h photoperiods but with no significant differences between the two photoperiods at 4^{o}C. There was no interaction between temperature and photoperiod in leaf dry weight. But the leaf dry weights at 4 and 8^{o}C were significantly lower than at 12 and 18^{o}C (Figure 2.3d). Sheath dry weight (Figure 2.3e) also increased with increase in temperature under 16 h photoperiod although the differences between 4 and 8^{o}C and 12 and 18^{o}C were not significant. Bulb development, leaf area and sheath dry weight were significantly promoted under 16 h photoperiod compared to 12 h (Figures 2.3a, b, c and e). Plants treated at 18^{o}C under 16 h matured before the end of the treatment period. Varieties Bonila F1 and Creation F1 had higher numbers of shoots than Ambition F1 and Matador F1 at 12 and 18^{o}C (Figure 2.3f). The difference between the first two varieties was not significant as was the difference between the latter two varieties.

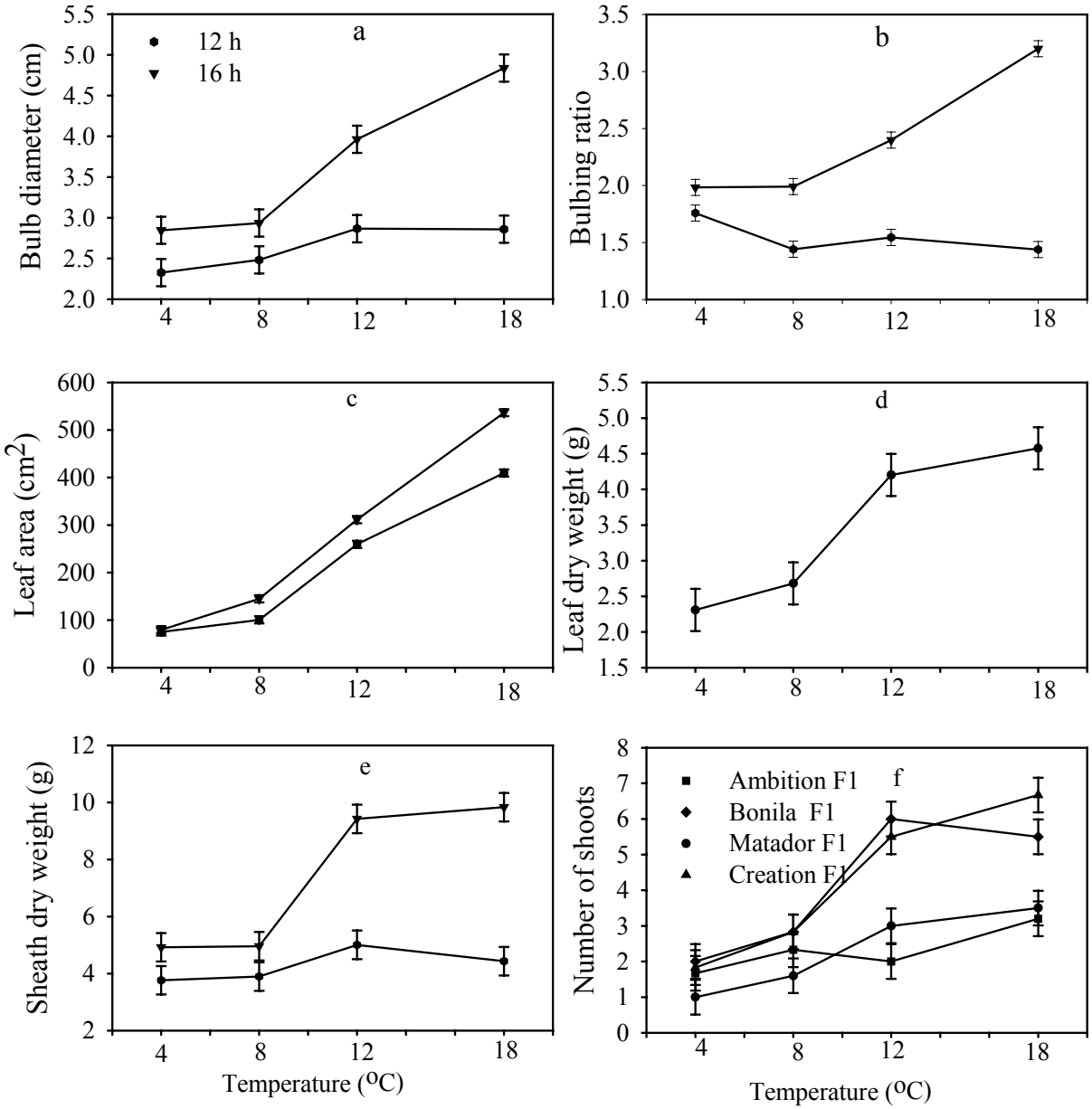

Fig. 2. 3 Vegetative growth of shallot plants at the four temperatures: a) bulb diameter, b) bulbing ratio, c) leaf area, d) leaf dry weight, and e) sheath dry weight - all under two photoperiods and across varieties (n=12) except d where means are across varieties and photoperiods (n=24), and (f) number of shoots of the four varieties at the four temperatures (means across photoperiods, n =6).

Reproductive parameter

Bolting

Among the varieties tested Bonila F1 and Creation F1 failed to bolt under any of the treatments. However, dissection of bulbs treated for 90 days revealed that some inflorescences

were initiated and developed to stage 5 (Figure 2.1d) which later on might have failed to bolt. Ambition F1 and Matador F1, the responsive varieties at the other temperatures, did not initiate inflorescences at 18^{o}C. At the three lower temperatures, they bolted at various levels depending on the photoperiod and the duration of treatment (Figures 2.4a to d). Matador F1 did not produce any inflorescence when treated at 4 and 12^{o}C for 30 days, but at 8^{o}C the proportion of the plants which produced inflorescences was 0.09. A considerable increase in bolting was observed with successive increases in treatment duration at 4 and 8^{o}C under both photoperiods, except when it was longer than 60 days under 12 h at the latter temperature. Increasing duration of treatment from 30 to 60 days at 12^{o}C did not increase bolting under both photoperiods whereas further increase to 90 days under 16 h photoperiod resulted in considerable decrease in bolting. Alike Matador F1, Ambition F1 did not bolt when it was treated at 4^{o}C for 30 days irrespective of the photoperiods. Nonetheless, treatment at 8 and 12^{o}C under 16 h for the same period increased the proportion of bolting up to 0.21 and 0.46, respectively. As the duration of treatment was extended from 30 to 60 days under 16 h photoperiod, the proportion of bolting increased to about 0.21, 0.71 and 0.66 at 4, 8 and 12^{o}C, respectively. The corresponding result under 12 h photoperiod was low. However, further increase in the duration of treatment to 90 days at 8^{o}C resulted in a slight decrease in bolting under 16 h photoperiod but showed no change under 12 h. Extension of the treatment from 30 to 60 days had favourable effect at 12^{o}C under both photoperiods, but further increase to 90 days incurred a decrease of quarter of the bolting under 16 h. Bolting did not differ much between 8 and 12^{o}C under 16 h photoperiod in both varieties.

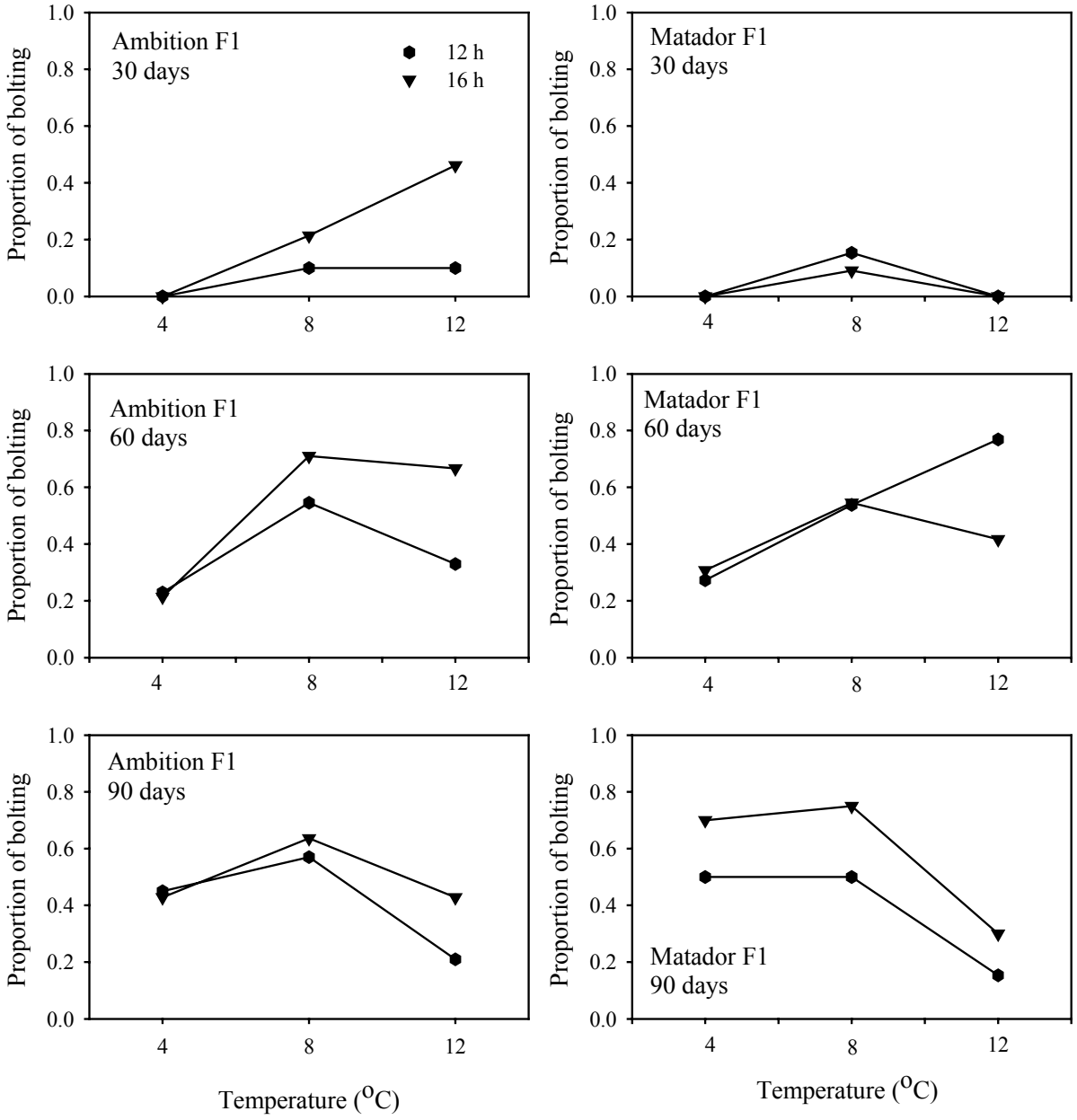

Fig. 2.4 Proportion of bolting (n=17) of the two responsive shallot varieties (Ambition F1 and Matador F1) treated at two photoperiods for 30, 60 and 90 days.

Prediction of bolting

Separate logistic models were used to predict the proportion of bolting of each variety based on the model selection criteria described in the materials and methods section. In Ambition F1, although models 1 to 2 had lower deviances compared to the rest of the models (Table 2.2), the interactions between temperature, photoperiod and duration, and between photoperiod and temperature were not significant ($p \leq 0.05$; Table 2.3). The simpler models had higher deviance and AIC values and non significant terms and hence model 3 was chosen

as the best model. The over all model fit was significant at $p<0.001$. The predicted probabilities were highly correlated ($r=0.97$) with the observed proportions. Hence, based on the selected model the logit of the probability of bolting (π) for Ambition F1 is estimated as:

$$\text{Logit}(\pi) = -18.613+0.248D+1.823T+0.180P-0.068T^2-0.001D^2-0.009D*T \quad (2.1)$$

D is duration, T is temperature, and P is photoperiod.

Tables 2.2 and 2.3 show that interactions between temperature and duration of vernalization, and all the main factors were significant ($p\leq0.05$). Plants vernalized at 4^oC had significantly lower proportion of botling compared to those treated at 8 and 12^oC (Figure 2.5). Moreover, plants treated for 30 days had the lowest proportion of bolting. The interaction between temperature and duration further revealed that plants treated for 90 days had higher proportion of bolting as compared to those treated for shorter periods except at 8^oC. Higher proportion of bolting was observed at the higher temperatures (12^oC) than at 4^oC after 4 weeks of vernalization.

Alike for Ambition F1, the model for Matador F1 was selected using the same procedure (Table 2.4). Models 1 to 4 had a lower deviances and all the interaction terms were not significant at $p \leq 0.05$ (Table 2.5). Models 6 to 9 had higher deviance and AIC values though all the main factors were significant, hence, model 5 was chosen as the best model. Consequently, the logit of the probability of bolting was given as:

$$\text{Logit}(\pi) = -11.803+0.203D+1.193T-0.082T^2-0.001D^2 \quad (2.2)$$

D, T and P are as described in equation 2.1

The predicted probabilities of bolting fit well with the observed proportions ($r=0.95$). Figure 2.6 shows that bolting is the highest at 8^oC and it increases as the duration of treatment increases. Plants treated for 30 days had very low proportion of bolting irrespective of the treatment temperature. The difference in bolting between plants treated for 30 days and those treated for 60 and 90 days is much higher than the difference between the latter two durations.

Table 2.2 Successively selected models using backward elimination of predictors based on deviance (-2Log L) and AIC, Ambition F1.

Model No.	Predictors	Deviance (-2Log L)	df	AIC	Models compared	Deviance difference
1	D*T*P[a]	238.80	217	258.80	-	-
2	D*T T*P[a]	239.53	219	255.52	(2, 1)	0.73
3**	D*T[a]	243.19	220	257.19	(3, 2)	3.66
4	D T T^2 D^2	261.63	222	271.63	(4, 3)	18.44
5	D T T^2	268.31	223	276.31	(5, 4)	6.68
6	D T	276.13	224	282.12	(6, 5)	7.82
7	D	279.94	225	283.94	(7, 6)	3.81
8	T	290.43	225	294.43	(8, 7)	10.49

[a] Only higher order terms are listed - the lower terms are included ; **selected model; D, duration ; T, temperature ; P, photoperiod; df, degrees of freedom

Table 2.3 Analysis of parameter estimates of the selected model, Ambition F1

Parameter	df	Estimate	se	Wald χ^2	$p>\chi^2$
Intercept	1	-18.6134	3.4894	28.4550	<0.0001
Duration (D)	1	0.2479	0.0610	16.4889	<0.0001
Temperature (T)	1	1.8126	0.4415	16.8555	<0.0001
Photoperiod (P)	1	0.1801	0.0790	5.1981	0.0228
T^2	1	-0.0684	0.0214	10.2527	0.0014
D^2	1	-0.0012	0.0004	9.1589	0.0025
D*T	1	-0.0088	0.0026	11.2205	0.0008

df, degrees of freedom; se, standard error; p, probability level

Table 2.4 Successively selected models using backward elimination of predictors based on deviance (-2Log L) and AIC, Matador F1

Model No.	Predictors[1]	Deviance (-2Log L)	df	AIC	Models compared	Deviance difference
1	D*T*P[a]	194.89	211	214.89	-	
2	D*T D*P[a]	196.21	213	212.21	(2, 1)	1.32
3	D*T[a]	197.24	214	211.25	(3, 2)	1.03
4	D T P T^2 D^2	199.78	215	211.78	(4, 3)	2.54
5**	D T T^2 D^2	203.03	216	210.03	(5, 4)	3.25
6	D T T^2	211.27	217	219.27	(6, 5)	8.24
7	D T^2	223.54	218	231.90	(7, 6)	12.27
8	D	229.63	219	233.63	(8, 7)	6.09
9	T	260.67	219	264.66	(9, 8)	31.04

[a] Only higher order terms are listed - the lower terms are included; **selected model; D, duration ; T, temperature ; P, photoperiod; df, degrees of freedom

Table 2.5 Analysis of parameter estimates of the selected model, Matador F1

Parameter	df	Estimate	se	Wald χ^2	$p>\chi^2$
Intercept	1	-11.803	2.3884	24.42	<0.0001
Duration (D)	1	0.203	0.0612	10.95	0.0009
Temperature (T)	1	1.193	0.3582	11.10	0.0009
Photoperiod (P)	1	0.163	0.0884	3.40	0.0652^{ns}
T^2	1	-0.082	0.0225	13.19	0.0003
D^2	1	-0.001	0.0005	7.29	0.0070
D*T	1	-0.005	0.0032	2.47	0.1163^{ns}

^{ns} non significant terms; df, degrees of freedom; se, standard error; p, probability level

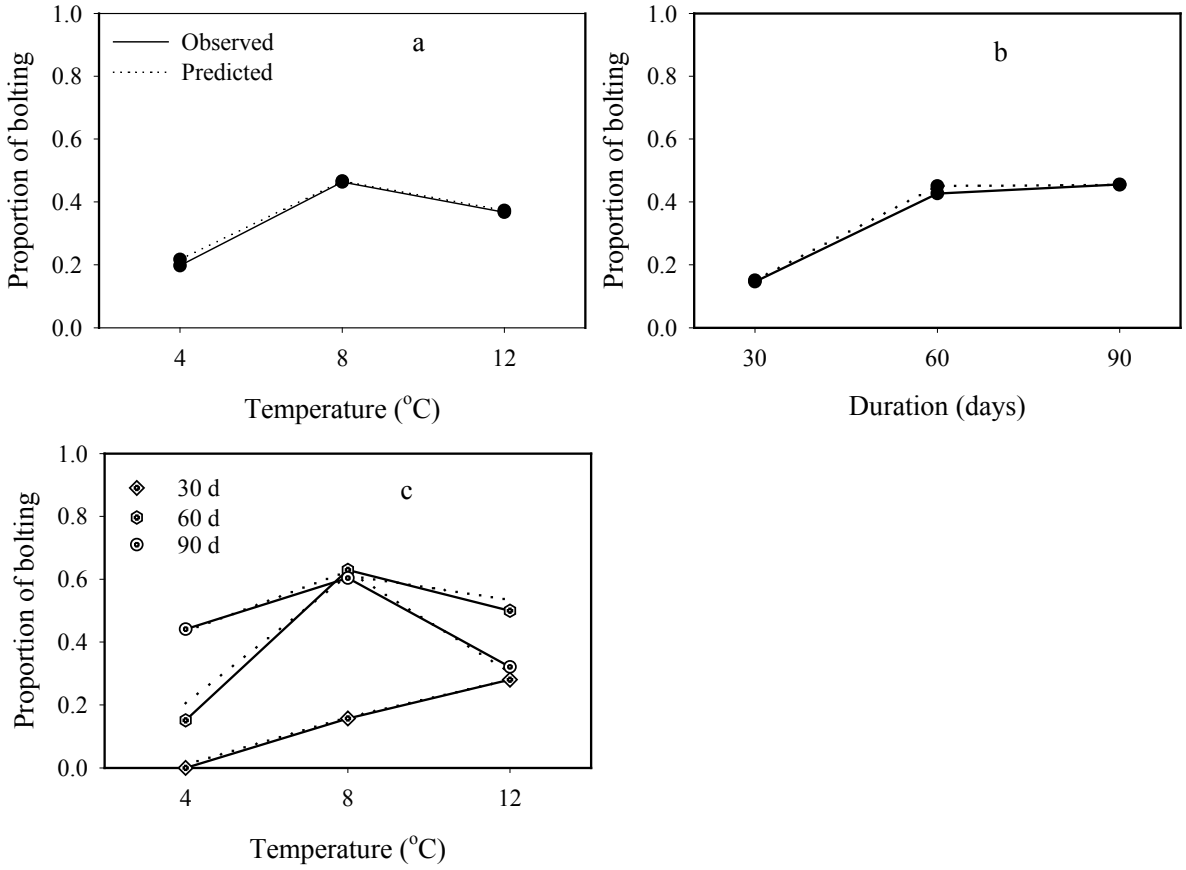

Fig 2.5 The observed proportions and predicted probabilities of bolting of Ambition F1 in response to vernalization: a) at the three temperatures (means across photoperiods and durations, n=102), b) treated for three durations (means across temperatures and photoperiods, n=102), and c) the interaction between duration and temperature (means across photoperiods, n=34).

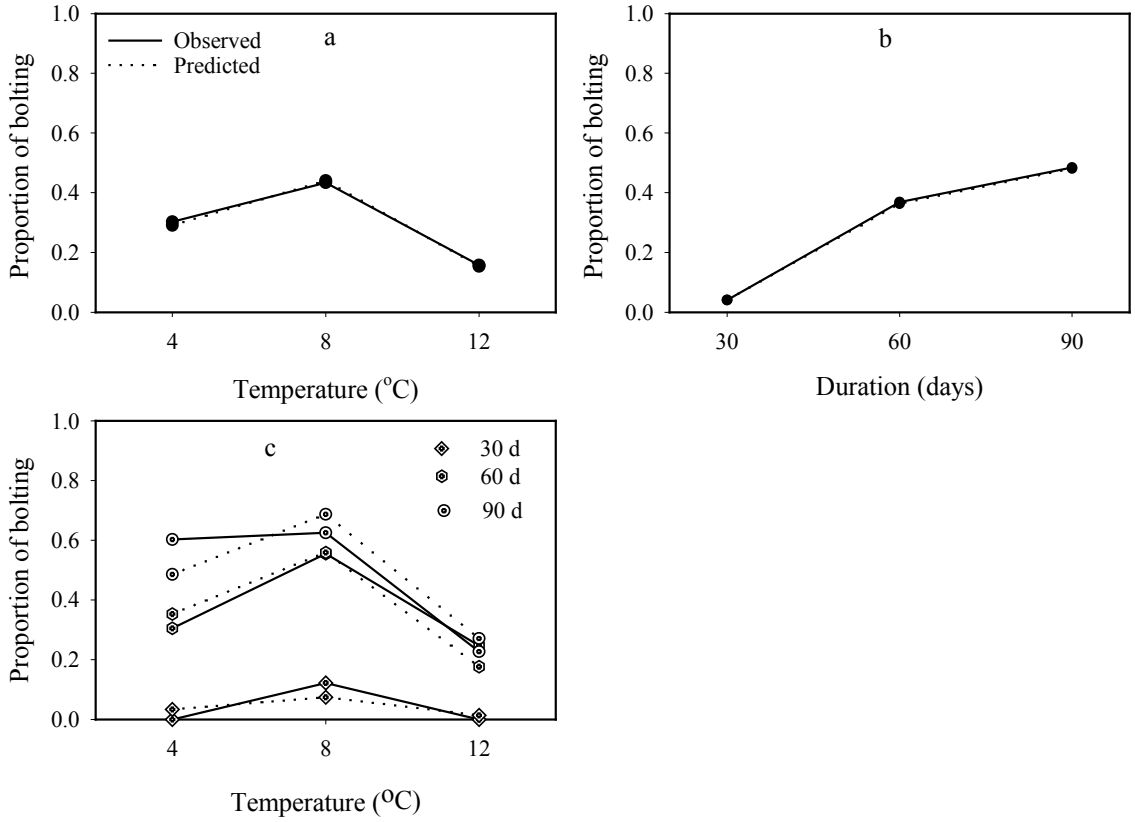

Fig 2.6 The observed proportions and predicted probabilities of bolting of Matador F1 in response to vernalization: a) at the three temperatures (means across photoperiods and durations, n=102), b) treated for three durations (means across temperatures and photoperiods, n=102), and c) the interaction between duration and temperature (means across photoperiods, n=34).

Days to bolting

Days to 50% of maximum bolting (DFMB), from final date of the treatment, were extrapolated from the graph of proportion of bolting plotted against number of days to bolting (data not shown). Differences were not observed between varieties and between photoperiods but differences were observed among the temperatures and among the durations. Treatment at 12°C gave earlier bolting (27 days) as compared to 8°C (38 days) and 4°C (48 days). Moreover, plants treated for 90 days showed earlier bolting (26 days) while those treated for 30 or 60 days had reached DFMB after 47 and 41 days, respectively.

Discussion

In contrast to onion, the transition to reproductive development in shallot had no inhibitory effect on further development of axiliary vegetative meristems (Krontal *et al*., 1998). Moreover, as all the growing points emerge on a compact stem (base-plate) through which physiological transactions are made within close proximity to each other at presumably faster rate than in plants with non-reduced stems, the floral bud(s) may face severe competition both from the adjacent and other neighbouring vegetative buds.

The vigorous vegetative growth in leaf number under longdays (LD) was in accordance with the results reported by Yamasaki *et al*. (2000). They found more leaves in Japanese bunching onion (*Allium fistulosum* L.) after 25 days of treatment under 16 h compared to 8 h photoperiod. LD also increased leaf sheath diameter but did not induce bulbing. In contrast, Konsin *et al.* (2001) reported that strawberry (*Fragaria x ananassa* Duch 'Korona') which flowers under 12 h at 18/16^{o}C, had reduced runner production and petiole length under LD.

Ambition F1 and Matador F1 had higher shoot dry weight, bulb diameter and bulbing ratio but fewer shoots than Bonila F1 and Creation F1 indicating the inherent behaviour of the latter two varieties in continuous initiation of shoot initials in stead of bulbing or initiating inflorescences. Such behaviour of the varieties could have led to diversion of much of their photosynthates to the initiation and growth of leaves than to the initiated inflorescences which were observed during the dissection. Therefore, this might be considered as an additional mechanisms of devernalization in these shallot varieties.

Plants treated at 18^{o}C under 16 h, did not bolt at all but quickly induced bulbing and matured before the treatment ended. The decrease in bolting observed in plants treated at 12^{o}C and 16 h for 90 days might also be due to the progress in bulbing which had attained a bulbing ratio of about 2.5 at the end of the treatment period (Figure 2.3b). Although an attempt to maintain post treatment photoperiod at 12 h could check the further advancement of bulbing, it did not revert the already decreased bolting. The high bulbing response under the long photoperiod and high temperature (Steer, 1980; Brewster, 1983) indicated the high possibility of competition from the growing bulb, which may suppress the growing inflorescence due to physical pressure and/or diversion of photosynthates resulting in devernalization (Rabinowitch, 1990). Van Kampen (1970) also reported that the promotive effect of

temperature and photoperiod on inflorescence growth will at some point be out-weighed by detrimental effects on inflorescence growth caused by competition from the bulbing they promote.

The responsive temperatures and photoperiod found effective in our studies are within the range reported by other researchers. Krontal *et al*. (2000) obtained high percentage of bolting in the temperature range of 5 to 12^{o}C and 16 h photoperiod whereas high (30^{o}C) and intermediate (13-20^{o}C) temperatures delayed the development of the inflorescence. In a related growth chamber experiment, however they found that seedlings of a shortday cv 66-1004 treated at 17/9 and 26/18^{o}C (day/night) temperatures resulted in 100 and 50% flowering, respectively. Brewster and Bulter (1989) reported that treatment of growing onion seedlings with temperature close to 9^{o}C, 4 to 6 h of photoperiod extension (94 μmol $m^{-2}s^{-1}$ PPFD) during chilling coupled with reduced N level to 15-40% of the normal, resulted in 20-30 days earlier and 100% bolting in some bolting resistant onion cultivars. Similar results were obtained in onion (*Allium cepa* var. *cepa* L.) cvs 'Rijnsburger' and 'Senshyu' at 9^{o}C under 16 h (Brewster, 1982; 1985). The optimum vernalization temperature for leek was 5^{o}C (Wiebe, 1994). Flowering in different genotypes of annual bluegrass (*Poa annua* var. *reptans*) was induced at 4 and 8^{o}C but failed at 12^{o}C when treated for 8 to 12 weeks (Johnson and White, 1997). Corms of *Achillea filipendulina* 'Parker' were induced to early and complete flowering when cooled at 4^{o}C for at least four weeks (Zoberi *et al*., 2003). *Allium cowanii* Lindl pre-exposed to 20-30^{o}C could bolt after three months of exposure to 9-20^{o}C (Kodairal *et al*., 2000).

Despite the relatively higher proportion of bolting at 8 and 12^{o}C compared to other temperatures, the maximum proportion of bolting attained was only about 0.73. This could be attributed to the juvenility of the plants since they were only 79 days old at the beginning of vernalization. Results in Chapter 4 show that plants of Matador F1 grown under similar light conditions should be 120 days old at the beginning of vernalization in order to get complete bolting.

In agreement with our study, photoperiod extension (16 h) improved bolting of cv 'Rawska' and European spring cv 'Rijnsburger' but the response was less in Japanese autumn cv 'Senshyu' and negative in cv 'Kaizuka' (Brewster, 1983). Extending the photoperiod from 8 to 20 h reduced days to inflorescence initiation of cv Rijnsburger from 86 to 38 (Brewster,

1983). Long photoperiods during chilling have been reported to increase the vernalizing effect of chilling in pea genotypes containing the Sn gene (Murfet and Reid, 1974) and in several strains of *Arabidopsis thaliana* (Napp-Zinn, 1985).

Despite the additional 4 to 6 lateral buds (Table 2.1; Figure 2.1a) formed during the treatment period at the optimum vernalizing temperatures (8 and 12^{o}C), the number of inflorescences per plant did not exceed two (Figure 2.1d). This shows that those buds that were present at the beginning of the treatment (Table 2.1) most likely responded to the treatments while the failure of the newly initiated buds might have been due to juvenility and/or insufficient vernalization.

The very low and late bolting at 4^{o}C when it was applied only for 30 days could be due to slow rate of initiation and/or extension of the inflorescences at this temperature (data not shown). Longer treatments at the same temperature resulted in higher bolting. Brewster (1983) reported that longer time is required for flower initiation under sub-optimal range of low temperature. Zemah *et al*. (2001) also reported a fully developed inflorescence following storage at 4^{o}C for 16 weeks in *Allium aflatunense*. Nonetheless, the care needed and the energy consumed during the extended period of treatment accounts for its disadvantage. Therefore, both 4 and 18^{o}C were found to be ineffective in vernalizing these varieties.

The local African cv 'Bawku' flowered satisfactorily where ambient temperatures were 15 to 21^{o}C whereas cvs from former north USSR had a temperature optimum of 3 to 4^{o}C (Rabinowitch, 1990). Van Kampen (1970) stored bulbs for 30 days either at 9 or 1^{o}C and obtained 73 to 84% and 10 to 16% bolting depending on locations. Inflorescence primordial formation was inhibited by temperature of about 0^{o}C; the longer the storage, the greater is the inhibitory effect on flower primordia development (Rabinowitch, 1990).

Plants treated at 4^{o}C both for 30 and 60 days had higher proportion of leafy inflorescences, but it was not observed in plants treated for 90 days at the same temperature. The disorder could be due to the excessive elongation of the last leaf homologue (the spathe) that envelops the apical meristem. Krontal *et al.* (2000) reported that 50% of the seedlings of a shortday shallot accession (66-1004) treated at 26/18^{o}C (day/night) temperature flowered, but 30% of the inflorescences had malformed umbels. Those treated at 17/9^{o}C (day/night) produced normal umbels. Such physiological disorders were also observed in onion (Rabinowitch,

1990), *Poa annua* var. *reptans* (Johnson and White, 1997), and *Sinapis* spp (Bernier *et al*., 1981) under sub-optimal vernalization treatments.

In conclusion, vernalization at 8 and 12^{o}C under 16 h photoperiod for 60 days can increase bolting at least in Ambition F1 and Matador F1 shallot varieties and had minimum interference from the negative effects of bulbing. However, the maximum proportion of bolting attained under this experiment was low (0.73) which could be due to juvenile plants which were only 79 d old at the beginning of the vernalization. Hence, more bolting may be achieved by considering older plants, as observed in Chapter 4.

Chapter 3

The influence of planting material and duration of bulb vernalization on bolting of shallot (*Allium cepa* L. var. *ascalonicum* Backer)

Abstract

Reproduction of shallot through bulbs has a number of disadvantages like the difficulties of bulb storage and disease carry-over. Propagation through seeds avoids these aforementioned shortcomings. Seed production in shallot can be achieved either through seed to bulb to seed or seed to seed methods by vernalizing bulbs or growing plants. In an attempt to investigate the relative performance of these planting materials, plants of Ambition F1 and Matador F1 regenerated from seeds (PS) and plants regenerated from bulbs (PB) were grown until they attained a post juvenile stage, and intact bulbs (B) above the critical size for juvenility were treated at 8 and 12^{o}C for 60 days. Each experimental unit comprised 25 plants and plant to plant variation was used for analysis. An attempt was also made to predict the probability of bolting using logistic regression. The probability of bolting of PS and PB was significantly higher than B. PS had significantly taller and thicker inflorescences, and more florets per umbel compared to PB and B. On average PB, PS and B produced about 6.0, 3.7 and 2.3 inflorescences per plant, respectively. Hence, PB had 90 and 75% more florets per plant than PS and B. Due to the low proportion of bolting (0.64) in vernalized bulbs an experiment was initiated to increase the proportion of botling by extending the duration of the vernalization period. Bulbs of Ambition F1 and Matador F1 and of seven other shallot genotypes that had been propagated vegetatively were treated at 8^{o}C for a duration of 30, 60 and 90 days. However, none of the vegetatively propagated shallot varieties could flower. The proportion of bolting in Ambition F1 and Matador F1 was about 0.6 irrespective of the duration of vernalization period. Therefore, vernalization of growing plants of post juvenile stage at 8 and 12^{o}C under 16 h for a period of 60 days was recommended for high bolting.

Introduction

Shallot (Alliaceae: *Allium cepa* var. *ascalonicum* Backer*)* is the most important subgroup of the Aggregatum group and the only one grown commercially (Rabinowitch, 1990). However, it is mainly propagated vegetatively by bulbs which requires large quantity for planting (1.2 ton ha^{-1}) of mother bulbs (Jackson *et al.*, 1985). Moreover, bulbs have poor keeping quality and may carry-over fungal diseases such as *Fusarium* spp. (Mengistu and Seid, 1990) and latent viruses (Proctor, 1987) from generation to generation. Thus, bulbs, are invariably less preferable as planting material. Alternatively, production of shallot through seeds was found to overcome these problems and is gaining momentum in recent years. Treatment of plants and bulbs of *Allium* spp. at vernalizing temperatures either in field or in growth chambers has been used as the major tool in achieving flowering for large scale seed production and breeding purposes, respectively.

Shallot, like some other *Allium* species, is a biennial plant that can be propagated generatively using two methods of seed production strategies, seed to seed and seed to bulb to seed are possible. The seed to bulb to seed method requires a two year cycle with one year being used to produce the bulbs which then produce seeding plants in the following year (Dowker, 1989). At the end of the first year, the bulbs need about three months of rest period to uniformly regenerate in the next season. This method has a long time requirement, but provides an opportunity for intensive selection for bulb quality. On the other hand, Brewster and Bulter (1989) were able to produce seeds in the seed to seed cycle within 12 months. Using this method, although seed production is achieved within a shorter period, selection for bulb quality is not possible. In the seed-bulb-seed method either bulbs or plants regenerated from bulbs need to receive cold treatment in the store and/or in the field (over-wintering). In the seed to seed method, seedlings should be vernalized. Onion (Brewster, 1985) and shallot (Krontal *et al*., 1998) seedlings and sets have been used to assess the effects of seedling stage and temperature on bolting but in separate sets of experiments.

Both plants in their growing and rest phases may be similarly induced to flower, provided they are beyond their juvenile stage (Rabinowitch, 1985; 1990). Onion seeds are not sensitive for vernalization (Heath and Holdsworth, 1948 cited by Rabinowitch, 1990). Under optimal vernalizing temperature, small bulbs show little or no bolting while sensitivity to bolting

increased with bulb size. The minimum number of leaves (including leaf initials and scales) required before flowering in onion bulbs, sets and growing plants was between 10 to 14 depending on genotype. A minimum size of 4 to 7 g fresh weight was required for inflorescence initiation of stored sets of onion cv 'Ailsa Craig' (Heath and Holdsworth, 1947 cited by Rabinowitch, 1990). Shishido and Saito (1976) reported that a minimum sheath diameter of 3.3 and 6 mm is required before flower initiation can take place in onion cvs 'Sapporoki' and Senshuki', respectively. Higher critical weights are required for bulbs, as compared to growing seedlings, before initiation of flowering can occur.

Brewster and Bulter (1989) reported that treatment of growing onion seedlings at 9^{o}C under 4 to 6 h photoperiod extension resulted in about 30 days earlier and completed bolting in some bolting resistant onion cultivars. Seedlings of spring sown 'Rijnsburger' and an autumn sown 'Senshyu Semi-Globe-Yellow' onion cvs that had shoot weights of 0.06 and 0.45 g, respectively, could initiate inflorescences after being treated at about 9^{o}C (Brewster, 1985). Krontal *et al*. (2000) found early and high bolting from shallot seedlings, sets and bulbs of three shortday shallot genotypes treated at 5 to 10^{o}C under Israeli field conditions. They also found that bulbs of a shortday shallot cv 66-1004 treated at 5 to 10^{o}C and planted in the field, bolted faster than those treated at 13^{o}C and higher. Large (>6 g fresh weight) sized bulbs bolted earlier and faster than the smaller ones. On the other hand, cv 977-1011 did not bolt at all irrespective of temperature or bulb size.

Vernalization studies on onion bulbs and plants indicated that shorter duration of treatment was required for flower initiation within the range of 5 to 12^{o}C, and that longer periods were required under both lower and higher temperatures (van Kampen, 1970; Shishido and Saito, 1975; Brewster, 1982; 1983; 1987; 1989). Shallot bulbs or young plants treated with temperatures ranging from 5-15^{o}C for a period of 70 to 90 days initiated flowers (Sinnadurai, 1970). A maximum bolting of 6 to 33% was obtained from bulbs of four tropical shallot cultivars vernalized at 10^{o}C for 4 or 8 weeks; however, extending the treatment to 12 weeks did not increase the percentage of bolting (Tabor, 1996).

Similarly, crops other than *Allium* spp. also can be vernalized with some variations. Yuan *et al*. (1998) reported that extending vernalization from 10 to 15 weeks (at 5^{o}C and at a minimum growth stage of eight nodes) increased flowering percentage of marginally mature plants of coreopsis (*Coreopsis grandiflora* cv. 'Sunray') but did not influence time to flower.

Extending treatment duration (at 18/16^{o}C day/night temperatures, and 12 and 13.5 h photoperiods) from 21 to 35 or 49 days increased the number of flowers by five folds in strawberry (*Fragaria x ananassa* Duch.'Korona'); however, the inflorescence produced more flowers in 21 and 35 days than in 49 days (Konsin *et al*., 2001). Johnson and White (1997) reported that annual bluegrass *(Poa annua L.)* genotypes did not flower after three weeks of cold treatment at 4 and 8^{o}C. After 8 to 10 weeks of the treatment, inflorescences emerged in all var. *reptans* genotypes; however, inflorescences were often deformed, spikelets were aborted and vestigial leaves often grew in their places. Extension of the treatment to 10 to 12 weeks resulted in normal fertile flowers that emerged quickly and uniformly. Vernalization did not occur at 12^{o}C and temperature did not compensate for the duration of vernalization, but inflorescence emergence was faster at 8^{o}C. High and early flowering have been reported with increase in the duration cold treatment in perennial ryegrass (*Lolium perene* L.; Aamlid *et al*., 2000), turnip (*Brassica rapa* L.; Takahashi *et al*., 1994), cultivated (*Cicer arietinum* L. cv. 'Hadas') and wild (*C. reticulatum* L. cv. Cr205) species of chickpea (Abbo *et al*., 2002), and fern-leaf yarrow (*Achillea filipendulina* 'Parker'; Zoberi *et al*., 2003).

Although numerous studies undertaken on the influence of environmental factors on bolting of bulbs, sets and growing plants on onion exist, none has simultaneously investigated the sensitivity of these planting materials to vernalization treatments. The present study, therefore, was initiated to investigate the bolting potentials of the three types of planting materials of shallot: intact bulbs, growing plants regenerated from seeds and those regenerated from bulbs, at two temperatures (8 and 12^{o}C) and 16 h photoperiod. The second experiment was initiated due to the relatively low proportion of bolting obtained from vernalization of bulbs in the preceding experiment. Its objective was to investigate whether or not increasing the duration of bulb vernalization at the effective temperature (8^{o}C) increases bolting of shallot bulbs.

Materials and methods

Experiment 1: The influence of planting material on bolting

Production of bulbs

Seeds of two shallot varieties (Ambition F1 and Matador F1 obtained from Bejo Zaden, b.v. Warmenhuizen, Holland) that were found to be responsive to vernalization treatments (Chapter 2), were sown on 4 February 2002 on trays on Potgrond P peat medium (Klassmann,

Geeste Gross Hesepe, Germany). They were placed in the greenhouse of the Institute of Vegetable and Fruit Sciences, University of Hannover, Germany until became ready for transplanting on 6 March 2002. The ambient temperature of the greenhouse was kept at 18/16^{o}C (day/night) and was vented at 20^{o}C. Supplemental light (85 μmol $m^{-2}s^{-1}$ PPFD) was provided by SON-lamps (400 W) from 5:30 to 8:30 and from 15:30 to 19:00. The seedlings also received weekly fertigation of 10 ppm Flory® 2 (Euflor GmbH, München, Germany) containing 15, 7, 22, and 6% N, P_2O_5, K_2O and MgO, respectively and micronutrients. They were transplanted to Ruthe Field Experimental Station (52^{o}2'N and 9^{o}4' E) on 11 April 2002. The plots were sprayed twice with 40 l ha^{-1} Alzodef (calcium cyanamide; SKW Trostberg AG, Germany) to control major weeds. The bulbs were harvested at maturity on 17 July at about 75% leaf-fall and were cured in the greenhouse of the Institute until mid-August (Annex 2.1). Healthy bulbs with tightly closed necks were selected and stored at 18±0.5^{o}C until required for planting or for vernalization.

Plants regenerated from seedlings and from bulbs

Seeds of cultivars Ambition F1 and Matador F1 were sown on 11 September 2002 and managed as described above. The seedlings were transplanted on 10 October 2002 to 1.5 l pots containing the same medium as described above. In addition, uniform and healthy bulbs of both varieties produced in the previous season were planted on 8 November 2002 in the same types of container and medium as for the seedlings. The average fresh and dry weights of the bulbs of the respective varieties were 39.6 and 4.5 g for variety Ambition F1, and 41.0 and 4.7 g for variety Matador F1. The plants regenerated both from seeds and bulbs were grown in the greenhouse maintained at about 18^{o}C and supplemented with light (400 W SON-lamps, 144±18 μmol $m^{-2}s^{-1}$ PPFD) from 5:00 to 9:00 and from 18:00 to 19:00. Twenty-five selected plants each raised from seeds (PS) and others raised from bulbs (PB), and 104 intact bulbs (B) that had been kept in the store were transferred to growth chambers set at 8 and 12^{o}C under 16 h photoperiod on 23 December 2002. The bulbs were put in well ventilated plastic baskets and were placed at the same level as the pots. In the chambers constant irradiance (266±26 μmol $m^{-2}s^{-1}$ PPFD) and CO_2 (320 ppm) were supplied. The status of PB and PS at the beginning of the experiment is shown in Table 3.1. The fresh and dry weights of Ambition F1 and Matador F1 bulbs at the beginning of the experiment were 42.95 and 43.71g and 5.54 and 4.99g, respectively. The temperature and radiation of the greenhouse during the study period are shown in Annex 3.1

Twenty-five plants from each combination of the varieties and the planting materials were retained in the greenhouse while about 40 bulbs were left in the store as controls. At the end of the 60 days treatment, the plants in the chambers were taken back to the greenhouse and the intact bulbs both from the store (control) and the chambers were also planted in the greenhouse in the same medium and size of pots as the seedlings. The plants also received weekly fertigation of Flory® 2 at 10 ppm along with irrigation water. Metasystox (S-[2-(ethylsulfinyl)ethyl]O,O-dimethyl phosphorothioate; 1ml l^{-1}), Neudosan (potash soap; 2 ml l^{-1}) and Perfection (O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate; 1 ml l^{-1}) were sprayed alternately against onion trips (*Thrips tabaci* Lind.) as deemed necessary.

Table 3.1 Vegetative parameters of plants regenerated from bulbs (PB) and from seeds (PS) of Ambition F1 and Matador F1 at the beginning of the treatment [1]

Treatments	Matador F1 PB	Ambition F1 PB	Matador F1 PS	Ambition F1 PS
No. of shoots	6.40±1.14	7.80±2.59	1.00±0.00	1.00±0.00
No .of visible leaves	36.00±9.11	46.75±12.74	8.20±1.30	9.20±1.30
No. leaves/shoot	5.83±0.49	5.33±0.99	8.20±1.30	9.20±1.30
Sheath base diameter (cm)	0.90±0.12	0.79±0.08	0.87±0.14	0.90±0.19
Sheath neck diameter (cm)	0.79±0.11	0.66±0.06	0.77±0.08	0.78±0.13
Leaf DW (g)	5.05±0.98	4.30±0.39	1.06±0.32	1.34±0.43
Sheath DW (g)	2.20±0.49	1.83±0.63	0.33±0.07	0.32±0.11
Shoot DW (g)	7.25±0.15	6.13±0.95	1.39±0.39	1.67±0.51

[1]Data are average of 5 plants ± sd; DW, dry weight.

Experiment 2: The influence of duration of bulb treatment

Bulbs of the two shallot varieties that had been used in the previous experiments (Ambition F1 and Matador F1) and of additional five varieties that are vegetatively propagated in Europe (Sante, Red Sun, Golden Gourmet and Pikant) were all obtained from the same source as those of the previous experiment. The bulbs were sorted for uniformity and kept in a store at 18°C. For each combination of variety and duration of cold storage, thirty-five bulbs were taken from the store and transferred to growth chambers maintained at 8°C and treated for 30, 60 and 90 days. The same number of bulbs from each variety was also left in the store as control. The longest treatment duration was transferred first and the others followed at the interval of 30 days. At the end of the vernalization, twenty selected bulbs from each treatment were planted at the same date. The fresh weight of bulbs at the time of the transfer is shown in

Table 3.2. The temperature and radiation of the greenhouse during the study period are shown in Annex 4.1

Table 3.2 Fresh weight (g) of bulbs of seven shallot varieties at the time of transfer to growth chambers for 90, 60 and 30 d of treatment [1]

Variety	90 days	60 days	30 days
Ambition F1	32.91±12.96	34.95±11.57	31.01±7.90
Golden Gourmet	33.20±11.34	27.17±4.19	25.16±7.38
Matador F1	41.75±11.31	37.80±6.40	31.34±5.30
Mikor	35.46±6.72	32.64±8.93	37.83±8.06
Pikant	26.72±6.13	27.44±4.32	21.30±3.89
Red Sun	24.89±5.90	23.38±3.95	22.19±4.03
Sante	22.42±4.62	20.68±4.14	21.43±2.78

[1]Data are average of five bulbs ± sd

Data collection

Prior to the transfer of the plants to the growth chambers, five plants (bulbs) per treatment were randomly sampled to determine shoot growth parameters (Table 3.1). Bolting (emergence of inflorescence 1 cm above the sheath neck) and flowering (opening of at least one floret per umbel) were recorded every other day. The number of days between the end of the treatment and the beginning of bolting and flowering were recorded both on plant and inflorescence basis. The number of days to 50% final bolting and flowering of inflorescences and of plants were determined by regressing the cumulative bolting and proportion of flowering on the number of days from end of the treatment to bolting and flowering, respectively. In addition, total number of shoots and number of floral shoots per plant among the bolted plants and inflorescence height (from the base of the plant to the tip of the umbel), diameter (about 10 cm above the neck of the sheath) as well as umbel diameter were recorded at flowering. Based on the observations made at the beginning of anthesis, all plants of Ambition F1 and 80% of Matador F1 were male-sterile. Hence, pollen from the fertile plants of Matador F1 were checked for fertility using karmic acetic acid and five plants (one umbel per plant) per treatment were hand-pollinated every three days. Percent seed set was recorded after harvesting individual umbels at maturity. The umbels were then sun-dried, threshed and the seeds were stored at 4^{o}C. Hundred seed weight and percent germination were recorded after about three months in the store.

Experimental design and data analysis

The first experiment was a three-factor factorial experiment comprising two varieties, two temperatures and three planting materials while the second experiment which was a two factor factorial experiment consisted of three durations of vernalization and seven varieties. Each experimental unit contained 25 and 20 plants in experiment 1 and 2, respectively. Treatment effects were tested against plant-to-plant variation. The data were subjected to both ANOVA and regression analysis. Binomial data, bolting (1) and non-bolting (0), with their respective frequencies were used as inputs to predict the probability of bolting. Variety and type of planting material were used as classification variables. The logistic regression model was used to estimate the factors which influenced bolting behaviour (Agresti, 1996). The PROC LOGISTIC procedure of SAS software (SAS Institute INC., 1999) was used for the analysis. The best-fit-model was selected by backward elimination of predictors based on the -2log likelihood ratio (-2Log L) or deviance and the Akaike Information Criterion (AIC; Agresti, 1996) as described in Chapter 2.

Only significant main effects or interactions ($p \leq 0.05$) were considered for presentation. Plants and bulbs retained in the greenhouse and in the store were used as indicators of extra-treatment conditions but were not considered as part of the data analysis.
Only bolted varieties were considered for logistic regression in Experiment 2.

Results

Experiment 1: The influence of planting material on bolting

Data collected before the plants were transferred to the growth chambers for treatment (Tables 3.1 and 3.2) show that the plants and the bulbs were in the post-juvenile stage (Shishido and Saito, 1976; Brewster, 1985; Wiebe, 1994)

All models except models 5 to 8 had deviance values less than model 9 (Table 3.3). However, because they involved more terms, their AIC values were higher than in model 9. Therefore, model 9, which contains only effects of planting material was selected as the best model. In addition, type III analysis of effects revealed that all terms, except planting material, were not significant in the other models. Hence, non-significant terms were eliminated from the model at 5% level of significance. Neither the main effects of variety and temperature nor all

interactions among the three factors were significant. Consequently, fitting the model to the type of planting material was found to be highly significant ($p \leq 0.0001$) and hence was able to predict the probability of bolting. The predicted probabilities of bolting that were computed using the selected model were also satisfactorily close to the observed proportions of bolting ($r=0.97$).

Table 3.3 Successively selected models using backward elimination of predictors based on deviance (-2Log L) and AIC

Model	Predictors[1]	Deviance (-2Log L)	df	AIC	Models compared	Deviance difference
1	V* T* PM	222.40	284	246.41	-	-
2	T*V V*PM T* PM	223.32	286	243.32	(2, 1)	0.92
3	V*PM T*PM	223.33	287	241.33	(3, 2)	0.01
4	V*PM	225.44	289	239.44	(4, 3)	2.11
5	V T PM	229.27	291	239.27	(5, 4)	3.83
6	V T	271.49	293	277.49	(6, 5)	42.22
7	V	271.52	294	275.52	(7, 6)	-0.03
8	T	272.01	294	276.01	(8, 6)	-0.52
9**	PM	227.97	293	235.97	(9, 6)	-43.52

[1]V, variety; T, temperature; PM, planting material. The models are symbolized by their highest order terms which includes main effects and interaction of the highest terms (in case of V*T*PM); * sign indicates interaction; **selected model.

Table 3.4 Analysis of maximum likelihood estimates of the selected model

Parameter	df	Estimates	se	Wald χ^2	p> χ^2
Intercept	1	1.999	0.231	74.594	<0.0001
Bulb (B)	1	-1.424	0.261	29.787	<0.0001
Plants from Bulbs (PB)	1	1.477	0.410	12.974	0.0003

df, degree of freedom; se, standard error; p, probability level

The data in Table 3.4 show the analysis of maximum likelihood estimates of the selected model. Hence, the logit of probability of bolting (π) is estimated by the following equation.

$$\text{Logit}(\pi) = 1.999 - 1.424B + 1.477PB \qquad (3.1)$$

Figure 3.1 shows that the probabilities of bolting in B was significantly lower (0.64) than that of PS (0.88) and PB (0.97), with no significant difference between the latter two. The corresponding odds ratios computed from the model indicated that bolting in B, PS and PB are 1.7, 7 and 32.8 times higher than non-bolting of the respective treatments.

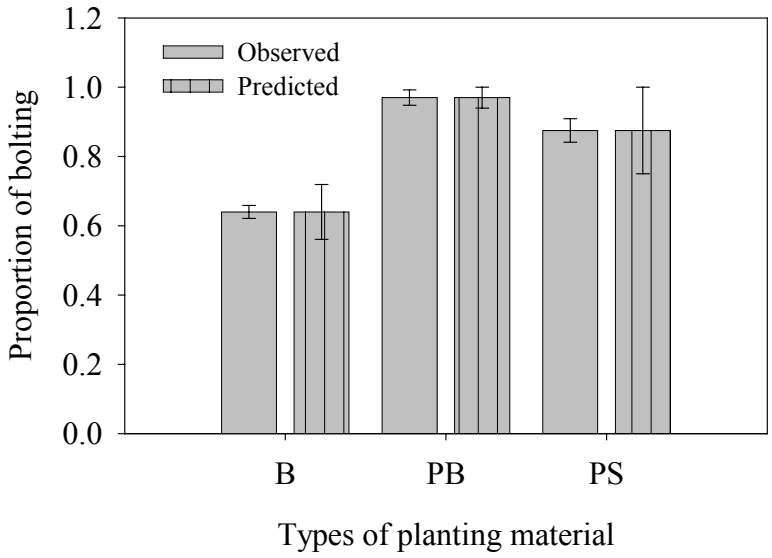

Fig. 3.1 Observed proportions and predicted probabilities of bolting of the three different types of planting materials (means across temperature and varieties, n=80): B, intact bulbs were treated before planting; PS, plants regenerated from seedlings were treated; PB, plants regenerated from bulbs were treated. Error bars indicate 95% confidence interval.

Inflorescence characteristics

Figure 3.2 shows that PS had significantly higher ($p \leq 0.05$) inflorescence height, inflorescence stem diameter, umbel diameter and number of florets per umbel as compared to PB and B. The difference between PB and B was not significant except in inflorescence stem diameter where B had thinner inflorescence stem than PB. The number of florets ranged from 195 in plants regenerated from bulbs of Matador F1 and treated at 8^{o}C to 1223 in plants of Ambition F1 regenerated from seedlings and treated at the same temperature. The average height of the inflorescences in this experiment did not exceed 1m. The observed interaction between the types of planting material and temperature in inflorescence height was due to significantly taller PS treated at 12^{o}C than the other treatments (Figure 3.2c). No interaction was observed among the treatments in the other parameters.

Proportions of shoots per plant

The results of this study also showed that PB produced a total of about eight shoots per plant on the average as compared to seven in B and four and half in PS (Figure 3.3). Among the

shoots of PB, 77% of them were floral shoots while the remaining 30% were barren (vegetative) indicating that presumably the majority of the shoots that were visible at the beginning of the treatment (Table 3.1) were vernalized.

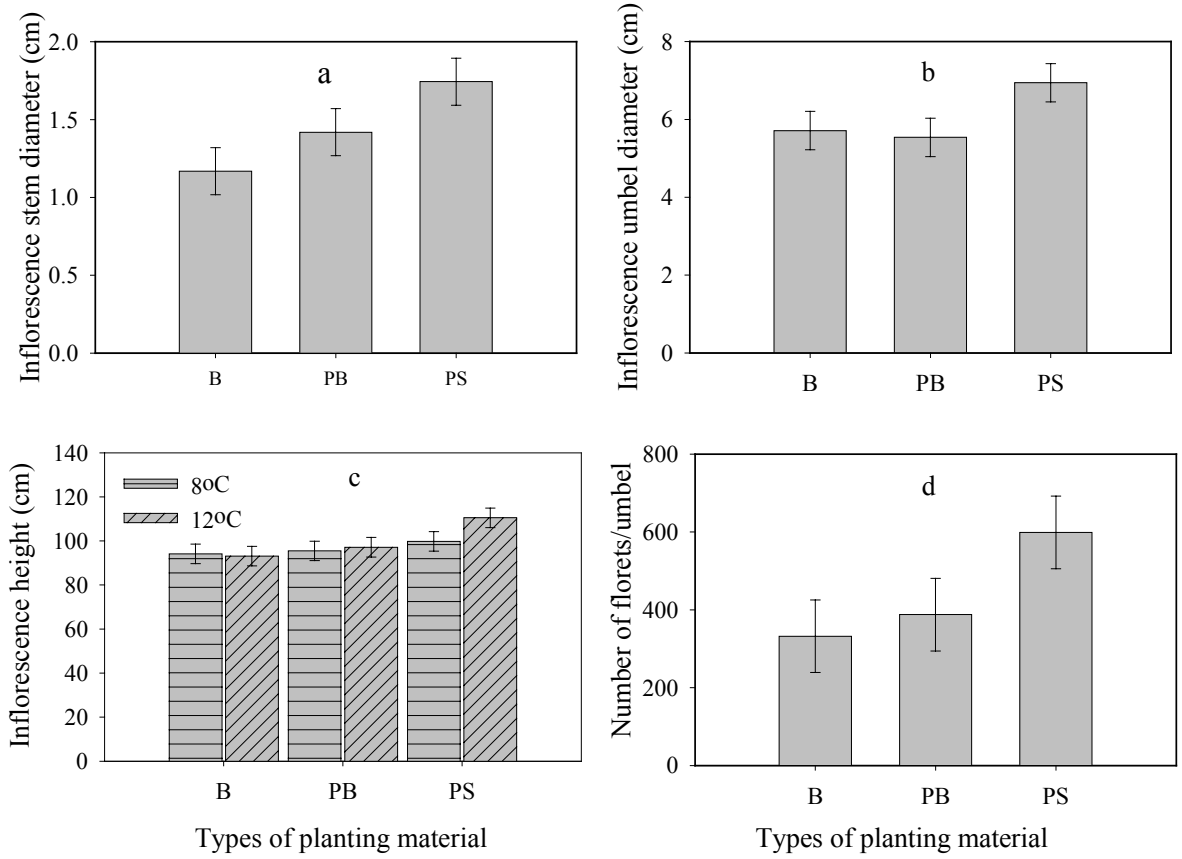

Fig. 3.2 Effects of types of planting material on inflorescence characteristics: a) inflorescence stem diameter, b) inflorescence umbel diameter, c) inflorescence height (means across varieties, n=6), and d) number of florets per umbel (a, b and d are means across varieties and temperatures, n=12). B, PB and PS as described for Figure 3.1. Error bars indicate ± two times standard errors.

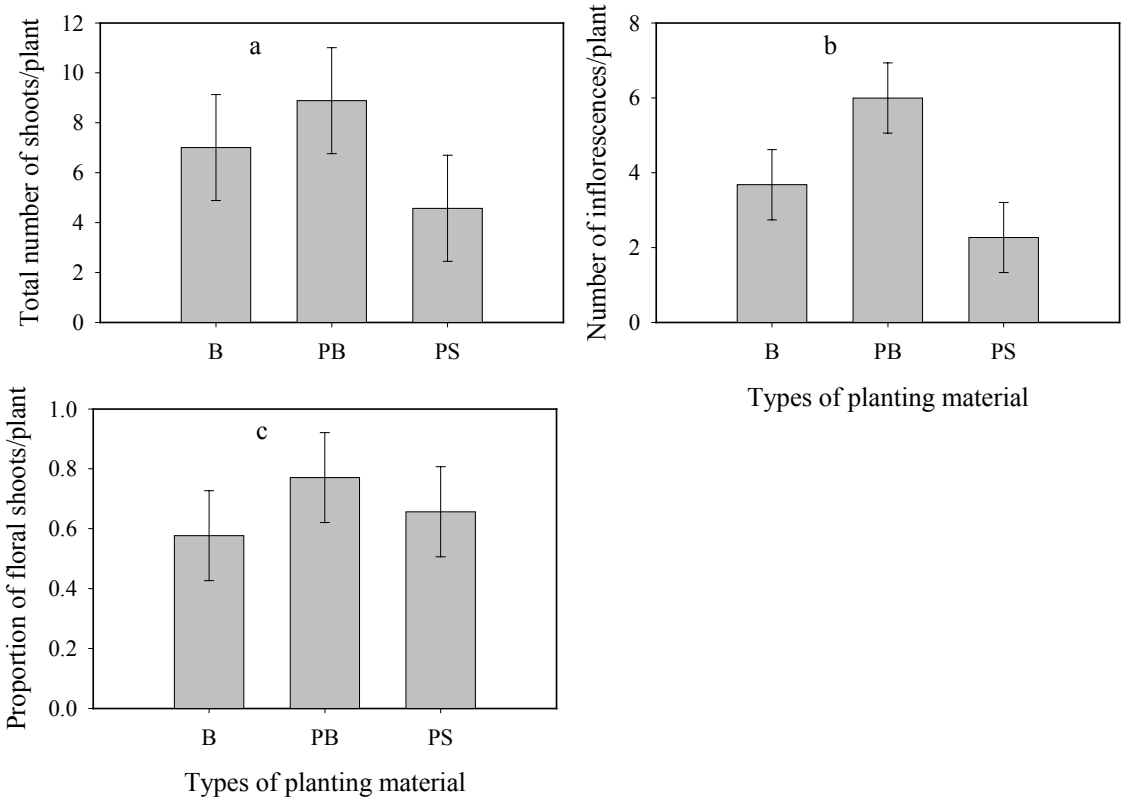

Fig. 3.3 Effects of planting material on the proportion of shoots among the bolted plants (means across varieties and temperatures, n=12): a) total number of shoots per plant, b) number of inflorescences per plant, and c) proportion of floral shoots per plant. B, PB and PS are as described for Figure 3.1. Error bars indicate ± two times standard errors.

Days to bolting and flowering

Figures 3.4 and 3.5 show cumulative number of bolted plants and inflorescences, respectively, during post treatment period. The average time of the beginning of bolting after the end of vernalization treatment in B, PB and PS was 45, 15 and 20 days, respectively (Table 3.4). Bolting was extended up to 77, 70 and 60 days after the end of the treatment respectively, i.e., it continued for a duration of about 32, 55 and 40 days in B, PB and PS. Despite the fact that planting of treated bulbs (B) was possible only after the end of the treatment, the short duration of bolting gave homogenous bolting, flowering and seed maturity within and among the plants. PS and PB took about 164 and 86 days, respectively, from planting to the end of the treatment. Thus, the average time from sowing (planting) to bolting in B, PB and PS ranged from 45-77, 101-156 and 184-224 days, respectively. The flowering behaviour of the plants followed the same trend as bolting; the range from the earliest to the latest flowering of

inflorescences being 70-99, 156-185 and 247-265 days for the respective treatments (data not shown). As a result of hand pollination only about 25% of the flowers were able to set seeds.

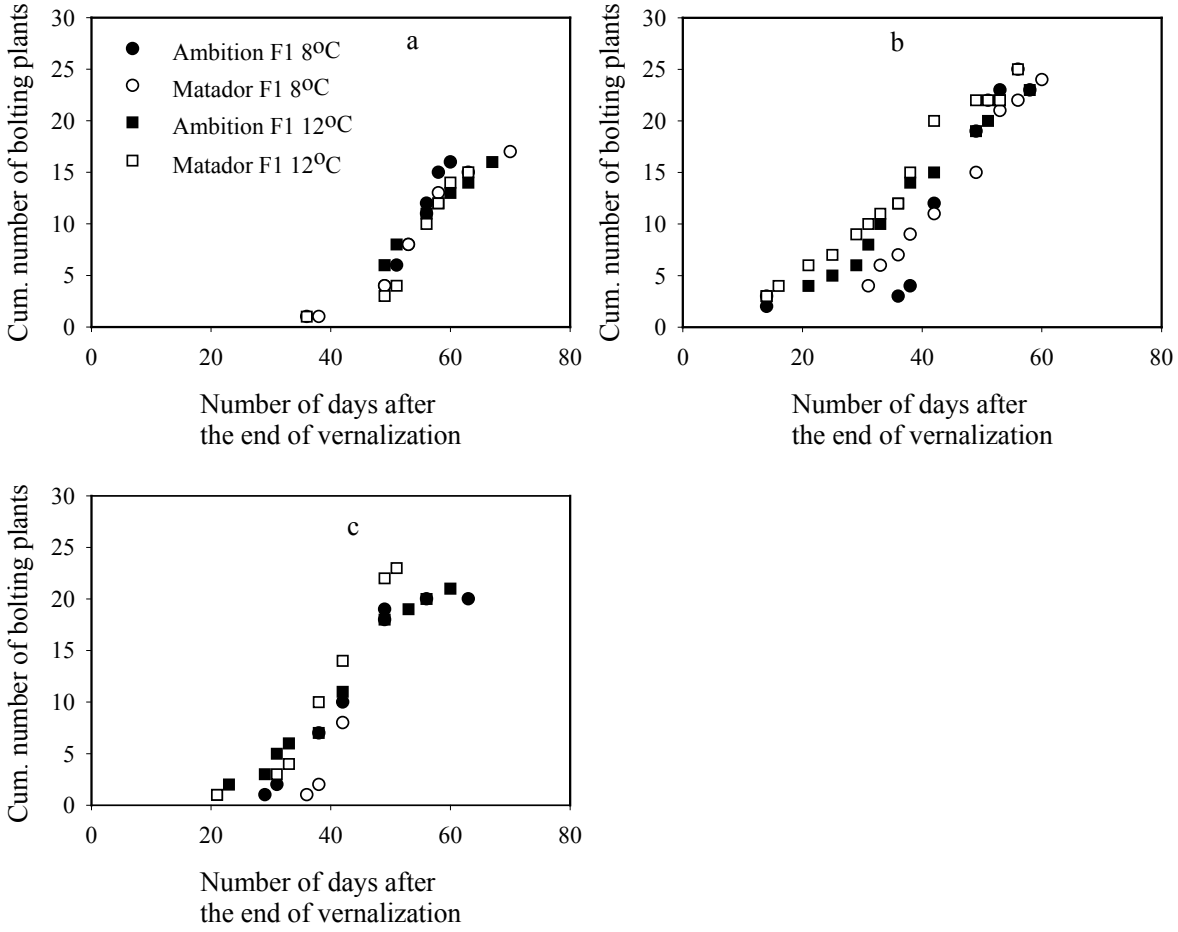

Fig. 3.4 Cumulative number of bolting plants of the three different types of planting materials after the end of the vernalization treatment (25 plants per treatment): a) intact bulbs, b) plants regenerated from bulbs, and c) plants regenerated from seeds.

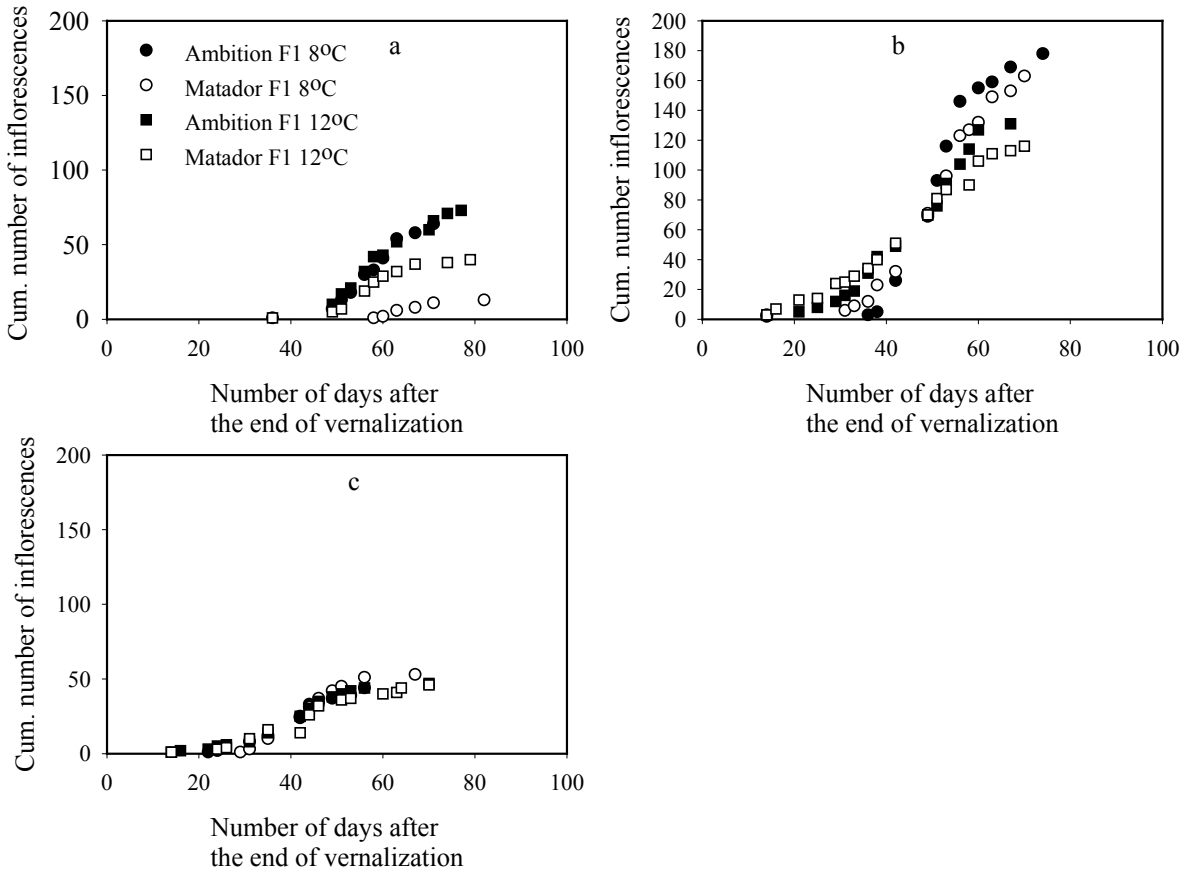

Fig. 3.5 Cumulative number of inflorescences of the three different types of planting materials after the end of the treatment (25 plants per treatment): a) intact bulbs, b) plants regenerated from bulbs, and c) plants regenerated from seeds.

Table 3.4 Range of days to bolting and days to 50% of the final bolting of the three different planting materials of Ambition F1 and Matador F1 vernalized at two temperatures.

Planting material	Variety	Temperature (°C)	Number of bolted plants	Number of inflorescences	Days to bolting (range)	Days to 50% of the final bolting
B	Ambition F1	8	16	56	36 - 71	56.4
B	Ambition F1	12	16	70	49 - 77	58.6
B	Matador F1	8	17	57	58 - 82	66.2
B	Matador F1	12	15	39	36 - 79	56.0
PB	Ambition F1	8	25	168	14 - 74	47.0
PB	Ambition F1	12	23	134	16 - 67	44.0
PB	Matador F1	8	24	156	14 - 70	48.7
PB	Matador F1	12	25	122	14 - 67	43.0
PS	Ambition F1	8	20	42	22 - 56	39.2
PS	Ambition F1	12	21	48	14 - 56	39.4
PS	Matador F1	8	20	54	29 - 56	42.0
PS	Matador F1	12	23	46	14 - 70	41.5

Experiment 2: The influence of duration of bulb treatment

The five vegetatively propagated varieties Sante, Red Sun, Golden Gourmet and Pikant failed to bolt irrespective of the duration of bulb vernalization. Hence the logistic regression analysis of bolting was done only for the two responsive varieties, Ambition F1 and Matador F1 (Table 3.5).

Table 3.5 Successively selected models using backward elimination of predictors based on deviance (-2Log L) and AIC.

Model No.	Predictors[1]	Deviance (-2Log L)	df	AIC	Models compared	Deviance difference
1	V*D	183.62	153	193.62	-	-
2	V D D^2	184.18	154	192.18	(2, 1)	0.56
3**	D D^2	184.43	155	190.43	(3, 2)	0.25
4	V	213.81	156	217.81	(4, 3)	29.38
5	D	196.15	156	200.15	(5, 4)	11.72
6	D^2	204.22	156	208.21	(6, 5)	19.79

D, Duration; V, variety; df, degrees of freedom; **selected model

Table 3.6 Analysis maximum likelihood estimates of the selected model

Paramer	df	Estimate	se	Wald χ^2	$p > \chi^2$
Intercept	1	-2.367	0.5290	20.014	<0.0001
Duration	1	0.089	0.0229	15.314	<0.0001
$Duration^2$	1	-0.001	0.0002	10.405	0.0013

df, degree of freedom; se, standard error; p, probability level

An attempt to establish the relationship considering duration of cold treatment to have a linear response resulted in a poor overall fit. Therefore, as in Chapter 2, the duration of the cold treatment was considered to have a quadratic response. Hence, the model selection was done based on the AIC and deviance as shown in Table 3.5. The selected model (model 3) was significant at $p< 0.0001$ level of probability. As in Chapter 2 and Experiment 1 in this chapter, no interaction was observed between duration of bulb vernalization and variety in the proportion of bolting plants. Duration of cold treatment was significant at ($p<0.001$); but the difference was only between the treated and the non-treated bulbs with no significant difference between 30, 60 and 90 days. A quadratic relationship between the logit of the probability of bolting and the duration of vernalization period, i.e., using model 3, was found. High correlation was also found between the predicted and observed values (0.99 and 0.98 for Ambition F1 and Matador F1, respectively). Based on the analysis of likelihood estimates of the selected model, the logit of the probability of bolting was predicted irrespective of varieties as follows:

Logit (π) = -2.367+0.089(D) -0.0007 $(D)^2$ (3.2)

D is the duration of vernalization in days.

As shown in Figure 3.6 the maximum proportion of bolting obtained in plants treated for 60 days was about 0.6 which is not significantly different from that of 30 and 90 d durations.

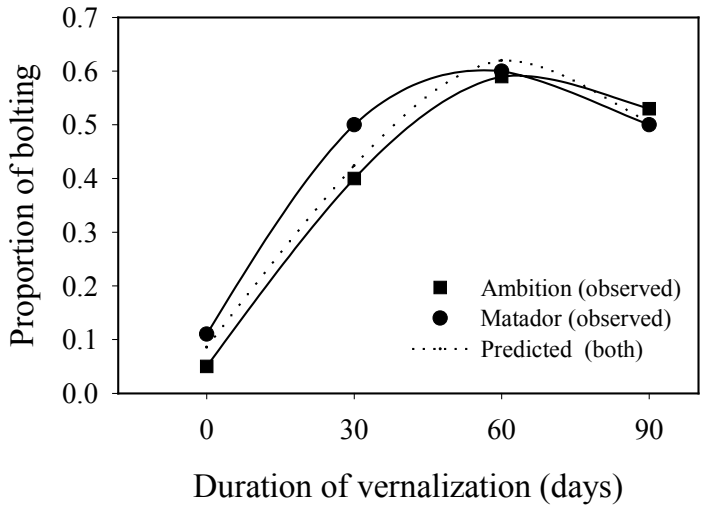

Fig. 3.6 Observed proportion and predicted probabilities of bolting of Ambition F1 and Matador F1 after vernalization of bulbs for 30, 60 and 90 days.

Inflorescence characteristics

No significant difference was observed between the varieties and among the durations of vernalization in the proportion of floral shoots of bolted plants and in the proportion of floral shoots of all plants in the treatment. However, bulbs of Ambition F1 stored for 60 and 90 days produced significantly more inflorescences per total number of shoots of all plants than Matador F1 (data not shown). No significant difference was observed among treatments in inflorescence diameter and height. 'Leafy inflorescences' (>10 cm) accounted for 18 and 12.5% of the inflorescences in Ambition F1 bulbs treated for 30 and 60 d whereas in Matador F1 even those treated for 60 and 90 d had 25 and 33%, respectively. Figures 3.7 and 3.8 show that in Ambition F1 bolting of plants from bulbs treated for 30 and 60 d were more extended than from those treated for 90 d but there was no difference among the treatments in Matador F1.

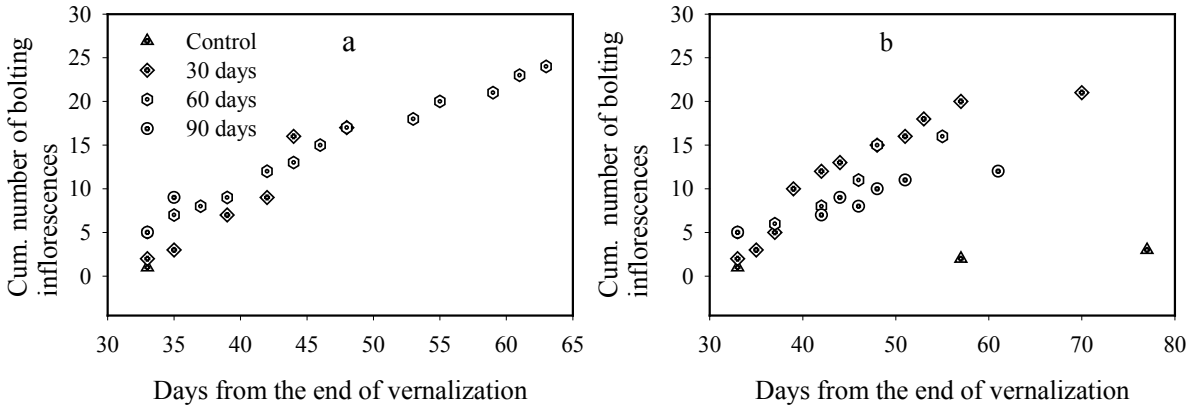

Fig. 3.7 Cumulative number of bolting of plants vernalized for different periods (20 plants per treatment): a) Ambition F1 and b) Matador F1.

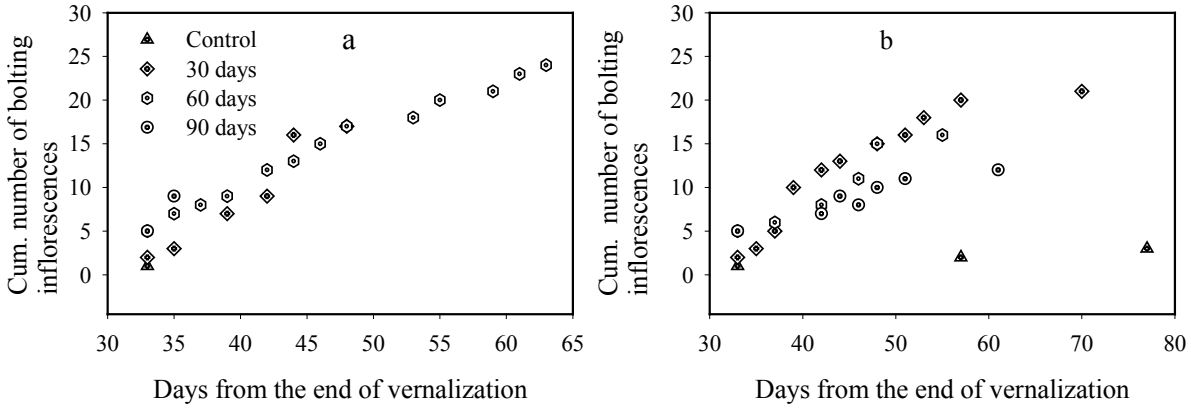

Fig. 3.8 Cumulative number of bolting inflorescences from bulbs vernalized for different periods (20 plants per treatment): a) Ambition F1 and b) Matador F1.

Discussion

Experiment 1: The influence of planting material on bolting

While comparing the different types of planting material, attempts were made to synchronize the stages of PB and PS by planting PB 28 days after transplanting PS which narrowed the difference in the number of leaves per shoot to about three (Table 3.1). The difference in total leaf number between the plants was much higher. The difference in the proportion of bolting plants between PB and PS, however, was not significant indicating little relevance of these differences once the plants are at the post-juvenile stage. The ability of bulbs to respond to vernalizing treatments is based on this concept although the probability of bolting in B was

significantly lower than that of PS and PB, with no significant difference between the latter two (Figure 3.1). Berghoef *et al.* (1992) also reported that floral initiation and development are scarcer in stored bulbs than in growing plants of ornamental onion (*Allium sphaerocephalon* L.).

The number of florets produced was within the common range (200-600) reported for onion (Ali *et al*., 1984 cited by Rabinowitch, 1990). Onion scape reaches about 1-1.8m in length even at spathe opening (Krontal *et al*., 1998), but the average height of inflorescences in this study did not exceed 1m. The number of total shoots per plant that ranged from 4.5 in PS to 8 in PB (Figure 3.3) was in the lower range of the values reported by Krontal *et al*. (2000). They reported that the number of laterals was highly influenced by growing temperatures ranging from 6 at 17/9^{o}C to 28 at 29/21^{o}C. However, from an other experiment they reported that the number of inflorescences per plant (cluster) in field grown shallot ranged from 1 to 3.5 in cv 66-1004 depending on the sowing date . The average number of floral shoots in PB, B and PS was about 6, 3.7 and 2.3, respectively.

Assuming a constant percentage seed set among the treatments, the amount of seeds that produced would be direct function of the number of florets. Consequently, the total number of florets per plant, taken as the product of the number of inflorescences and the number of florets, PB would produce 90 and 71% more florets and seeds than B and PS, respectively. However, under field conditions the number of inflorescences per unit area in PS can be increased by reducing its spacing. On the other hand, due to the low proportion of floral buds (0.64) coupled with the already high number of shoots per cluster reducing the spacing between plants in B might not able to fully compensate for the low yield. Hence renders B as a less preferred method of seed production. But ease of treatment of bulbs while they are in storage and the possibility of selection for bulb quality still compromise the feasibility of the method for use in breeding programs and where field temperatures are not sufficient to vernalize field grown plants. Hence, further investigation to increase the proportion of bolting and the number of inflorescences per plant as well as to invigorate the umbels for more florets and seeds is required.

Experiment 2: The influence of duration of bulb treatment

The vegetatively propagated shallot cultivars apparently have been selected for resistance to bolting, thus they failed to bolt regardless of the vernalization treatments. Similarly, Krontal

et al. (2000) also reported that a clonally propagated shallot cv from Nepal failed to flower even after it was treated at 5 to 30^{o}C for 21 days. Tabor (1996) also obtained very only 6% bolting in bolting resistant shallot cultivars which were selected for vegetative bulb production. Sheldon *et al.* (2000) reported that even genome wide demethylation, which prompted flowering in vernalization responsive *Arabidopsis* ecotypes and mutants failed to induce flowering in non-vernalization responsive plants.

As opposed to the various reports (Takahashi *et al*., 1994; Johnson and White, 1997; Yuan *et al*., 1998; Konsin *et al*., 2000), extension of the duration of cold temperature treatment from 30 to 60 and 90 d did not increase bolting significantly even in responsive varieties, Ambition F1 and Matador F1. Tabor (1996) also found no significant increase in the bolting percentage of tropical shallot cultivars as the duration of temperature was extended from 8 to 12 weeks.

The proportion of leafy inflorescences was very high reaching up to 25%. Leafy inflorescences, i.e., the development of the spathe into a leaf-like structure, has been reported as the sign of partial (insufficient) vernalization (Bernier *et al*., 1981). It prohibited normal development and opening of florets; the florets were deformed as a result of compaction and opened while they were inside the spathe. Consequently, seed setting of such florets is presumed to be significantly reduced.

In conclusion, vernalization of growing plants regenerated either from seeds or bulbs at 8 and 12^{o}C under 16 h for 60 days can give higher bolting compared to vernalization of bulbs. Bulb vernalization will be an alternative where facilities are not available and field temperature is not effective to vernalize growing plants. If the field temperature is found to be optimum for vernalization, planting the bulbs by synchronizing the time of optimum vernalization temperature with the right stage of the plants (Krontal *et al*., 2000) should be assessed to increase the percentage of bolting in bulbs.

Chapter 4

The influence of juvenility on bolting of shallot (*Allium cepa* L. var. *ascalonicum* Backer)

Abstract

In an attempt to produce shallot through seeds, vernalization of growing plants was found to be effective. However, young shallot plants failed to flower before they attained a certain critical stage of development. To identify the stage of development at which the plants flower satisfactorily, twenty plants of each of the three shallot varieties: one long-day (LD) variety, Matador F1 and two shortday (SD) varieties, Tropix F1 and Rox F1, were grown in two separate greenhouses maintained at about 18^{o}C and 16 and 12 h photoperiods, respectively for a period of 120, 90, 60, 30 and 0 (imbibed seeds) days. The seedlings and the imbibed seeds were then vernalized in growth chambers at 8^{o}C for 60 days. Logistic regression was used to predict the probability of bolting plants and plant-to-plant variation was used to detect the difference in inflorescence characters among the treatments. Complete bolting was obtained in plants treated at six-leaf stage (after 90 d) in Tropix, at 17 leaf-stage (120 d) in Rox F1 and at 12 leaf-stage (120 d) in Matador F1 or after they attained 7.8, 9.8 and 15.5 g/100 g of sheath DW of TSCHO. However, the proportion of bolting in Rox F1 and Matador F1 vernalized after 90 d of growth was only 0.75 and 0.60. Apart from the high proportion of bolting, plants which attained sufficient size also had high proportion of floral shoots and bolted shortly after the end of vernalization. An increase of about one inflorescence per plant was obtained as the number of days before treatment was increased from 90 to 120 d in the two SD varieties. In addition, plants of the three varieties vernalized after 120 d, on the average, produced about 4.4, 1.38 and 0.52 inflorescences per day per treatment (20 plants) whereas those vernalized after 90 d and 60 d produced about one inflorescence per day and one to two inflorescences per 10 days, respectively. No interaction was found in inflorescence characters but the stages were significantly different. Moreover, thicker and taller inflorescences were obtained from the high bolting treatments. A strong positive correlation was also observed between the content of total soluble carbohydrates (TSCHO) in the sheath and the proportion of bolting. The study revealed that vernalizing shallot seedlings at the age of 60 d or younger did not result in sufficient bolting. The proportion of bolting increased with increase in plant age at the beginning of treatment and thus to obtain complete bolting, plants should attain the leaf stages and the sheath TSCHO specified above.

Introduction

The juvenile phase is a period during early development in which the plant is totally insensitive to conditions which later promote floral transition (Bernier *et al*., 1981). Plants may flower at a very small size or may do so only after a protected period of vegetative growth and attainment of a minimal size. At the completion of the juvenile phase, the plants become sensitive to conditions which eventually cause flower initiation. This change in phase is determined by mechanisms intrinsic to the shoot apex itself, rather than by the conditions prevailing within the differentiated parts of the plants (Robinson and Wareing, 1969; Bernier *et al*., 1981).

Dry seeds cannot be vernalized, but imbibed seeds of many species are responsive. Seeds of many cereals can be imbibed with water as a pre-requisite for vernalization (Purvis, 1961 cited by Michaels and Amasino, 2000). In some species, such as rye, embryos can be vernalized prior to seed desiccation. In other plants, however, imbibed seeds or young seedlings cannot be vernalized, but must reach a critical stage before vernalization can occur (Michaels and Amasino, 2000). Meristematic cells of such plants must first be competent to respond to developmental signals that evoke them into a florally determined state (McDaniel *et al*., 1992).

Bulb onions (*Allium cepa* L.) can not be induced to flower during the juvenile phase (van Kampen, 1970). Attempts to vernalize onion seeds failed (Heath and Holdsworth, 1948 cited by Rabinowitch, 1990). However, floral induction may occur both during the growth of plants and during the storage of bulbs, provided they have completed their juvenile phase. After completion of the juvenile phase, receptivity of onion to inductive temperature and photoperiod increases with age and size (Rabinowitch, 1979). The minimum number of leaves (including leaf initials and scales) required before flowering in onion bulbs, sets and growing plants was between 10 to 14 depending on genotype (Rabinowitch, 1985; 1990). The spring sown 'Rijnsburger' and an autumn sown 'Senshyu' onion cvs raised at 17^{o}C and 600 µmol $m^{-2}s^{-1}$ PPFD had to produce six to ten leaf initials, respectively, before they could initiate inflorescence at 9^{o}C. The stages correspond to 60 and 450 mg shoot dry weights and 3.8 and 8.5 mm shoot diameters (Brewster, 1985). Shishido and Saito (1976) reported a minimum sheath diameter of 3 and 6 mm in cvs 'Sapporoki' and 'Senshuki', respectively. Similarly, shallot seedlings initiated inflorescence at six or more leaves, including leaf

primordial (Rabinowitch, 1990; Krontal *et al*., 1998). However, Krontal, *et al*. (2000) indicated that the length of the juvenile phase is highly dependent on environment. Their study revealed that seedlings grown in a phytotron at 17/9^{o}C and 26/18^{o}C day/night temperatures produced 13 to 16 and 18 leaves, respectively, before the first inflorescence became visible. Early sown plants accumulated more mass and produced earlier and more axiliary buds and side shoots, had high (>90%) and early flowering and three inflorescences per plant while the late sown plants had low (55%) bolting, late flowering with single inflorescences per plant (Krontal *et al*., 2000). Brewster and Bulter (1989) reported that in bolting resistant onion cvs 'Rawska' and 'Kaizuka' sown early under favourable growing medium (Perlite), supplemental light (94 μmol $m^{-2}s^{-1}$ PPFD) and extended photoperiod (4 to 6 h) had accelerated growth of seedlings and attained the critical size for vernalization within a shorter period of time. Consequently, complete and early flowering was attained after treatment between 7 to 11^{o}C compared to their counter parts which flowered late and only up to 20-30%.

It is assumed that higher critical weights are required for bulbs as compared with seedlings. Large bulbs (>6.0 g fresh weight) of a shortday shallot cv 66-1004 treated at 5-10^{o}C bolted earlier and faster than smaller ones. In contrast, cultivars like 977-1011 did not bolt at all irrespective of temperature and bulb size (Krontal *et al.*, 2000). A minimum size of 4 to 7 g fresh weight was required for inflorescence initiation of stored sets of onion cv 'Ailsa Craig' (Heath and Holdsworth, 1947 cited by Rabinowitch, 1990).

Many other plant species such as leek (*Allium porrum* L.; Wiebe, 1994), *Delphinium* spp. (Kikuchi *et al*., 2000), coreopsis (*Coreopsis grandiflora* L. cv.'Sunray'; Yuan *et al.*, 1998), chicory (*Cichorium intybus* L.; Bernier *et al*., 1981), annual bluegrass (*Poa annua* L. var. *reptans;* Johnson and White, 1997), winter rape (*Brassica napus* L. var. *oleracea*; Dubert and Filek, 1994) and turnip (*Brassica rapa* L.; Takahashi *et al.*, 1994) are also known to have juvenile phase.

Low percentage and delayed flowering is often found to be associated with low and profuse and early bolting with high carbohydrate level. Ito *et al*. (2001) reported that promotive or inhibitory effects of sugar on flowering depend on the concentration and time of addition of sugar as well as the genetic background of plants. In *Sinapis* spp. it is supposed that changes in cell proliferation preceding flowering require large expenditure of energy supplying

compounds (Bernier *et al*., 1981). Arabidopsis can flower in complete darkness if the aerial portion of the plant is supplied with sucrose or glucose (Araki and Komeda, 1990). Treatments such as supplementary irradiation and low N treatments (Shishido and Saito, 1976; Brewster, 1983; 1985; Brewster and Bulter, 1989) increased the soluble carbohydrate content in onion plants and resulted in high bolting. Plants with low sugar reserves flowered late (Brewster, 1985).

Early and transient increase in the flux of sucrose in the phloem sap during floral transition in longday (LD) induced plants of *Sinapis alba* (Lejuene *et al.*, 1991) and increased sucrose export from leaves of LD or displaced shortday (DSD) *Arabidopsis thaliana* plant (Corbesier *et al.,* 1998). The amplitude of the increase in export of sucrose reflected the efficiency of floral induction by either LD or DSD. Strong competition for photosynthates by the vegetative lateral buds also led to a suppression of reproductive buds (Rabinowitch, 1990). On the other hand, although the export of sucrose out of the leaves and its content in the phloem sap increased in *Xanthium strumarium* L. (Houssa *et al.,* 1991) and in *Trifolium pratense* (Jones, 1990) exposed to inductive conditions, it did not suffice to trigger the complete sequence of floral evocation. Bodson and Outlaw (1985) suggested that the sucrose that accumulates soon after photoinduction originates not from increased photosynthesis, but from mobilization of reserve carbohydrates.

Shallot is characterized by continuous initiation of lateral buds through most of its growth period that results in vast differences in developmental stages among the buds (apices) of a plant. Since juvenility of an apex influences its sensitivity to vernalization treatments, apices of different stages may respond differently (McDaniel *et al*., 1992). Vernalization treatment given for a certain duration thus may induce flowering in older buds while it partially or fully fails to do so in younger ones. The buds which failed to be induced proceed to bulbing while those which succeed tend to bolt and produce seeds. Thus, producers of shallot seeds and bulbs should cautiously synchronize the plant growth stages to growing weather conditions depending on the intended purpose of production. Such information is not available for shallot. The objective of the present study is, therefore, to determine the optimum growth stages at which flowering can be induced in some ecologically different (LD and SD) shallot varieties and to relate these stages to growth parameters such as number visible leaves and total soluble carbohydrate contents of the seedlings at the beginning of vernalization treatment.

Materials and methods

A longday variety (Matador F1) which was used in the previous experiments and two shortday (SD) varieties (Rox F1 and Tropix) were obtained from Bejo Zaden, bv. Warmenhuizen, Holland. The seeds of these varieties were sown in monthly intervals between 21 July and 21 November 2003 in order to establish plants that are 120, 90, 60 and 30 days (d) old. Imbibed seeds (IS) were taken as 0 d treatment. Seeds of the LD and SD varieties were sown in separate greenhouses that were maintained at 18^{o}C and ventilated at 20^{o}C and were grown for one month. The seedlings were then transplanted to 1.5 l pots and kept in the same greenhouses. The LD variety was supplemented with additional light from 6:00 to 8:00 and 18:00 to 20:00. The light intensity in the greenhouses was about 112 μmol m^{-2} s^{-1} PPFD. At the end of growing for the above periods, twenty plants were selected from each growth stage and transferred to two growth chambers maintained at 8^{o}C on 21 November and vernalized for a period of 60 days. The same number of plants was also kept in the greenhouses as untreated checks. The SD and LD varieties were supplied with 114 and 118 μmol m^{-2} s^{-1} PPFD for 12 and 16 h, respectively and CO_2 was kept constant at 320 ppm. The plants also received a weekly fertigation of 10 ppm Flory® 2 (Euflor GmbH, München, Germany) containing 15, 7, 22 and 6% N, P_2O_5, K_2O and MgO, respectively and micronutrients. Metasystox (S-[2-(ethylsulfinyl)ethyl]O,O-dimethyl phosphorothioate; 1ml l^{-1}) and Perfection (O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate; 1 ml l^{-1}) were sprayed alternately against onion trips (*Thrips tabaci* Lind.) as deemed necessary.

Total soluble carbohydrates (TSCHO) analysis

Duplicate samples of leaves (from the second fully expanded leaf) and leaf-sheaths were collected. Moreover, about 0.6 g of seeds of each variety was imbibed with distilled water for 36 h at room temperature (18^{o}C). The samples were ground using an electric mortar after adding twenty times (w/w) distilled water of the fresh weight. The samples were shaken on a vertical-shaker for 5 minutes, centrifuged for 10 minutes at 7000 rpm and then filtered through a filter paper (Schleicher and Schuell No. 593, Dassel, Germany). A sample of 500 μl of the supernatant was mixed with 500 μl of solution of anthrone ($C_{14}H_{10}O$) reagent (200 g anthrone dissolved in 100 ml of 95% H_2SO_4). Then it was incubated for 15 minutes in a water bath heated to 95°C and later cooled down to 20°C. Total soluble carbohydrates were measured using a Lambda 2S spectrophotometer (Perkin Elmer, USA) at 578 nm. Distilled water was used as a reference (Scherz and Bonn, 1998) .

Data collection

Before transfer to the growth chambers five plants were harvested from each of the developmental stages. Numbers of leaves and shoots were counted, shoot fresh and dry weights measured. Total soluble carbohydrates were determined from leaves and sheaths. Bolting, emergence of inflorescences (about 1 cm) above the sheath neck, and flowering (opening of at least one floret per umbel) were recorded every other day.

Statistical analysis

The LD and SD varieties were considered as two separate sets of experiments.

Treatment effects were tested against plant-to-plant variation. Vegetative data were subjected to analysis of variance (ANOVA) and regression analysis. Binomial data, bolting (1) and non-bolting (0) with their respective frequencies were used as inputs to predict the probability of bolting using a logistic regression model (Agresti, 1996). PROC LOGISTIC procedure of SAS software was used (SAS Institute INC., 1999) for the analysis.

Separate logistic regression models were fitted to each variety taking plant age as a continuous variable. Assuming a linear relation between growth stages and bolting resulted in poor overall fit but a quadratic relation improved the fit. Separation of means ($p \leq 0.05$) was done using confidence intervals calculated from the best-fit model. Moreover, the relation between the proportion of bolting, and the number of leaves and sheath TSCHO were used for curve fitting in order to determine the points at which no bolting, 50 and 100% bolting can be achieved.

Results

Vegetative development

There was an increase in the number of shoots, number of leaves and total shoots, and sheath and leaf dry weights as the age of the plants increased (Figure 4.1). The difference between plants grown for 30 d and 60 d was negligible; however, a notable increase was observed in those which were grown for 90 d and above**.** As indicated by the bulbing ratio (BR), the two SD varieties (Tropix and Rox F1) did not show any indication of bulbing ($BR \leq 2.0$) whereas the LD variety Matador F1 showed a very low level of bulbing ($BR \geq 2.0$) after 120 d growth.

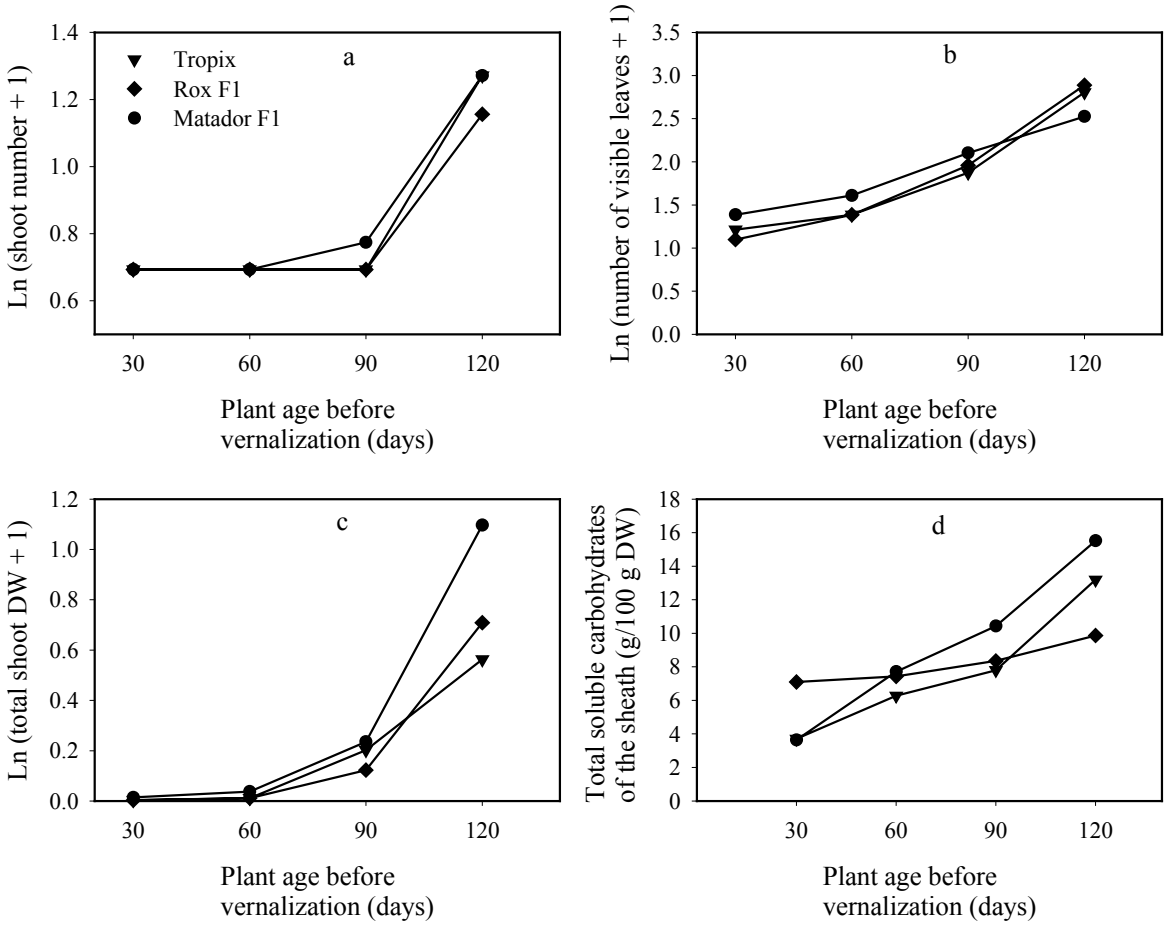

Fig. 4.1 Vegetative characteristics and TSCHO of plants of the three shallot varieties at different ages (n=5): a) number of shoots, b) number of visible leaves, c) shoot dry weight, and d) sheath total soluble carbohydrates.

Bolting percentage

As there was no other variable, the logit of the probability of bolting of each variety was estimated using logistic model consisting of plant age (Equations 4.1 to 4.3). See Table 4.1 for the estimates.

$$\text{Logit } (\pi)_{\textbf{Tropix}} = -4.17+0.0009S^2 \quad (4.1)$$

$$\text{Logit } (\pi)_{\textbf{Rox F1}} = 0.833+0.049S+0.0008S^2 \quad (4.2)$$

$$\text{Logit } (\pi)_{\textbf{Matador F1}} = -5.215+0.0007S^2 \quad (4.3)$$

S is age of the plant in days

Short day varieties

Figure 4.2 shows that the predicted proportions of bolted plants were close to the observed values in all the three cultivars ($r \geq 0.97$). Plants of Tropix vernalized after 120 and 90 d of growth bolted 100% each of them producing 2.9 and 1.9 inflorescences, respectively (Figures 4.2a and 4.3a). Those vernalized as IS and after 30 d of growth had significantly lower proportion of bolting as compared to those treated at older stages whereas those treated after 60 d bolted significantly lower than the ones treated after 90 and 120 d. However, the earliest two and the latest two growth stages performed similarly in terms of bolting. Plants that were grown for 90 d developed about 6 visible leaves, 3 mm sheath base diameter and 240 mg of shoot dry weight whereas the corresponding values for the same parameters in plants treated for 120 d were 16 visible leaves, 15 cm and 760 mg, respectively (Figure 4.1).

Alike Tropix, the proportion of bolting plants of Rox F1 which were vernalized after 90 d and 120 d of growth were 0.75 and 1.00, respectively, while those vernalized after 60 d or earlier had significantly low bolting (Figure 4.2b). In addition, the average number of floral shoots increased from 1.2 to 2.1 and 3.4 as the age of the plants before vernalization increased from 60 to 90 and 120 d, respectively. The 90 d old plants had about 6 visible leaves, 3.4 mm sheath base diameter and 130 mg of shoot dry weight. The corresponding values for plants treated after 120 d were 17 visible leaves, 8 mm and 140 mg, respectively (Figure 4.1).

Longday variety

Plants of Matador F1 vernalized after 90 d of growth or older bolted significantly more than those vernalized after 60 d or earlier (Figure 4.2c). After 90 d of growth, the plants had about 7 visible leaves, 4.4 mm sheath base diameter and 270 mg of shoot dry weight; however, the maximum proportion of bolting attained was only 0.6 where as those plants grown for a period of 120 d attained 12 visible leaves, 13.5 mm sheath base diameter and 1.99 g shoot dry weight (Figure 4.1) and bolted completely.

After 90 d of growth Tropix and Rox F1 had bulbing ratios of 1.2 and 1.4, respectively, which also remained nearly the same even at the age of 120 d. The corresponding total soluble carbohydrates (TSCHO) for both varieties were 7.79 and 8.35 g per 100 g of sheath dry weight which increased to 13.19 and 9.86 at the age of 120 d, respectively (Figure 4.1d; Figure 4.6a). After 90 d growth Matador F1 had a bulbing ratio of 1.51 whereas at 120 d it increased to about 2.1, i.e., the plants started bulbing and had 1.99 g of shoot dry weight. The

TSCHO increased from 10.43 to 15.55 as the plant age increased from 90 to 120 d (Figure 4.1).

Table 4.1 Analysis of the maximum likelihood estimates of the logistic models of the three shallot varieties vernalized at different ages*

Cultivar	Parameter	df	Estimates	se	Wald χ^2	p> χ^2
Tropix	Intercept	1	-4.1698	0.9142	20.80	<0.0001
	S	1	-0.0216	0.0580	0.14	0.7095
	S^2	1	0.0009	0.0002	17.82	<0.0001
Rox F1	Intercept	1	0.8333	0.4737	3.09	0.0786
	S	1	0.0491	0.0244	4.05	0.0442
	S^2	1	0.0008	0.0003	9.40	0.0022
Matador F1	Intercept	1	-5.2154	1.1820	19.47	<0.0001
	S	1	0.0142	0.0190	0.14	0.7057
	S^2	1	0.0007	0.0002	19.90	<0.0001

*Separate logistic models were fitted for each variety; df, degrees of freedom; se, standard error; S, plant age; p, probability level

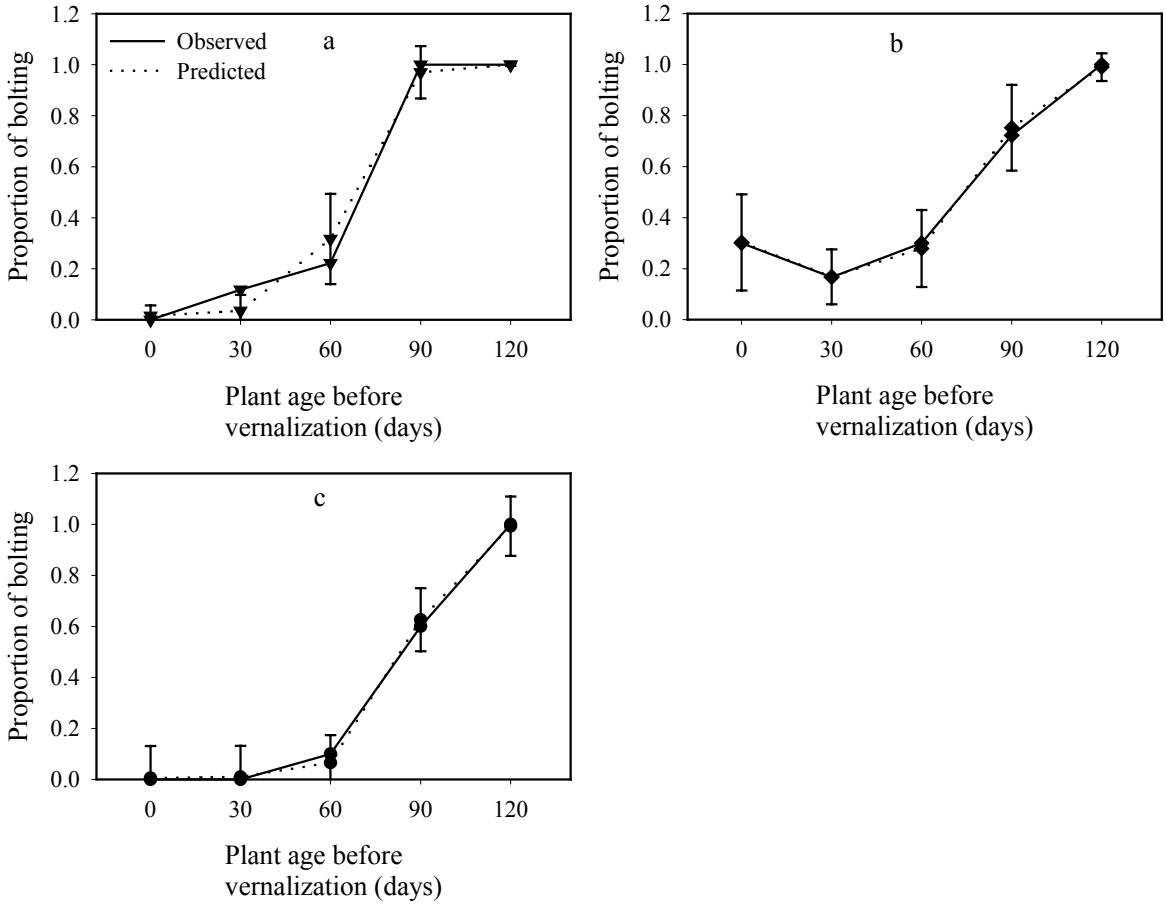

Fig. 4.2 Observed proportions and predicted probabilities of bolting of shallot varieties vernalized at different plant ages (20 plants per treatment): a) Tropix, b) Rox F1 and c) Matador F1. Error bars indicate 95% confidence interval.

Proportions of shoots

Shortday varieties

The analysis of variance of total number of shoots, floral shoots and proportion of floral shoots to total shoots showed significant differences among plant ages but the interaction between plant age and variety was not significant.

The highest and the lowest number of total shoots were observed in the oldest and the youngest ages, respectively. The three intermediate stages had significantly different number of total shoots from both extremes but with no significant difference among themselves. Moreover, the oldest plants produced the highest number of inflorescences and proportion of bolting shoots per plant (Figure 4.3), and taller and thicker inflorescences followed by the 90

d treatment. The three youngest treatments had the least of these parameters. In all the varieties the shoots started to divide at the age of 120 d.

Rox F1 had significantly more (5.9) total shoots than Tropix (3.8; Figure 4.1c). However, there was no significant difference between the two varieties both in the number of inflorescences per plant and in the proportion of bolting shoots. It also had significantly taller (73.8 cm) inflorescences than Tropix (67.6 cm) but in both cases the inflorescence stalk had a diameter of about 9 mm (data not shown).

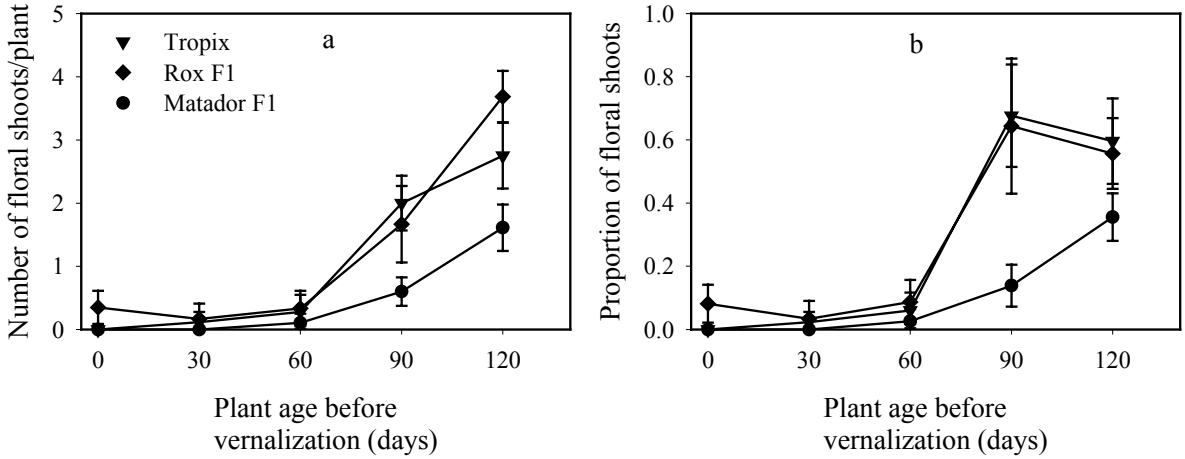

Fig. 4.3 Number of floral shoots per plant (a), and proportion of floral shoots to total shoots (b) of plants vernalized at different ages of the three varieties.

Longday variety

Plants of Matador F1 treated at different ages did not differ from each other in the total number of shoots per plant except that plants treated as IS had the lowest value. However, alike the other varieties, plants treated after 120 d produced the highest number of floral shoots and proportion of bolting shoots followed by those treated after 90 d; those treated at younger stages had the lowest values of both parameters and did not differ from each other (Figure 4.3). No significant difference was observed in inflorescence characters among bolted plants treated after 60, 90 and 120 d.

Time of bolting

Cumulative number of bolting plants showed that, plants of Tropix vernalized after 120 d of growth stared bolting 9 d after the end of vernalization (DAV) and all plants bolted within 14 DAV (Figure 4.4a). More inflorescences continued to emerge until 21 DAV where each plant

on the average produced 2.9 inflorescences (Figure 4.3a). Plants vernalized after 90 d, started bolting 14 DAV and continued until 32 DAV. The emergence of inflorescences continued until 75 DAV attaining an average of 1.9 per plant. However, only a few plants treated after 60 and 30 d of growth bolted 70 DAV whereas those subjected to vernalization as IS, did not bolt at all.

Plants of Rox F1 treated after 90 and 120 d began bolting at the same time 14 DAV (Figure 4.4b). Almost all plants that were treated after 120 d bolted within 29 DAV while only 65% of plants which were treated after 90 d bolted until 32 DAV. In the former treatment, the emergence of new inflorescences continued until 50 DAV and finally each plant on an average produced 3.4 inflorescences (Figures 4.3a and 4.5b). Those treated after 60 d or earlier began bolting at the same time as the corresponding treatments in Tropix, but the proportion of bolting plants was much more than those plants that had received the same treatments in Tropix.

Plants of Matador F1 treated after 120 d of growth began bolting at the same time as those in Rox F1, however, complete bolting took about two more months, i.e., 75 DAV (Figure 4.4c). All inflorescences emerged within the same period although the mean number of inflorescences per plant was limited to only one and a half. The proportion of bolting in plants that were vernalized after 90 d started 25 DAV and continued until 50 DAV was only 0.65 each with only one inflorescence. Only a few plants of those treated after 60 d of growth flowered 36 d DAV.

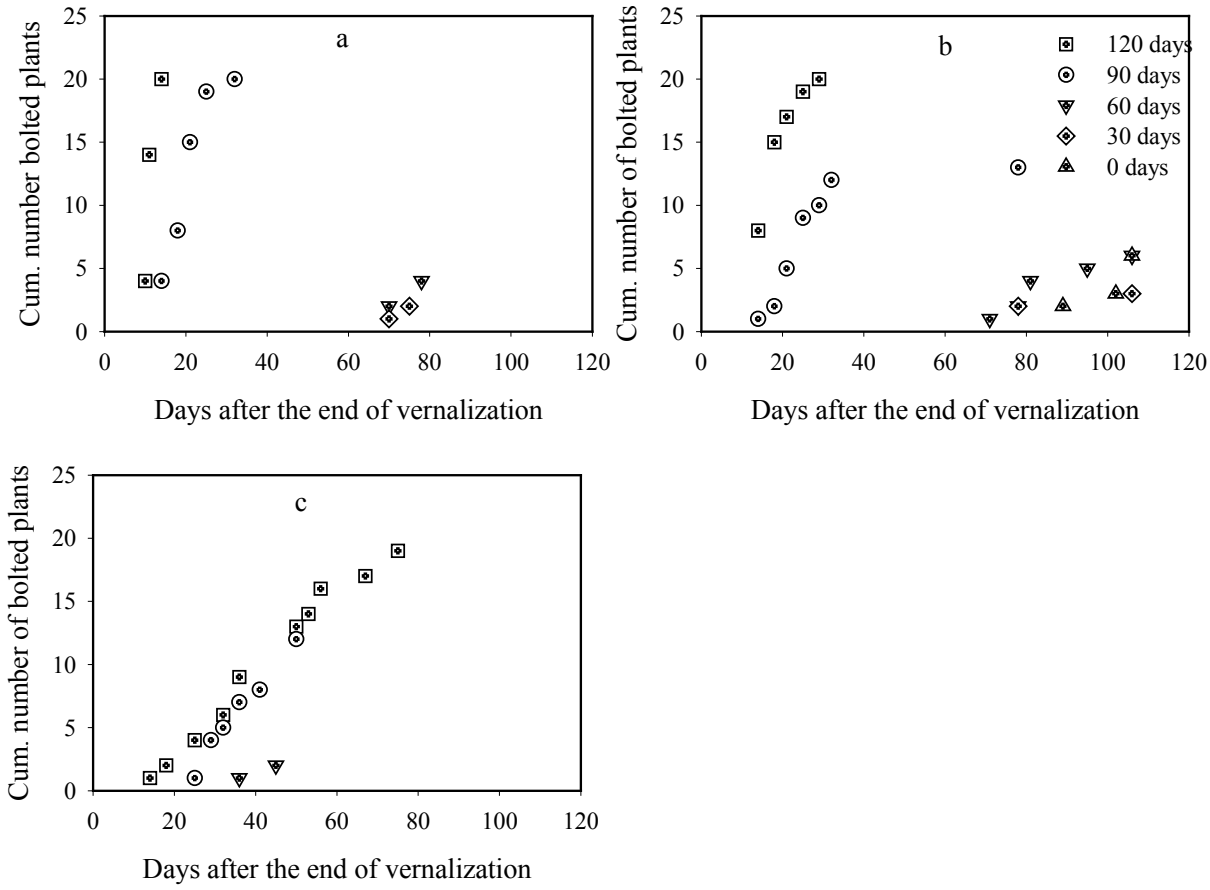

Fig. 4.4 Cumulative number of bolted plants of the three varieties after the end of vernalization: a) Tropix, b) Rox F1 and c) Matador F1

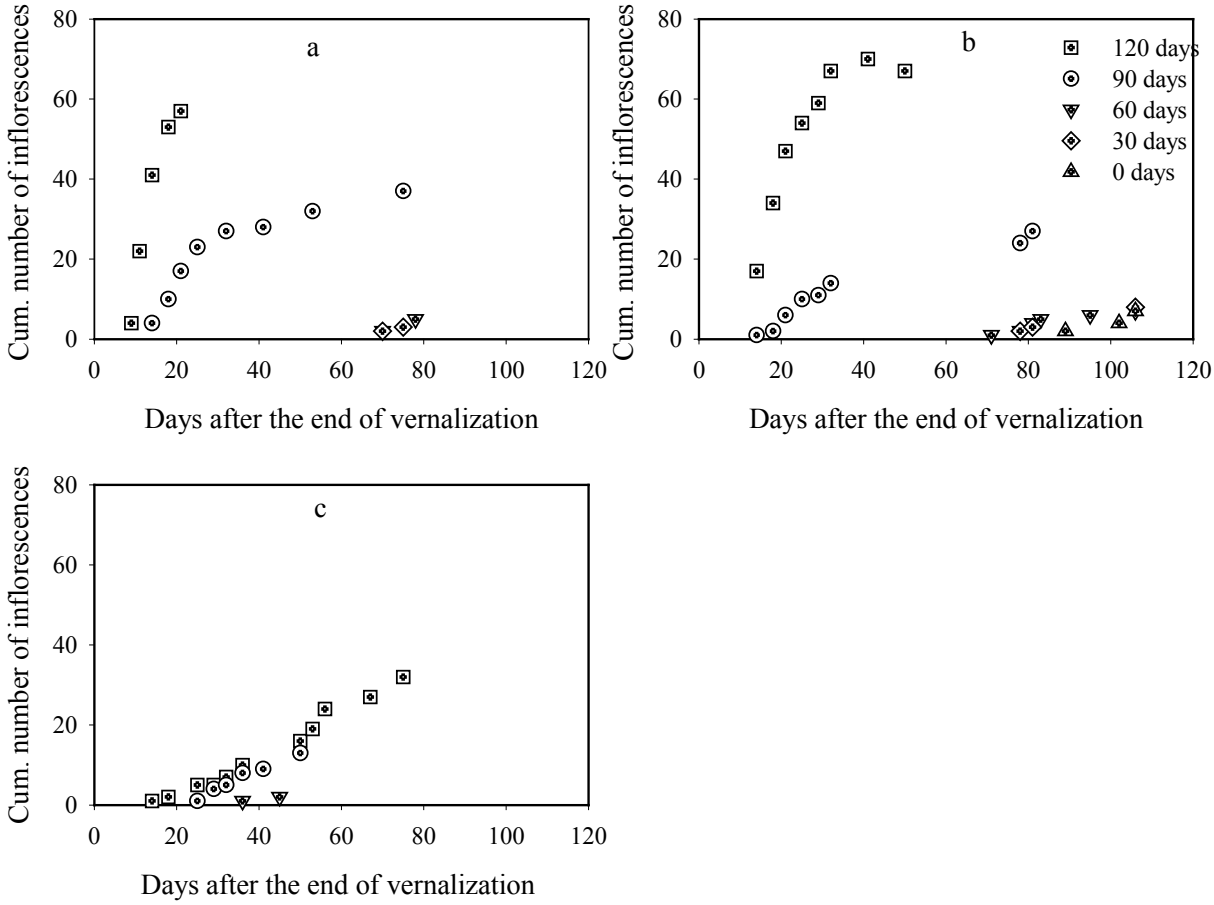

Fig. 4.5 Cumulative number of inflorescences of the three varieties after the end of vernalization (20 plants per treatment): a) Tropix F1, b) Rox F1 and c) Matador F1

TSCHO and flowering

In all the three cultivars (Tropix, Rox F1 and Matador F1) a strong positive correlation was observed between TSCHO and the proportion of bolted plants (r= 0.93, 0.92 and 0.98), the number of inflorescences per plant (r = 0.84, 0.91 and 0.98) and the proportion of floral shoots per plant (r = 0.81, 0.89 and 0.97), respectively. However, as shown in Figure 4.6 the difference between the varieties in the amount of TSCHO at the beginning of vernalization required to attain their maximum bolting was considerably high. About 8 g of TSCHO/100g sheath dry weight was sufficient to have complete bolting in Tropix at the beginning of vernalization but further increase in the number of inflorescences per plant seems to require more carbohydrates. On the other hand, plants of Rox F1 which had similar amounts of TSCHO also had lower proportion of bolting. An additional 2 g TSCHO/100 g sheath dry weight was needed to attain the same amount of bolting in Rox F1. Likewise, in Matador F1,

the amount of TSCHO the plant accumulated in the sheath had to almost double the amount in Tropix to attain maximum proportion of bolting. In all the varieties the number of inflorescences per plant increased with the increase in sheath TSCHO. On the other hand, except for Matador F1, there was no significant increase in the proportion of floral shoots although the TSCHO increased beyond what was sufficient for maximum bolting. A second degree polynomial function fitted to the proportion of bolting and TSCHO showed that bolting did not occur in all the varieties at 3.5 to 3.7 g TSCHO /100g sheath DW (Table 4.2). The LD variety (Matador F1) needed to accumulate more TSCHO to attain 50 and 100% bolting as compared to the SD varieties, Rox F1 and Tropix.

A similar function fitted to the number of leaves estimated that plants of any of the varieties with only one true leaf can not bolt at all whereas four, five and seven visible leaves were required to reach 50% bolting and eight, 17 and 11 visible leaves were required to achieve 100% bolting in Tropix, Rox F1 and Matador F1, respectively (Table 4.2).

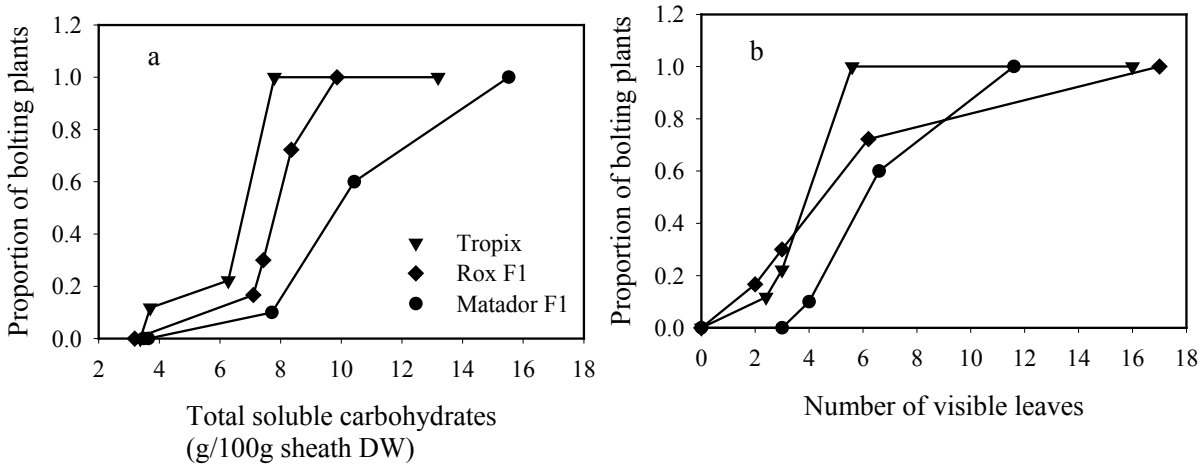

Fig. 4.6 Proportion of bolting plants in relation to a) sheath total soluble carbohydrates and b) number of leaves produced

Table 4.2 TSCHO (g/100g sheath DW) and number of visible leaves of the three shallot varieties required to attain no bolting, 50 and 100% bolting; data were generated by fitting the parameters to the proportion of bolting.

Variety	Parameter	r^2	Proportion of bolting		
			0	0.50	1.0
Tropix	TSCHO	0.83	3.50	5.93	9.31
	Leaf number	0.85	0.77	3.60	8.00
Rox F1	TSCHO	0.91	3.66	7.23	8.20
	Leaf number	0.87	0.00	4.60	16.70
Matador F1	TSCHO	0.95	3.70	10.57	15.22
	Leaf number	0.93	1.28	7.29	11.30

TSCHO, total soluble carbohydrates of the sheath; DW, dry weight

Discussion

The results of the present study re-affirm the existence of juvenility in both LD and SD shallot varieties as all failed to respond to vernalizing temperature before the plants attained a certain stage of growth regardless of their respective ecological adaptations as temperate and tropical plants.

There was an increase in the proportion of bolting plants and inflorescences per plant with increase in the size of plants. The extent of bolting in shallot seedlings started as IS or those grown for less than 90 d before vernalization was negligible while a profuse bolting was recorded in plants grown up to 90 d or above. The corresponding stages at which all plants bolted in Tropix, Rox F1 and Matador F1 were six, 17 and 12 visible leaf-stages, respectively. The results for Tropix were close to the five visible leaf-stage reported by Wiebe (1994) in leek but higher than the six leaf initials in SD Thai shallot variety (Krontal *et al*., 1998) and six leaf initials in onion cv 'Rijnsburger' (Brewster, 1985). The sheath diameter and shoot dry weight were closer to 'Rijnsburger' but it was only half of what was reported for cv 'Senshyu'. However, results of Matador F1 and Rox F1 were about twice as specified for the above-mentioned *Allium* spp. Krontal *et al.* (2000) also reported that shallot seedlings grown in a phytotron at 17/9^{o}C and 26/18^{o}C day/night temperatures produced 13 to 18 leaves before the first inflorescence became visible. The large difference in growth, despite only 30 days of difference between the treatments, could partly be due to the division of the shoots each of which independently initiated leaves and partly due to the exponential behaviour of growth and difference in natural light intensity during the pre-vernalization growth period.

Apart from the high proportion of bolting, early bolting was attained as the age (size) of the plants before vernalization increased. Plants grown for 120 d began bolting in less than two weeks after vernalization, but the time for all the plants to have at least one inflorescence was 14, 29 and 75 days in Tropix, Rox F1 and Matador F1, respectively. In addition, within the period of bolting, plants of the three varieties vernalized after 120 d, on the average, produced about 4.4, 1.4 and 0.5 inflorescences per day per 20 plants while those vernalized after 90 d and 60 d produced about one inflorescence per day and one to two inflorescences per 10 days, respectively. Ito *et al*. (2002) found a positive correlation between sugar catabolizing enzymes and the rate of floral bud growth in Japanese pear (*Pyrus pyrifolia* Burm.; Nak.). The rate of inflorescence emergence has an implication on the uniformity in maturity and seed harvest. In this regard, it is anticipated that vernalizing Tropix in particular and all the varieties in general after 120 d of growth may result in uniformity in seed maturity and harvest. The beginning of bolting in plants grown for 90 days was also similar to those of 120 d, except Matador F1 which delayed by 11 d. This indicates that once satisfactory growth is attained, the difference in the inception of bolting may be low. However, the difference in the inception of bolting between the two oldest stages and the 60 d treatment, was as high as their difference in their sowing dates.

The older the plants were at the beginning of vernalization, the higher were the proportion of floral shoots per plant. There was an increase of one inflorescence per plant as the time of growth before vernalization increased from 90 to 120 d in Tropix; the increase was even more (1.3) in Rox F1, but it was only 0.5 in Matador F1, i.e., half of the bolted plants had one more inflorescence. This could be attributed to the capacity of the plant to produce axiliary shoots early which become receptive to cold temperature stimulus. Krontal *et al.* (2000) also reported that field sown SD shallot varieties well ahead of vernalizing temperature produced high (90%) and early bolting plants with more than three inflorescences as compared to those sown shortly before vernalization. The latter had low (55%) and late bolting plants with single inflorescence.

The increase in both the proportion of bolted plants and the number of inflorescences per plant could partly be due to the increase in the TSCHO in the sheath, which might have increased the amount of available energy for active cell division and differentiation into floral shoots. The increase in bolting with the increase in the amount of TSCHO at the beginning of

vernalization was inline with the findings of Brewster and Bulter (1989) and Brewster (1983; 1985). However, no definite relation was observed between proportion of bolting and inflorescence per plant, and the leaf TSCHO indicating the dependence of the bolting process on the stored carbohydrates rather than on that immediately produced in the leaves. This is inline with the findings of Bodson and Outlaw (1985) who suggested that the source of sucrose accumulation at the apex soon after photoinduction are reserve carbohydrates such as starch from leaves and stems. On the other hand, although Matador F1 grown for 120 d had the highest TSCHO, it did not produce more inflorescences per plant indicating that carbohydrates may not have an overriding (triggering) effect on precautious bolting of the juvenile buds.

In shallot, which has multiple growing points (buds) that emerge sequentially, juvenility seems to be associated with individual buds rather than to the whole plant. As a result some buds tend to bolt while others remain vegetative and grow into bulbs. This may also indicate the non-transmissibility of floral stimulus from flowering to non-flowering buds within the same plant (Heide, 1994) or its ineffectivity on juvenile buds. Conversely, had juvenility been a whole plant character in such plants or had the flowering factors been transmissible and could be effective in causing precautious flowering even in juvenile buds, all buds of a plant could flower.

Krontal *et al*. (2000) and Wurr *et al*. (1994) reported that juvenility, as defined by leaf number, is highly dependent on environment. Moreover, Wiebe (1972) showed that low light intensity extended the juvenile phase in cauliflower (*Brassica oleracea* var *botrytis*). Hence, the low light intensity during this experiment might have lead to the extended juvenile phase. Conversely, had the plants been grown under high light intensity, the plants could have attained the same optimum size and concentration of sheath TSCHO within a shorter period of time.

In general, the present study has suggested the possibility of manipulating the behaviour of the three shallot varieties either to produce flowers or bulbs. It is recommended that plants of Tropix, Rox F1 and Matador F1 meant for seed production should be subjected to cold temperature at six, 17, and 12 leaf-stages or when they attain 7.8, 9.8 and 15.5 g TSCHO /100 g of sheath DW for the best results either artificially in growth chambers or by synchronizing

with weather conditions in the field. On the other hand, plants meant for bulb production should not be exposed to vernalizing temperatures at these stages or later.

Chapter 5

Final discussion

The high proportion of bolting at 8 and 12^{o}C and failure of bolting at 18^{o}C was in agreement with the findings of other researchers. Krontal *et al*. (2000), Brewster (1982; 1985) and Brewster and Bulter (1989) obtained high percentage of bolting in the temperature range of 5 to 12^{o}C compared to those in the range of 13-20^{o}C. However, the maximum proportion of bolting attained in Chapter 2 was only 0.73. This was attributed to the juvenility of the plants at the beginning of vernalization which were only 79 days old compared to the 120 days required for complete bolting in Matador F1 (Chapter 4). In line with the findings of Murfet and Reid (1974) and Brewster (1983) an increase in photoperiod at these temperatures slightly increased bolting. But with extended treatment, especially at 12^{o}C, a decrease in bolting was incurred which might be due to devernalization (Brewster, 1990). The very low bolting in plants treated at 4^{o}C for 30 days and its increase with increase in duration of treatment could be due to the gradual accumulation of the 'flowering substances'. Brewster (1983) reported that longer time is required for initiation under sub-optimal range of low temperature. In contrast, Zemah *et al*. (2001) obtained a fully developed inflorescence following storage at 4^{o}C for 16 weeks in *Allium aflatunense*. Nonetheless, the care needed and the energy consumed during the extended period of treatment under this temperature accounts for its disadvantage. Therefore, both 4 and 18^{o}C were found to be ineffective in vernalizing the tested varieties. Based on these results it can be suggested that plants intended for seed production should be exposed to 8 or 12^{o}C temperatures for 60 days at their post juvenile stages but those intended for bulb production should not.

As shown in Chapter 3, the probability of bolting in plants treated as intact bulbs (B) was significantly lower than those plants regenerated from seeds (PS) and from bulbs (PB), with no significant difference between the latter two (Figure 3.1). In line with the result of this study Berghoef *et al.* (1992) reported that floral initiation and development is scarcer in stored bulbs than in growing plants of ornamental onion (*Allium sphaerocephalon* L.). However, the number of inflorescences per plant was the highest in PB followed by B and PS.

Assuming a constant percentage seed set among the treatments, the amount of seeds that could be produced would be a direct function of the number of florets. Consequently, the total

number of florets per plant, taken as the product of the number of inflorescences and the number of florets, PB would produce 90 and 71% more florets and seeds than B and PS, respectively. However, under field conditions the number of inflorescences per unit area in PS can be increased by reducing its spacing. On the other hand, due to the low proportion of floral shoots (0.64), coupled with the already high number of shoots per plant, reducing the spacing between plants in B might not able to compensate for the low yield; hence rendering B as a less preferred method of seed production. In contrast to the results of other researchers (Takahashi *et al*., 1994; Jonson and White, 1997; Yuan *et al*., 1998; Konsin *et al*., 2000) extending the duration of vernalization (Chapter 3 Experiment 2) could not increase bolting. On the other hand, ease of treatment of bulbs while they are in storage and the possibility for selection of bulb quality still compromise the feasibility of bulb vernalization especially for application in shallot breeding and where field temperatures are not sufficient to treat growing plants. Hence, investigations that increase the proportion of bolting plants, number of inflorescences per plant and invigorate inflorescences (umbels) for more florets and seeds are required.

The vegetatively propagated shallot cultivars had been selected for resistance to bolting, thus they failed to bolt regardless of the treatments. Similarly, Krontal *et al*. (2000) reported that a clonally propagated shallot cv from Nepal treated between 5 to 30^{o}C for 21 days failed to bolt. Tabor (1996) obtained very low (6%) bolting in bolting resistant shallot cultivars which were selected for vegetative bulb production. Sheldon *et al.* (2000) reported that even genome wide demethylation which prompted flowering in vernalization responsive *Arabidopsis* ecotypes and mutants failed to induce flowering in non-vernalization responsive plants. The results, therefore, suggest the need for investigation of the genetic basis of flowering in shallot genotypes.

There was an increase in the proportion of bolting plants and inflorescences per plant with increase in the age (size) of plants. The extent of bolting in shallot seedlings started as imbibed seeds (IS) or those grown for less than 90 d before vernalization was negligible while a profuse bolting was obtained in plants grown up to 90 d or above. The corresponding stages at which all plants bolted in Tropix, Rox F1 and Matador F1 were six, 17 and 12 visible leaf stages or when they accumulated 7.8, 9.8 and 15.5 g TSCHO/100 g of sheath DW, respectively. Apart from the high proportion of bolting, more number of inflorescences per

plant and earlier bolting were obtained as the age of the plants before vernalization increased which resulted in uniform flowering and seed maturity.

The increase both in the proportion of bolting plants and number of inflorescences per plant could partly be due to the increase in the TSCHO in the sheath, which might have increased the amount of available energy for active cell division and differentiation into floral shoots. The result was inline with the findings of Brewster and Bulter (1989) and Brewster (1983; 1985). However, no definite relationship was observed between the proportion of bolting and number of inflorescence per plant and the leaf TSCHO, indicating the dependence of the bolting process on the stored carbohydrates than on what is immediately produced in the leaves (Bodson and Outlaw, 1985). On the other hand, although Matador F1 grown for 120 d had the highest TSCHO, it did not produce more inflorescences per plant indicating that carbohydrates may not have an overriding (triggering) effect on the juvenile buds to cause precautious bolting.

In shallot, which has multiple growing buds that emerge sequentially, juvenility seems to be the behaviour of individual buds rather than of the whole plant. As a result, some buds tend to bolt while others remain vegetative and grow into bulbs. Results of our studies (Figure 2.1a and d; Table 3.1; Figure 3.1) indicated that those buds that were present at the beginning of the treatment were the most likely to receive and respond to the treatments while the failure of the newly initiated buds might have been due to juvenility and/or insufficient vernalization. It also indicates the non-transmissibility of floral stimulus from flowering to non-flowering buds within the same plant or its ineffectivity on juvenile buds. Conversely, had juvenility been a whole plant character in such plants or had the flowering factors been transmissible and could be effective in causing precautious flowering even in juvenile buds, all buds within the treated plant could have flowered. This is inline with the assumption in *Lolium perenne* L. that the low temperature or SD stimulus is local in induced tillers and can not be transferred to later formed daughter tillers on the same plant (Heide, 1994).

Krontal *et al*. (2000) and Wurr *et al.* (1994) reported that juvenility, as defined by leaf number, is highly dependent on environment. Moreover, Wiebe (1972) showed that low light extended the juvenile phase in cauliflower (*Brassica oleracea* var. *botrytis*). The low light intensity during this experiment might have also lead to the need for extended time to attain the specified periods; had the plants been grown under high light intensity, the plants could

have attained the same ‘critical’ size and concentration of sheath TSCHO within a shorter period of time.

In summary, the present study revealed that vernalization of Ambition F1 and Matador F1 at 8 and 12°C under 16 h photoperiod and of Tropix and Rox F1 at 8°C under 12 h for a period of 60 days is effective. Vernalization of growing plants of the two former varieties regenerated either from seeds or bulbs yield high proportion of bolting. In addition, vernalization of Tropix, Rox F1 and Matador F1 under 16 h for 60 days when they attain six, 17 and 12 visible leaf-stages or older, i.e., when they accumulated 7.8, 9.8 and 15.5 g TSCHO/100 g of sheath DW, respectively, gave high proportion of bolting and number floral shoots per plant. Therefore, a producer of shallot seeds has to carefully synchronize the genotypes, the temperatures and the photoperiods and the growth stages for higher seed yields.

It is also suggested that future studies should investigate the causes of failure of initiated inflorescences to bolt. Moreover, cheaper and large scale methods of vernalization such as field ‘overwintering‘ of selected genotypes based on local climatic data and integration of these methods with bulb vernalization should be given emphasis. Molecular genetic and physiological basis of flowering should be studied to reveal the causes of differences in magnitude and timing of bolting among different shallot genotypes.

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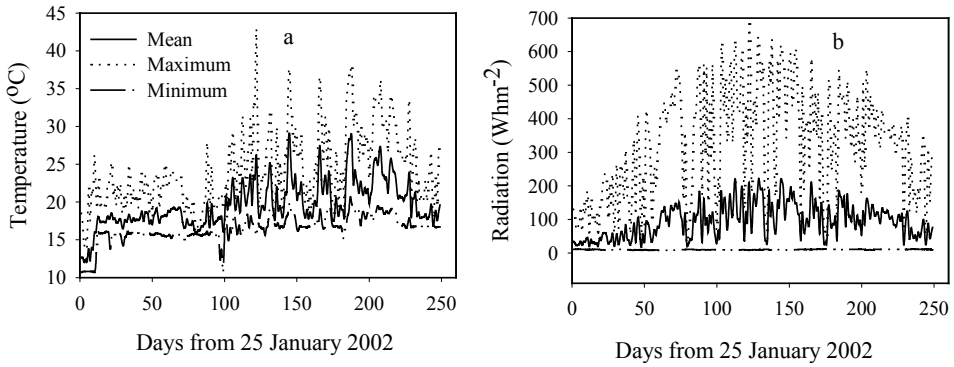
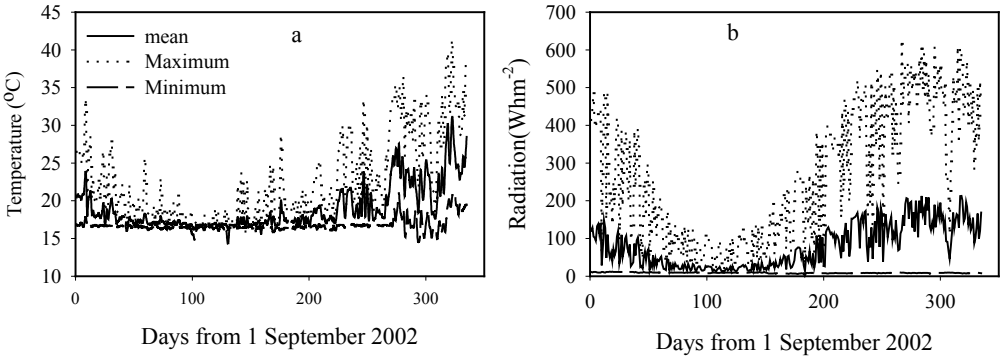
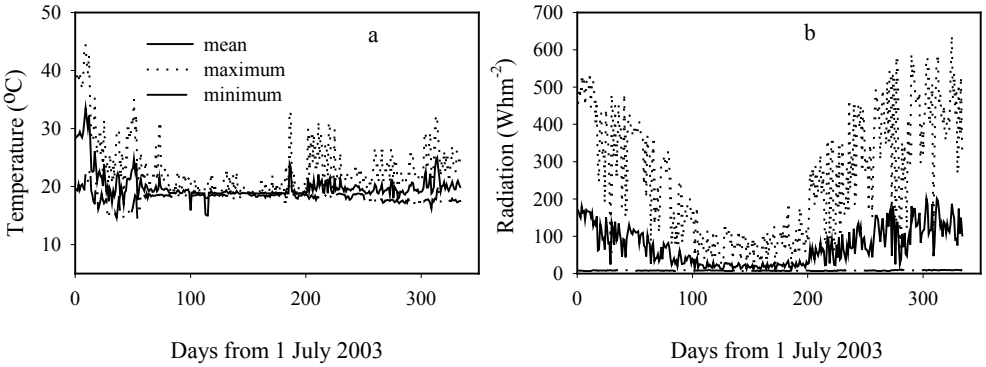
Annex



Annex 2.1 Mean, maximum and minimum temperature (a) and radiation (b) during the experiment in 2002 (Chapter 2).



Annex 3.1 Mean, maximum and minimum temperature (a) and radiation (b) during the experiment in 2002/2003 (Chapter 3 Experiment 1)



Annex 4.1 Mean, maximum and minimum temperature (a) and radiation (b) during the experiment in 2003/2004 (Chapter 4 and Chapter 3 Experiment 2).

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Eidesstattliche Erklärung

Heirmit erkläre ich an Eides Statt, daß ich die vorliegende Arbeit selbständig angefertigt habe und keine anderen als die angegebenen quellen und Hilfsmittel benutzet habe sowie daß diese Arbeit noch nicht als Dissertation oder andere Prüfungsarbeit vorgelegt worden ist.

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