Genetic and physiological aspects of postharvest flower longevity in Asiatic hybrid lilies (*Lilium* L.)

Genetic and physiological aspects of postharvest flower longevity in Asiatic hybrid lilies (*Lilium* L.)

J.J.M. van der Meulen-Muisers

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. ir. L. Speelman,
in het openbaar te verdedigen
op woensdag 27 september 2000
des namiddags te half twee in de Aula.

Stellingen

1. De keuze voor het gebruik van gedeïoniseerd en gedestilleerd water als uniforme vaaswater-controles in studies naar de naoogst fysiologie van snijbloemen, zoals in 1980 afgespoken op het "2nd Symposium on postharvest physiology of cut flowers", dient heroverwogen te worden.
    Reid, M.S. and A.M. Kofranek, 1980. Recommendations for standardized vase life evaluations. Acta Horticulturae 113: 171-173.
    Van Meeteren, U., H. van Gelder and W. van Ieperen, 2000. Reconsideration of the use of deionized water as vase water in postharvest experiments on cut flowers. Postharvest Biology and Technology 18: 169-181.

2. Het feit dat de consument houdbaarheid als belangrijkste factor ervaart van snijbloemen oefent onvoldoende druk uit op veredelingsbedrijven om houdbaarheid als belangrijk kenmerk mee te nemen in de selectie.

3. De nadruk bij de huidige kwaliteitsnormen voor sierteeltproducten ligt nog te sterk op het uiterlijk van het product en te weinig op de inwendige kwaliteit.

4. De grote genetische variatie binnen het lelie-assortiment maakt het mogelijk de voor de afzetketen gewenste kwaliteit via veredelingstechnieken in te bouwen waardoor het gebruik van milieubelastende chemicaliën in deze gewasgroep gereduceerd kan worden.

5. De kwaliteit van indirecte selectie met behulp van genetische merkers is sterk afhankelijk van de kwaliteit van de waarnemingen bij de kartering van het betreffende kenmerk.

6. Geen veredeling zonder plantenfysiologie.
    J.C. van Oeveren.

7. Bij het te gelde maken van een uitvinding is de formulering van het octrooi belangrijker dan de uitgevonden formule.

8. Als werkervaring belangrijker wordt geacht dan promoveren, wordt voorbij gegaan aan het feit dat een proefschrift schrijven gewoon werk is.
    I. van der Neut, 1993. De meerwaarde van een promotie. Onderzoeksbureau Research voor Beleid, Leiden.

9. Allergie is een ziekte van de moderne beschaving.
    I. Wolffers, 1996. Allergie en overgevoeligheid. Consumentenbond, Den Haag.

10. Aangezien individuele verschillen op velerlei vlak in grote mate genetisch zijn bepaald is de gegeven omschrijving van "opvoeden" in de "Dikke van Dale" een te ruime interpretatie van dit begrip.
    M. Roele. De illusie genaamd opvoeden. Intermediair 18-2-1999.
    Van Dale Groot Woordenboek der Nederlandse Taal, 12e herziene druk, 1995.

11. Afval bestaat niet.
    Stichting Global Action Plan Nederland, Ecoteam Programma 1998/1999.

12. Het is een gemiste kans om de achterzijde van je proefschrift onbenut te laten.

Stellingen behorende bij het proefschrift "Genetic and physiological aspects of flower longevity in Asiatic hybrid lilies (*Lilium* L.)", J.J.M. van der Meulen, 27 september 2000.

Euveral leet gelök verborge,
want dit is weer eine nieje morge.
Wat daen daag ouk bringe maag,
ik hald van de bloome, ik hald van
vandaag.

Hannelore Winter
Uit: Gelök leet euveral verborge....

Aan mijn ouders

Ter nagedachtenis aan Joop van Oeveren

Contents

Chapter 1

General introduction

The lily

The lily (*Lilium* L.) is a perennial, leafy-stemmed, bulbous ornamental, belonging to the subclass *Monocotyledonae* and the family of *Liliaceae.* The primary gene center of *Lilium* is located in the Himalayan region, but it has become native to Europe, Asia, and North America (Comber, 1949). Within the genus *Lilium* about 80 species can be classified into seven sections. The worldwide commercially important lily cultivars, Asiatic hybrids, Oriental hybrids and cultivars of *Lilium longiflorum*, originate from only three of the seven sections. The species of these three sections originate mainly from China and Japan. The Asiatic hybrids were obtained after complex interspecific hybridization between (at least 12) species of the *Sinomartagon* section. Crosses between five species of the section *Archelirion* have resulted in the Oriental hybrids, whereas *Lilium longiflorum* belongs to the section *Leucolirion.* Tissue culture techniques (Van Creij et al., 1993; Van Tuyl et al., 1991) have permitted hybridizations originating from intersectional crosses. Crosses between *L. longiflorum* and 'Asiatic' lilies have resulted in 'LA' hybrids, and crosses between *L. longiflorum* and 'Oriental' types have produced the 'LO' hybrids. Especially the difficult crosses between Oriental hybrids and Asiatic hybrids ('OA' hybrids), a combination of the two commercially most important lily groups, are a break-through in lily breeding (Van Tuyl et al., 2000). Cross pollination barriers have been overcome by polyploidization techniques restoring fertility of sterile interspecific diploid hybrids (Van Tuyl et al., 1989; 1992). The intersectional crosses have considerably increased the availability of genetic variation in *Lilium* for important traits.

The acreage of lily bulb production in The Netherlands has increased during the last 35 years from less than 100 ha in 1965 to over 4200 ha in 1999, with the Asiatic hybrids accounting for about 45% of this area, the Oriental hybrids for about 40% and the cultivars of *L. longiflorum* for about 5%. The relatively new *L. longiflorum* x Asiatic ('LA') hybrids are already responsible for about 7% of the acreage of lily production. In 1999, in The Netherlands in total more than 1200 million lily bulbs were grown, which were used worldwide for year-round forcing. From the total Dutch lily bulb production, about 85% were exported, mainly for cut flower production. The remaining 15% of the bulbs were used for the own year-round flower production. About 80% of the flowers produced were exported. The turnover for bulb production was over 180 million Euro and for cut flower production over 140 million Euro. The lily is the fourth most important cut flower in the Netherlands (Source: BKD/CBS/ PT/VBN, 2000).

Although the lily is a cross-breeding crop, genotypic characteristics are perpetuated by vegetative propagation. The perennial bulb (Fig. 1.1) exists of scale-like leaves attached to a compressed stem, the basal plate, and is used as a storage organ. The scales are commonly used to propagate the lilies (Griffiths, 1933). The basic production cycle for lily bulbs requires one to three years when propagated
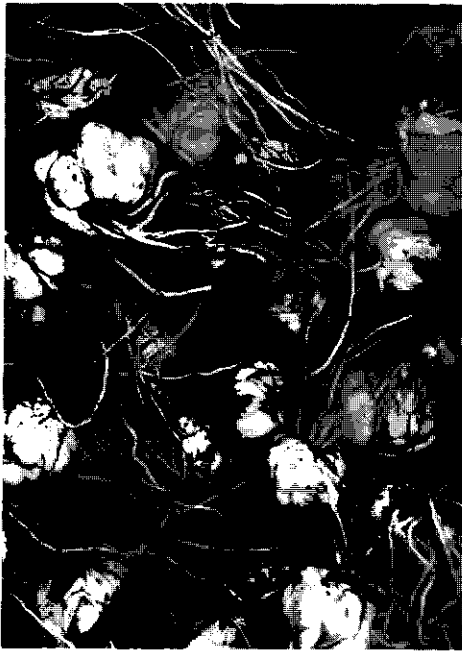
Fig. 1.1 Scale-type bulbs of hybrid lilies.

Fig 1.2 Inflorescence of the Asiatic hybrid lily 'Orlito'.

from scales. Seed propagation is usually limited to certain species and is especially used by plant breeders. The bulblets harvested from seeds are usually grown one to four years before they flower (Le Nard and De Hertogh, 1993).

The lily flower contains two alternating whorls of perianth segments (tepals). The individual flowers are usually arranged in an inflorescence, which contains several flowers in different stages of development (Fig. 1.2). Flower development proceeds acropetally. The number of flowers per inflorescence depends on the bulb size. At the commercial bulb size of 12 to 14 cm in circumference, many forced cultivars contain 3 to 8 flowers per inflorescence.

Lily flowering

Since lilies are grown primarily for their flowers, flowering is the most important event in the growth and development cycle. Before bulbs achieve the capacity to flower, they must reach a certain physiological stage. In lily, this stage is reached after a juvenile period of 6 months to 3 years (Le Nard and De Hertogh, 1993; J.M. van Tuyl, personal communication). Bulb size is the major and most easily measured

factor that determines the capacity to flower. The critical size is dependent on the species and cultivar (Rees, 1966) and can also vary with the environmental conditions (Hartsema, 1961). In lily the minimum flowering size of the bulbs usually ranges from 5 to 12 cm in circumference (Le Nard and De Hertogh, 1993).

Langhans and Weiler (1968) divided the visible aspects of flowering into four stages: 1. Flower initiation (conversion from vegetative to reproductive growth); 2. Organogenesis (differentiation of floral parts); 3. Maturation and growth of the floral parts; 4. Anthesis. The lily bulbs are harvested from late August to December in the Northern Hemisphere. They are cooled at 1-2°C for 6-12 weeks, depending on the cultivar, before they are forced in a greenhouse. However, if longer storage is required, the temperature must be lowered to 0°C for storage up to 6 months, and to -1 to -2°C for storage longer than 6 months. Flower initiation takes place either before or after the bulbs are harvested, depending on the cultivar (Beattie and White, 1993). Flower organogenesis is usually completed in 1.5-2 months (Baranova, 1972). Throughout lily forcing, the temperature has the greatest influence on the rate of growth and development (Rivière, 1978), whereas plant quality is increased and height is usually reduced with naturally high light levels or with supplemental high intensity lighting (Boontjes et al., 1975). Increasing light intensity increases photosynthesis, which increases the rate of flower development and the number of flowers formed, reduces bud abortion and enhances the total flower potential (Wilkins and Dole, 1997). Most of the assimilates synthesized in the vegetative organs are directed to the stem apex during flower evocation (Wang and Breen, 1984), and next to the flower buds throughout their development as lily flowers are strong carbohydrate sinks until anthesis (Wang and Breen, 1986ab).

Lily flower quality

Preharvest. In an inflorescence-type flower like lily the ornamental value is often based on the number of flowers produced per plant. A loss of even one or two flowers might be an economic problem, especially for the cultivars that have an inherent low number of flowers per plant. Relatively much research has been done to prevent or reduce bud loss in lilies. Bud loss due to abscission was reported to be influenced by storage time and storage temperature of the bulbs and is probably correlated with ethylene production by the buds (Durieux et al. 1982/83; Roh, 1990c). Abscission occurs only during a critical stage of development of the flower buds, which coincides with a peak in the endogenous production of ethylene by the buds at the end of the meiotic phase of the stamens (Durieux et al. 1982/83). Further, high temperatures during forcing promote bud loss in hybrid lilies (Boontjes, 1982; Roh, 1990b), whereas light shortage is crucial for bud loss to take place (Durieux, 1975; Kamerbeek and Durieux, 1971; Durieux et al., 1982/83), likely due to changes in the

distribution of assimilates causing depletion of the soluble carbon source in small buds (Roh, 1990c; Van Meeteren, 1981).

Postharvest. Like other inflorescence-type flowers, lilies are normally harvested with a large variation in developmental stages of the buds within the inflorescence. The life of the inflorescence is a function both of the life of individual flowers, and of the postharvest expansion and opening of the buds. Ideally many of the individual flowers on the inflorescence should open before senescence of the bottom flower. Because senesced flowers are unattractive, the longevity of the bottom flower is an important factor of the commercial life of the inflorescence. Despite its important contribution to the commercial value of the inflorescence, hardly any research has been reported on the flower life of individual lily flowers.

The postharvest development of the floral buds is largely dependent on the harvest stage of the inflorescence. The more mature the floral buds at harvest, the more flowers develop and reach anthesis after harvest (Swart, 1980). This is likely due to an increase in the tepal carbohydrate amount as lily flower development proceeds (Clément et al., 1996). Van Meeteren et al. (2000) reported that the carbohydrate amount in inflorescences of the Asiatic lily hybrid 'Enchantment' at harvest largely affected the number of buds that developed and reached anthesis, whereas vase water sucrose especially enhanced the development of the young lily buds. Similar results were reported by Nowak and Mynett (1985), where sucrose in the vase water improved both the percentage flowers that reached anthesis and the longevity of individual flowers in the Asiatic hybrid 'Prima'.

Nowak and Mynett (1985) also found a beneficial effect on percentage flowering buds and flower longevity of sucrose in combination with silver thiosulphate (STS), an inhibitor of ethylene action, even after storage periods up to 4 weeks at 1°C. Another beneficial effect of STS was found on the reduction of bud abscission in the Asiatic hybrid lily 'Enchantment' by Van Meeteren and De Proft (1982). They associated bud abscission with increased sensitivity to and a subsequent rise in biosynthesis of ethylene by small (2.0-3.5 cm) flower buds following dark treatment. In the Dutch flower auction system, lily growers were required to treat Asiatic hybrid lilies with an ethylene antagonist before auctioning (Van Doorn and Woltering, 1991). At the beginning of the 1990's was started with testing the lily assortment for the effectiveness of such an ethylene antagonist and several cultivars are cleared from its use (A. Ruting, personal communication).

In 1999, Elgar and co-workers, screened several lily cultivars of the three commercial important groups for their postharvest response to ethylene. The vase life (defined by them as 'the days taken for 50% of the buds which eventually opened on each stem to reach senescence') of most Oriental hybrids and *Lilium longiflorum* cultivars was unaffected by ethylene exposure; however, some Asiatic hybrids showed minor responses. For those Asiatic hybrids responding to ethylene exposure, the main effects were caused by high ethylene levels, with no full expansion of the

tepals so the flower did not entirely open and with a reduced percentage of buds which opened. Effects on individual flower longevity were not tested. No climacteric increase in ethylene or CO_2 production of lily buds and flowers (from a bud length of 40 mm until senescence) was detected during 7 days at 20°C. Ethylene production by flowers and response of flower petals to ethylene is often observed to vary depending on the physiological age and state of the tissue at the time of exposure (e.g. Halevy et al., 1984), which could explain the contrasting results concerning ethylene production and sensitivity to ethylene in lily.

Flower longevity and breeding

The production of ornamentals is increasing rapidly and due to the growing supply on the international market the competition is becoming stronger, making ornamental quality a key factor. Longevity is the most important quality requirement for ornamental products from the consumers' point of view (Van de Genugten, 1984). The longevity that potentially can be reached is largely a characteristic of the crop and the cultivar itself and is genetically determined. De Jong (1986) showed that in *Gerbera* 78% of the differences in longevity between crosses could be contributed to genetic factors. Knowledge of the potential longevity of existing and new cultivars is, therefore, of great importance. The use of reliable tests to determine the potential longevity is essential. For this reason, the development of so called 'reference-tests' was started in The Netherlands in the late 1980's (De Gelder, 1989). In these tests the longevity of new cultivars was meant to be determined by strict rules and compared with some well-known cultivars. However, due to conflicting interests and expected high costs, development and introduction of such tests were hampered in several crops, among them the lily. Nevertheless, discussion on the subject has made lily breeders aware of the importance of selection for flower longevity and more attention is paid to the rejection of genotypes with inferior flower longevity (G. Beentjes, personal communication).

Although longevity is the most important quality requirement of ornamental products, internationally, relatively few breeding research activities for improving ornamental longevity take place (e.g. Wernett et al., 1996). Improvement of flower longevity can be achieved by breeding, when genetic variation for flower longevity is available. Large genetic differences in longevity between cultivars have been claimed in, for example, *Gerbera* (Harding et al., 1981; De Jong and Garretsen, 1985) and tulip (Benschop and De Hertogh, 1969; Van Eijk et al., 1977; Van Eijk and Eikelboom, 1986).

Breeding efforts have mainly been focused on broadening of the assortment and increase of the production. This might be explained by the fact that flower longevity is a particularly difficult genetic character to assess, since it is markedly affected by

growing conditions prior to harvest, stage of flowering at harvest and environmental conditions during distribution and after sale. It can be divided into a large number dependent and independent components together determining longevity as a whole. Components in this context are characteristics that perhaps can be combined by breeding in order to improve longevity. Important components might be e.g. the in potential reachable longevity under standardized conditions, polyploidy, stress tolerance, sensitivity to ethylene, water balance and carbohydrate status (reviewed by Halevy and Mayak, 1979; 1981).

Furthermore, compared to longevity of fruit and vegetables longevity of ornamentals is complex, because it is determined by two conflicting processes: (1) Promotion of flower bud growth and anthesis; (2) Retardation of metabolic processes leading to senescence (Halevy and Mayak, 1979). Therefore, besides knowledge of genetic aspects also insight in the physiological regulation (e.g. flower bud development, flower bud opening, flower senescence) is needed for the improvement of flower longevity.

Petal senescence

The flower is a complex organ composed of many different tissues, all of which senesce at different rates. In the commercial use of flowers, it is usually the life span of the petals that determines the effective flower life. The senescence process of flower petals is mediated by a series of highly coordinated physiological and biochemical changes such as increased activity of specific enzymes (e.g. peroxidases, RNAses, DNAses and hydrolases of cell wall polysaccharides), degradation of carbohydrate, changes in protein and nucleic acid content, loss of cellular compartmentation, and a climacteric surge in respiration. These changes are associated with changes in gene expression and *de novo* synthesis of proteins (reviewed by: Halevy and Mayak, 1979; Borochov and Woodson, 1989).

The rate at which petal senescence proceeds directly determines the longevity of the cut flower, and as a result, this process has been widely studied. Most research has concentrated on the regulation of petal senescence in ethylene-sensitive flowers, where senescence is accelerated by the presence of ethylene. Considerable effort has been addressed to understanding the molecular basis of the rise in ethylene biosynthesis (Rottman et al., 1991), and the events that ethylene induces (Lawton et al., 1989). As a result the ethylene biosynthesis route is now completely known. By genetic modification using anti-sense transformation (Hamilton et al., 1990), the expression of genes responsible for the production of enzymes that play a role in the biosynthesis of ethylene could be inhibited. This has resulted in the improvement of flower longevity in several crops such as carnation (Michael et al., 1993) and *Gerbera* (Elomaa, et al., 1993).

Relatively little is known regarding the initiation and progression of senescence in petals of ethylene-insensitive cut flowers including most of the important geophytes (Woltering and Van Doorn, 1988; Reid and Wu, 1992). In the last decade most research concerning petal senescence of ethylene-insensitive flowers has been reported on the bulbous ephemeral daylily flower (*Hemerocallis*), starting in 1989 by Lukaszewski and Reid. They concluded that daylily petal senescence is an active metabolic process and they hypothesized that gene regulation, mRNA and protein synthesis are probably involved. Further studies showed that floral senescence of daylilies requires protein synthesis, and is associated with rapid hydrolysis of cellular proteins (Lay-Yee, et al., 1992). The molecular basis of the senescence of this ethylene-insensitive flower is under investigation. Onset of flower senescence appears to be associated with the up-regulation of specific genes, involved in the hydrolysis of corolla proteins (Valpuesta et al., 1995). An overall decrease in cell protein levels has been found during both ethylene-sensitive, and ethylene-insensitive flower senescence (Van Doorn and Stead, 1994). In *Iris*, another ethylene-insensitive geophyte, a sharp increase in tepal leakage preceded the first visible senescence symptoms (Celikel and Van Doorn, 1995). The data reported indicated that one or more proteins, synthesized *de novo*, were responsible for the increase in leakage, as cycloheximide, an inhibitor of protein synthesis, was found to delay tepal leakage. Delay of senescence due to cycloheximide has been reported before, both in ethylene-sensitive flowers like carnation (Wulster et al., 1982) and in ethylene-insensitive flowers like *Hemerocallis* (Lukaszewski and Reid, 1989), *Gladiolus, Narcissus* and *Iris* (Jones et al., 1994).

In conclusion, despite many studies on the mechanisms involved in flower petal senescence, knowledge of the genetic and physiological events that lead to vase life termination in cut flowers is still limited, especially in ethylene-insensitive flowers.

Carbohydrates and postharvest flower quality

Carbohydrate is generally known to be used during respiration to maintain normal functioning of the tissue. Furthermore, it is used during growth for both structural biomass synthesis and osmotic adjustment during cell expansion. Due to low light intensities during storage, transportation and at the consumer, cut flowers largely depend on the presence of carbohydrates at harvest. Therefore, the amount of substrate in cut flowers is limited. Especially in inflorescence-type ornamentals with flowers in different stages of development competition for carbohydrate may occur and if the amount is insufficient, this may result in failure of floral bud development, smaller flowers, and/or a shorter flower life depending on the developmental stage at harvest (Eason et al., 1997). A carbohydrate source is commonly used in cut flower preservatives because of its beneficial effect on flower life. However, the mechanism by which senescence is delayed by additional carbohydrate is still poorly understood.

Many speculations have been made about its possible action in flower senescence e.g. substrate for respiration, maintenance of mitochondrial structure and function, and general maintenance of membrane function, protein synthesis and water status (reviewed by Halevy and Mayak, 1979). More recently carbohydrate has been reported to be involved in regulation of gene expression related to flower development and senescence (Eason et al., 1997).

Outline of the thesis

The aim of the research described in this thesis was to investigate the potential of plant breeding as an effective environmentally friendly tool for improving postharvest longevity of bulb flowers. Although the research started on tulips and on the three commercially important lily groups, it was focused gradually on one lily group, the Asiatic hybrids. Part of the results obtained on Asiatic hybrids are presented in this thesis.

The expression of genetic characters depends on the trial conditions. Cut flower longevity is a difficult genetic character to assess, since it can be affected by conditions prior to harvest, the developmental stage of the flowers at harvest, and postharvest conditions. In order to improve cut flower longevity by breeding several steps are needed. First, a reliable screening test has to be developed. Because lily flower longevity can be defined in terms of inflorescence longevity as well as in terms of individual flower longevity, special attention has to be paid to the measurement of flower longevity. A parameter is needed that can be used to discriminate among longevity levels of a large group of genotypes in a consistent way (Chapter 2). To optimize the screening procedure, standardized conditions in climate-controlled chambers are preferred, by which an accurate estimation of the longevity level of the flowers can be achieved. For standardization, understanding the parameters which influence flower longevity is necessary (Chapter 2, 3).

Next, the success of breeding depends on finding genetic variation for flower longevity. Variation was determined in (old) cultivars, species, and seedling clones (Chapter 4). Results obtained using standardized conditions should be similar between experiments (years) and comparable with results obtained after greenhouse forcing, such as commonly used in practice. Comparisons were made to study the validity of the standardized test conditions (Chapter 4).

Finally, knowledge about the inheritance of flower longevity is a prerequisite for the successful use of this trait as a selection criterion. The genetic analysis of postharvest flower longevity is described in Chapter 5. Besides individual flower longevity other important characters of lily postharvest performance have to be taken into account (e.g. number of buds per inflorescence, percentage of flowering buds). Knowledge about associations between flower longevity and other desirable plant

characters can lead to an improvement in selection efficiency for postharvest performance (Chapter 4 and 5).

Besides insight in the genetic aspects of flower longevity also knowledge of the physiological processes determining postharvest flower longevity is important. Flower carbohydrate status at harvest is known to be a major (internal) factor in determining postharvest flower performance. In lily cultivars differing in flower longevity the role of tepal carbohydrate in floral bud development (Chapter 6) and flower senescence (Chapter 7), was studied to investigate the mechanisms involved and to look for possible causal associations.

The main lines of the results of the study presented in this thesis are discussed in Chapter 8, and suggestions for further research are given.

Chapter 2

Influence of variation in plant characteristics caused by bulb weight on inflorescence and individual flower longevity of Asiatic hybrid lilies after harvest

J.J.M. van der Meulen-Muisers and J.C. van Oeveren

Abstract. Nongenetic variation in cut flower longevity due to plant characteristics was investigated in whole inflorescences and individual flowers of Asiatic hybrid lilies (*Lilium* L.). To distinguish this variation from genetic variation, plant characteristics of five cultivars were varied by using bulbs of three significantly different weight classes per cultivar. Inflorescence longevity depended on total number of floral buds, number of buds opening and variation in bud length. Variation in individual flower longevity per cultivar appeared to be small, despite a larger number of buds per stem with increasing bulb weight. Plant characteristics caused only small nongenetic variation in individual flower longevity when compared to inflorescence longevity. Therefore, individual flower longevity appears to be the best criterion to discriminate among longevity levels for a lily breeding program.

Introduction

Lily (*Lilium* L.) is one of the major bulb crops in The Netherlands, with the Asiatic hybrids as an commercially important group for cut flower production. In December 1990, a program was initiated at CPRO-DLO (part of the current Plant Research International) to investigate the possibilities of improving flower longevity of Asiatic hybrid lilies by breeding and selection. For that purpose, it was necessary to make an inventory of the components involved and to investigate genetic variation.

Flower longevity is determined by a large number of genetic and nongenetic components (Halevy and Mayak, 1979). To improve the selection response of lily genotypes for individual genetic components of longevity, sources creating nongenetic variation must be minimized. Lily bulbs are propagated vegetatively and can, therefore, be considered genetically identical within genotypes.

Plant and flower quality in lilies may be influenced by bulb weight, which has a direct impact on plant characteristics, such as plant length, plant weight, and number of buds (Beattie and White, 1993; Miller, 1993). In breeding trials, variation in bulb weight among genotypes is expected, because for each genotype there is a minimum bulb size and weight for flowering (Rees, 1966). Therefore, bulb weight could be a possible source of undesirable variation in selection trials on longevity.

In breeding research on improvement of longevity, a parameter is needed that can be used to discriminate among longevity levels of a large group of genotypes. Because lilies produce several flowers per stem, longevity can be defined in terms of inflorescence longevity as well as in terms of individual flower longevity.

The objective of this research was to investigate the influence of variation in plant characteristics caused by bulb weight, on inflorescence and individual flower longevity of Asiatic hybrid lilies. Based on the results the use of a definitive selection parameter for flower longevity among genotypes is proposed.

Materials and Methods

Plant materials. Bulbs of five Asiatic hybrid lily cultivars (Bright Beauty, Fashion, Harmony, Orlito and Yellito) were obtained from commercial growers in The Netherlands and from the CPRO-DLO cultivar collection. The cultivars were chosen based on differences in plant growth and flowering characteristics, such as forcing time and number of flower buds. Three sizes of bulbs varying in circumference from about 10 to 18 cm were used for each genotype. Per genotype, bulbs were provided by one grower in order to reduce influence of bulb origin on plant characteristics (Van der Meulen-Muisers et al., 1992). The bulbs were stored at −2°C for about six months until used. Within each size class, variation in bulb weight was further reduced by selection

for approximately similar weights. Three significantly different bulb weight classes per genotype were obtained (Fig. 2.1A).

Cultural conditions. Standard conditions were used during the preharvest, harvest and postharvest stages (Van der Meulen-Muisers et al., 1992). Plants were grown in a growth chamber of the CPRO-DLO Selektron (Smeets, 1986), with a constant air temperature of 17°C, relative humidity (RH) 60%, and with a 16-h photoperiod. Photosynthetically active radiation (PAR, 400 to 700 nm) at the top of the plants, was kept at a photosynthetic photon flux density (PPFD) of about 112 $\mu mol.m^{-2}.s^{-1}$, by high-pressure metal halide lamps (HPI-T 400W, Philips). Plants were grown individually in 2.5-liter plastic pots using standard prefertilized commercial potting soil. No additional fertilization was applied. Irrigation frequency was daily.

Harvest conditions. Lily stems were harvested at anthesis of the most mature floral bud by cutting the stems at the soil level. Stems were harvested within four hours after onset of the photoperiod. Flower buds were counted, stem were weighed, and stem length (distance between the stem base and the pedicel base of the basal flower bud), inflorescence length (distance between the pedicel base of the basal and apical flower bud) and bud length were measured. Bud length was rounded off to units of 5 mm at the time of measuring. The leaves on the basal 15 cm were removed and individual stems were placed in 1-liter glass flasks containing about 500 ml tap water.

Postharvest conditions. Cut stems were held at a constant air temperature of 17°C, 60% RH, and a 12-h photoperiod. PAR (400 to 700 nm) at the top of the stems, was kept at a PPFD of about 14 $\mu mol.m^{-2}.s^{-1}$ by fluorescent lamps (TL-D84 36W, Philips). Each individual flower was observed daily, within 4 to 6 hours after onset of the photoperiod. Flower longevity was measured as the time between bud anthesis and deformation of the flower, which was usually due to withering of the tepals. Individual flower longevity was calculated as the mean flower longevity per stem. Longevity of the whole inflorescence was defined as the time between anthesis of the most mature floral bud of a stem and deformation of the last flower.

Completely randomized designs were used. Number of plants per treatment was 20. Data sets were analyzed as factorial analysis of variance with two-way treatment structures, using the Genstat 5 statistical package (Rothamsted, U.K.).

Results and Discussion

Response to bulb weight. There were significant differences among bulb weight classes in plant weight (Fig. 2.1B), inflorescence length (Fig. 2.1C), and number of buds and flowers (Fig. 2.1D), but stem length was not influenced (Fig. 2.1C). The relationship between bulb weight and plant characteristics was not linear as the contribution of each unit of bulb weight to an alteration in plant characteristics decreased with increasing bulb weight. Therefore, variation within plant characteristics
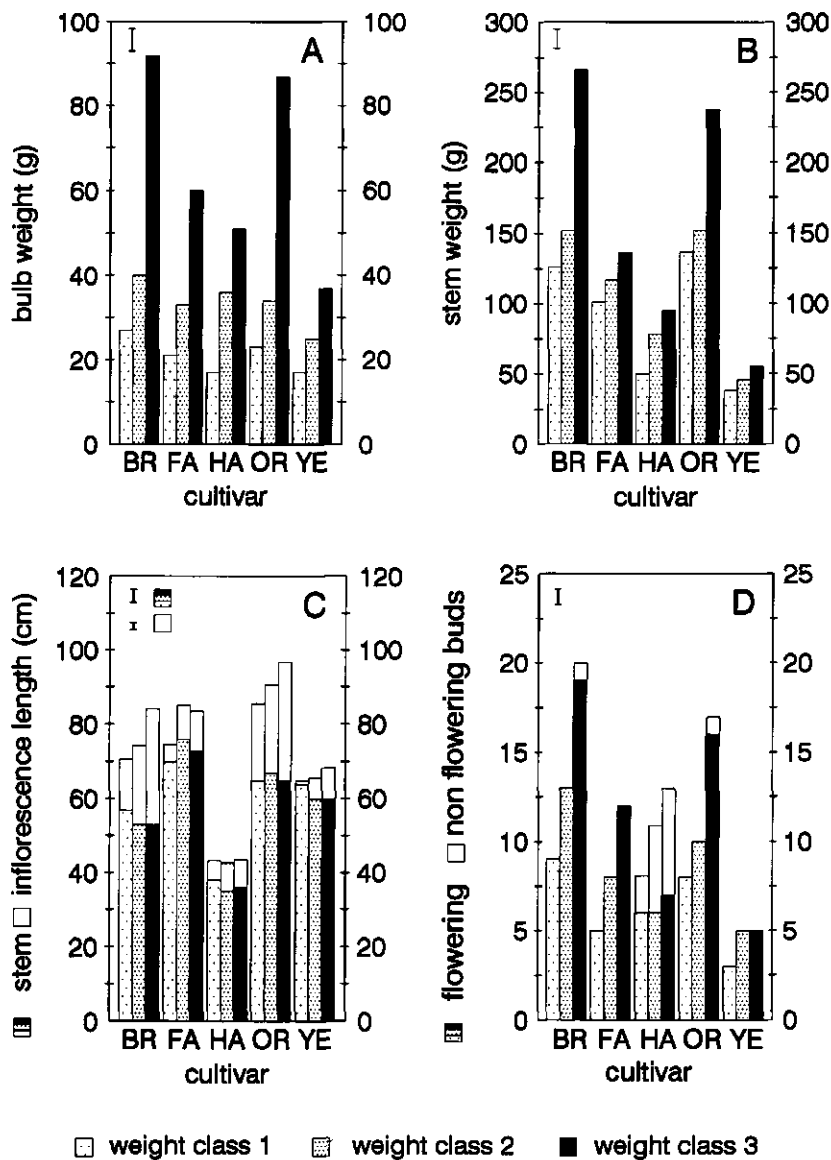
Fig. 2.1 Distribution of bulb weight per weight class (**A**) and effect of bulb weight on stem weight (**B**), stem length and inflorescence length (**C**), and number of flowering and non-flowering buds (**D**) of the Asiatic hybrid lilies 'Bright Beauty' (BR), 'Fashion' (FA), 'Harmony' (HA), 'Orlito' (OR), and 'Yellito' (YE). Bars represent LSD values at $P = 0.05$.

Fig. 2.2 Effect of bulb weight on inflorescence longevity (**A**) and individual flower longevity (**B**) of the Asiatic hybrid lilies 'Bright Beauty' (BR), 'Fashion' (FA), 'Harmony' (HA), 'Orlito' (OR), and 'Yellito' (YE). Bars represent LSD values at $P = 0.05$. Bulb weight classes are defined in Fig. 2.1A.
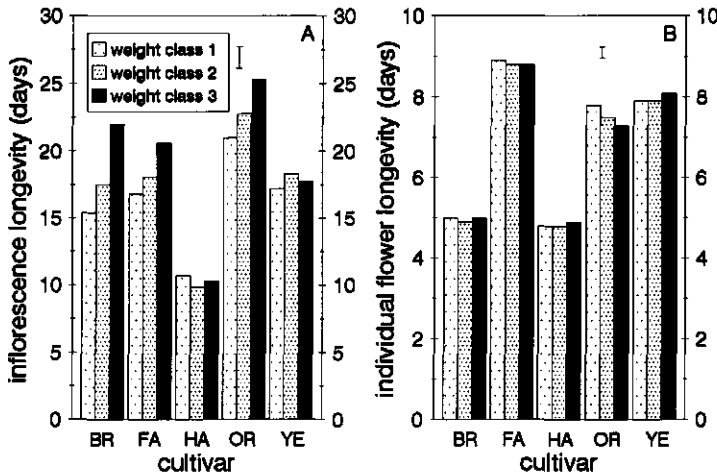
was relatively smaller than expected on the basis of the variation created among bulb weight classes. As for 'Yellito', the number of floral buds was constant when bulb weight increased from weight class 2 to weight class 3 (Fig. 2.1D). Within 'Harmony', inflorescence length and number of flowers was unaffected in spite of a significant increase in number of buds and, therefore, internodes with an increase in bulb weight (Fig. 2.1C, D). Within 'Harmony', failure of floral bud opening occurred in stems obtained from bulbs of all three weight classes, which increased with increasing bulb weight (Fig. 2.1D). Within 'Bright Beauty' and 'Orlito', stems from bulbs of weight class 3 exhibited some failure of flower opening.

Most of the varying plant characteristics we found in our study, were cited earlier by Beattie and White (1993) and Miller (1993), summarizing studies with different bulb weights in several lily species and hybrids. In our study bulb weight classes differed among cultivars, partly due to genetic differences in variation of bulb weights (Rees, 1966). Because of different production areas (growers) of the cultivars, differences in plant characteristics among genotypes can be expected (Van der Meulen-Muisers et al., 1992). Therefore, no comparisons of variation in plant characteristics among cultivars were made. Variation in plant characteristics among genetically identical plants was used to study the importance of bulb weight as a source of undesirable nongenetic variation in breeding for longevity.

Floral longevity. Inflorescence longevity increased with bulb weight in 'Bright Beauty' and 'Orlito', while for 'Fashion' this effect was found only between bulb weight class 2 and 3 (Fig. 2.2A). Inflorescence longevity of stems of 'Harmony' and 'Yellito'

was independent of the bulb weight. Individual flower longevity was not influenced by bulb weight in all cultivars except 'Orlito', in which longevity decreased slightly with an increase of bulb weight (Fig. 2.2B). The ranking of the genotypes based on longevity values differed comparing inflorescence longevity and individual flower longevity. Inflorescence longevity was the longest within 'Orlito' and the shortest within 'Harmony'; whereas, individual flower longevity was the longest within 'Fashion' and the shortest within both 'Bright Beauty' and 'Harmony' (Fig. 2.2). Therefore, by using either inflorescence longevity or individual flower longevity as a selection criterion in breeding research on longevity, different selection results would be obtained. Differences in inflorescence longevity between plants with different bulb weights can be expected because of the effect of bulb weight on number of buds and flowers produced per stem. When the number of buds or number of flowering buds did not increase with bulb weight, as found within 'Yellito' and 'Harmony', respectively (Fig. 2.1D), there was no increase in inflorescence longevity (Fig. 2.2A). A larger number of floral buds per stem is expected to lead to an increase in the competition for the metabolites available. Nevertheless, a reduction in individual flower longevity did not occur. So presumably all cultivars had adequate metabolites to maintain flower longevity at a constant level.

Floral bud distribution. Within all cultivars increases in bulb weight resulted in increases in number of floral buds per bud length class (Fig. 2.3). 'Harmony' and 'Yellito' showed little differences in bud distribution per bud length class between bulb weight class 2 and 3 (Fig. 2.3C, E). Within 'Bright Beauty', 'Fashion' and 'Orlito', additional smaller buds were formed (Fig. 2.3A, B, D). Those three cultivars also showed an significant increase in inflorescence longevity with bulb weight (Fig. 2.2A); whereas in 'Harmony' and 'Yellito no differences in inflorescence longevity were found among inflorescences from different bulb weight classes.

Time to anthesis. For each bud, time to anthesis decreased with an increase of bud length at the time of harvest. Per bud length, the time to anthesis was not influenced by bulb weight, except in 'Harmony' in which time to anthesis in weight class 1 was shorter than at the same bud length in the bulb weight classes 2 and 3. Differences in time to anthesis among cultivars were due to differences in bud length at the time of anthesis. Flower buds of 'Bright Beauty' and 'Orlito' reached a length of about 90 mm at the time of anthesis; while flower buds of 'Fashion' reached about 85 mm and flower buds of 'Harmony' and 'Yellito' about 80 mm (Fig. 2.3). Within 'Harmony', differences in time to anthesis among bulb weight classes were also due to differences in bud length at the time of anthesis. In weight class 1, anthesis occurred at a bud length of 75 mm; whereas, in weight classes 2 and 3 anthesis took placed at a bud length of 80 mm (Fig. 2.3). Time to anthesis was hardly influenced by bulb weight in spite of an increase in number of buds per length class with increasing bulb weight and, therefore, in the number of competitors for the available metabolites.
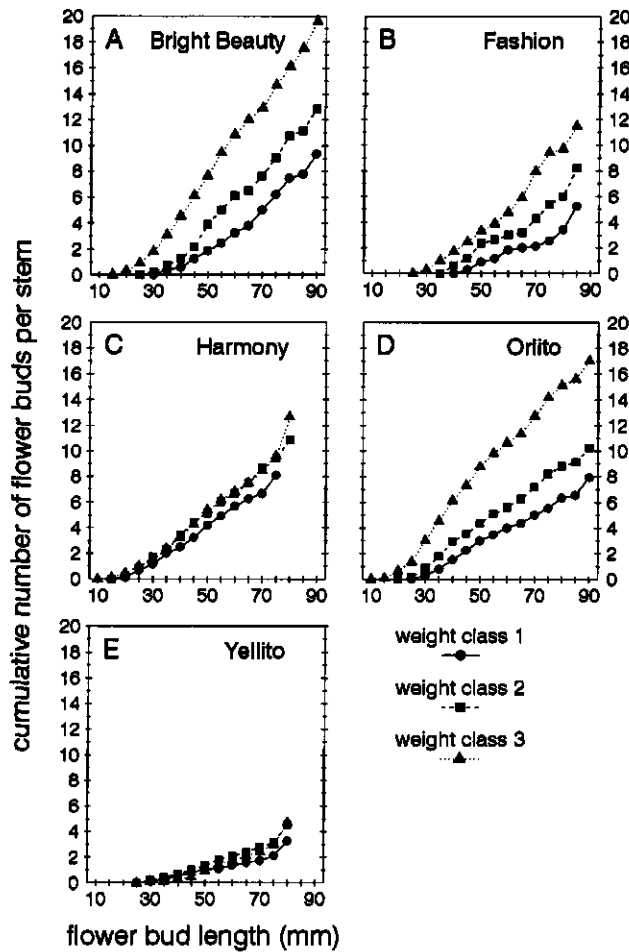
Fig. 2.3 Effect of bulb weight on the cumulative distribution of flower bud length per stem of the Asiatic hybrid lilies 'Bright Beauty' (**A**), 'Fashion' (**B**), 'Harmony' (**C**), 'Orlito' (**D**) and 'Yellito' (**E**). Represented are the cumulative numbers of buds until a given maximal bud length. The largest bud length class reached represents the tepal length at anthesis. Bulb weight classes are defined in Fig. 2.1A.

Flower opening. Number of open flower per day differed among plants with different bulb weights (Fig. 2.4), which was due to a varying number of buds per length class. There was an increase in the number of open flowers per day with an increase of bulb weight. Within 'Harmony' and 'Yellito', only small differences in number of open flowers per day were found. This was due to small differences in bud distribution among bulb weight classes (Fig. 2.3C, E). Within all cultivars, the highest number of open flowers per stem was reached one day before termination of longevity of the first flower (Fig. 2.2B, Fig. 2.4).
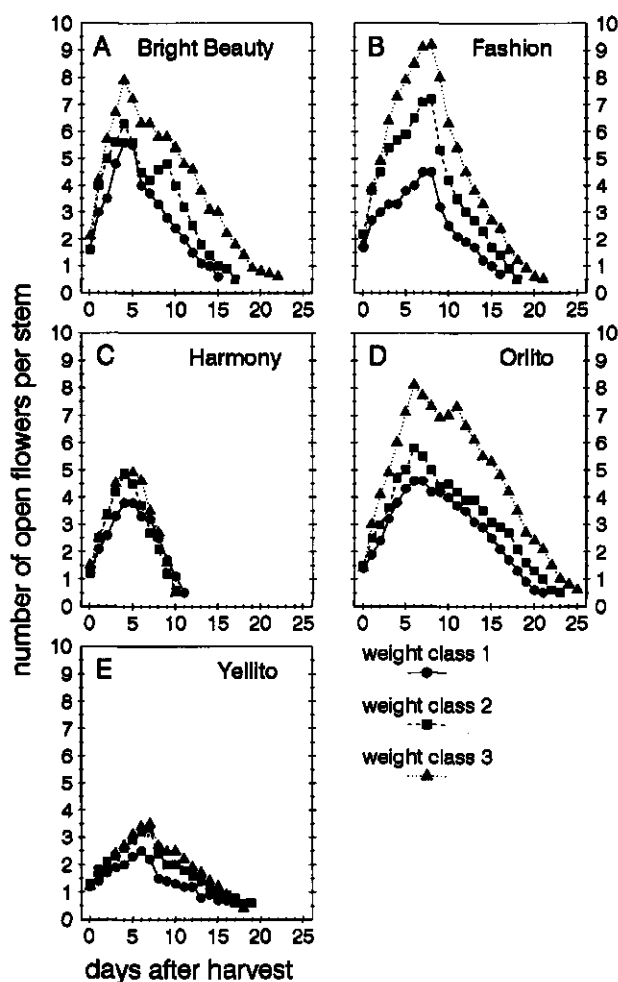
Fig. 2.4 Effect of bulb weight on the number of open flowers per day of the Asiatic hybrid lilies 'Bright Beauty' (**A**), 'Fashion' (**B**), 'Harmony' (**C**), 'Orlito' (**D**) and 'Yellito' (**E**). Bulb weight classes are defined in Fig. 2.1A.

Floral buds of 'Harmony' failed to open from a bud length smaller than 45 mm at the time of harvest in bulb weight class 1, and from a bud length smaller than 50 mm in bulb weight class 2 and 3. Within 'Bright Beauty' and 'Orlito', flowers did not open from a bud length at harvest smaller than 30 mm and 25 mm, respectively. These stages of small buds only occurred in weight class 3 (Fig. 2.3). As floral bud opening often is mediated by sugar supply (Han, 1992; Spikman, 1989) failure of bud opening could be due to carbohydrate depletion, that can be expected to occur with a large increase in number of buds per inflorescence as within 'Bright Beauty' and 'Orlito'. Also differences

in carbohydrate status among cultivars can be expected, that could explain the relatively low number of floral buds that reach anthesis within 'Harmony'.

Inflorescence. The influence of variation in plant characteristics on inflorescence longevity was based on the effect of bulb weight on number of buds. However, the extension of inflorescence longevity with an increase of bulb weight appeared to be smaller than expected on basis of number of additional buds per bulb weight class (Fig. 2.1D, Fig. 2.2A). In general, an increase in number of buds per bud length class (Fig. 2.3) also reduced the effect of an increase in total number of buds. This resulted in more flowers per day instead of an increase in inflorescence longevity (Fig. 2.4).

Within 'Bright Beauty', 'Fashion' and 'Orlito', the time period during which open flowers were present differed among stems of different bulb weight classes; whereas, in 'Harmony' and 'Yellito' hardly any differences were found (Fig. 2.2A). In the three former cultivars also additional smaller buds occurred when bulb weight increased (Fig. 2.3). Because the time period to flowering increased with a decrease of bud length at the time of harvest, the time period after harvest during which flowers will be present will only increase with bulb weight when additional smaller buds are present.

The final effect of bulb weight on nongenetic variation in inflorescence longevity was due to the additional smaller buds, their size and, therefore, the time necessary to reach anthesis.

Small buds can vary in number within and among cultivars. This variation is due not only to variation in bulb weight but also to differences in sensitivity of genotypes to factors such as duration of bulb storage, and growing conditions (Beattie and White, 1993; Durieux, et al., 1982/83; Roh, 1990bc). Although in our experiment no abortion and abscission of small buds occurred during cultivation, this might occur under less optimal preharvest conditions. Therefore, nongenetic variation in inflorescence longevity can be expected, even if bulbs of the same weight class are being used.

Individual flower. The constant longevity of individual flowers within inflorescences which was observed (Fig. 2.2B) has also been observed in *Liatris* (Han, 1992), *Gladiolus* (Yamane et al., 1993), and *Freesia* (Spikman, 1989). In *Freesia* individual flower longevity remained constant even when sucrose was supplied (Spikman, 1989). *Liatris* individual flower heads required sucrose for complete opening, after which longevity was not further influenced by the amount of sucrose supplied (Han, 1992). In both cases, an artificial sugar supply increased the number of floral buds opening and, therefore, longevity of the inflorescence. These results suggest that longevity of individual flowers of bulbous crops is maintained at a constant level within inflorescences, even after a physiological change such as an increase in internal sugar content.

From these results, it is concluded that individual flower longevity is a very stable parameter in longevity evaluation. In contrast, this is not the case for inflorescence longevity. Therefore, in our breeding program for improvement of flower longevity, individual flower longevity will be used as a selection parameter.

Acknowledgements

Chapter 3

Influence of bulb stock origin, inflorescence harvest stage and postharvest evaluation conditions on cut flower longevity of Asiatic hybrid lilies

J.J.M. van der Meulen-Muisers and J.C. van Oeveren

Abstract. To improve the ability to discriminate between Asiatic hybrid lilies (*Lilium* L.) with regard to cut flower longevity in breeding trials, sources creating nongenetic variation during the preharvest, harvest or postharvest phases were identified. The bulb stock origin (grower) and evaluation temperature caused only small nongenetic variation in individual flower longevity. In contrast, the developmental stage of floral buds, when cut, produced significant nongenetic variation in flower longevity. This variation could be reduced by delaying harvest. An evaluation temperature of 17°C was optimal to discriminate between longevity levels, compared to 14 and 20°C. Flower deformation due to withering of the tepals was an improved criterion for the termination of flower longevity and was preferred instead of loss of turgor of the tepals. Standard conditions for screening and selecting Asiatic hybrid lilies for individual flower longevity after cutting are proposed.

Introduction

In The Netherlands, lily cultivation has increased from about 250 ha to about 3600 ha in the past 25 years. About two-third of the bulbs is exported whereas about one-third of the bulbs is used for year-round flower production in greenhouses. Currently, the lily is the second bulb crop, after tulip for cut flower production in The Netherlands with the Asiatic hybrid lilies as an important group. Flower longevity is a primary limiting character of cut flower quality. To prolong the longevity of Asiatic hybrid lilies, pretreatment with silver thiosulfate (STS) is obligatory at the Dutch auctions. Alternative methods for improving longevity of the lily flower must be developed to reduce environmental pollution with the heavy metal silver. Although STS can retard senescence in lilies (Nowak and Mynett, 1985; Swart, 1980), the possibilities for extending flower longevity in the currently available genetic stocks are limited. Breeding and selection techniques to improve the genetic potential could be a less-polluting alternative for improving lily flower longevity.

The expression of genetic characters depends on the trial conditions. Flower longevity is a difficult genetic character to assess, since it can be affected by conditions before harvest, the developmental stage of the flowers at harvest, and postharvest conditions (Halevy and Mayak, 1979). To optimize the screening and selection procedures, environmental variance should be reduced. Therefore, a standardized test with a minimum variation in flower longevity within genotypes and a high degree of variation among genotypes is needed. For standardization, understanding the parameters which influence flower longevity is necessary.

Lilies produce several flowers per stem, and longevity can be defined in terms of inflorescence longevity as well as individual flower longevity. Individual flower longevity appeared to be a preferable parameter for longevity evaluation in breeding trials compared to inflorescence longevity because it showed less nongenetic variation (Van der Meulen-Muisers and Van Oeveren, 1996). Although lily inflorescence longevity has been object of many studies, to our knowledge, information about individual flower longevity is lacking.

Lily bulbs are propagated vegetatively and are genetically identical within genotypes; however, bulb growing conditions can cause physiological changes in lily flower bulbs (Van der Boon and Niers, 1986) and potentially affect flower longevity. Plant forcing conditions and season influence lily inflorescence longevity (Swart, 1980) and likely affect individual flower longevity. To reduce nongenetic variation experiments using controlled growth chambers are preferred over greenhouse experiments.

In preliminary research, developmental stage of the floral buds, at the time of harvest greatly influenced the longevity of lily inflorescences, whereas individual flower longevity hardly varied per genotype (Van der Meulen-Muisers et al., 1992). In practice, lilies are harvested in the bud stage; the stage normally depends on the cultivar assessed. In breeding trials, it is desirable to define a harvest stage more

comparable among and within genotypes to reduce environmental variance and to improve the ability to discriminate between genotypes with regard to individual flower longevity.

In postharvest research, standard conditions for flower evaluation have been proposed (Reid and Kofranek, 1980). These conditions simulate consumer environments and are based on the determination of the commercial display life of marketable genotypes. In contrast, only one or a few plant characteristics per evaluation are considered in selection trials to develop new genotypes. Other evaluation conditions may be more discriminative for detecting genetic differences within the genotypes (Van Eijk and Eikelboom, 1986). Various interpretations of the definition of flower longevity influence the data and selection results (Van Eijk and Eikelboom, 1977). In a breeding program, criteria for indicating the start and end of flower life have to be uniform to discriminate among longevity levels of a large group of genotypes in a consistent way.

The objective of the present research was to determine screening conditions with sufficient sensitivity to discriminate between different longevity levels in Asiatic hybrid lilies. The influence of bulb stock origin, inflorescence harvest stage, and evaluation conditions on environmental variance in individual flower longevity were examined.

Materials and Methods

Plant materials. Bulbs of Asiatic lily hybrids, 12 to 16 cm in circumference, were obtained either from commercial growers in The Netherlands or from the CPRO-DLO (part of the current Plant Research International) lily collection, in two successive years. The choice of the genotypes was based on known differences in individual flower longevity. The bulbs were stored in moist peat at −2°C for about five months until planted.

Forcing conditions. Plants were forced in a growth chamber of the CPRO-DLO Selektron (Smeets, 1986), with a constant 17°C air temperature, 60% relative humidity (RH), and a 16-h photoperiod. Photosynthetically active radiation (PAR) (400 to 700 nm) at the top of the plants was kept at a photosynthetic photon flux density (PPFD) of about 112 $\mu mol.m^{-2}.s^{-1}$ using high-pressure metal halide lamps (HPI-T 400W, Philips). Plants were grown individually in 2.5-liter plastic pots using a standard prefertilized commercial potting soil. No additional fertilization was used and plants were irrigated daily.

Harvest conditions. Lily inflorescences were harvested at anthesis of the most mature floral bud by cutting the stems at the soil level within four hours after onset of the photoperiod. The leaves on the basal 15 cm were removed and individual inflorescences were placed in 1-liter glass flasks, containing about 500 ml tap water.

Postharvest conditions. Cut inflorescences were held at a constant 17°C air temperature, 60% RH, and a 12-h photoperiod. PAR (400 to 700 nm) at the top of the inflorescences, was about 14 μmol.m^{-2}.s^{-1} using fluorescent lamps (TL-D84 36W, Philips). Each individual flower was observed daily, within 4 to 6 hours after onset of the photoperiod. Flower longevity was recorded as the time between anthesis and deformation of the flower, which was usually due to visual withering of the tepals. Individual flower longevity, calculated as the mean flower longevity per stem, was used as a parameter for screening (Van der Meulen-Muisers and Van Oeveren, 1996).

Bulb stock origin (Expt. 1). To ascertain the influence of bulb stock origin (grower) on individual flower longevity, 10 cultivars were used (Table 3.1). Bulbs of a varying number of origins were obtained per cultivar within the same year. Plants were forced, harvested and evaluated for longevity as described above. For each cultivar, individual flower longevity data of nine inflorescences per bulb stock origin were used for statistical analyses.

Inflorescence harvest stage (Expt. 2). To determine a harvest stage with minimum variation in flower longevity within genotypes after harvesting and allowing maximum variation between genotypes, 16 cultivars were used (Table 3.2). Plants were forced and evaluated for flower longevity as described above. Three stages of floral bud development at the time of cutting were used. The first two stages were based on color gradation of the most mature floral bud: stage 1, just starting to color; stage 2, fully colored; stage 3, at anthesis of the most mature floral bud. Data on time to anthesis of the first two harvest stages and data on flower longevity and percentage of flowers that reached anthesis of all three harvest stages were used for statistical analyses. Seven inflorescences were used per treatment combination.

Evaluation conditions (Expt. 3). Thirty-five lily genotypes consisting of twenty-five cultivars, eight tetraploid genotypes, and two species were used in the experiment (Table 3.3). These genotypes were examined to determine a criterion for end of flower life and to ascertain an evaluation temperature for screening on individual flower longevity in lily breeding. Plants were forced as described above and harvested at anthesis of the most mature floral bud. Two criteria were compared to define the end of the life of each flower: (1) loss of turgor of the tepals and (2) flower deformation. The loss of turgor was determined by tactile sense, while flower deformation was determined by visual detection of withering, which usually started at the tip of the tepals. All flowers were evaluated using both criteria. Three air temperatures (14, 17 and 20°C) were used during the evaluation of flower longevity. In each genotype, flower longevity data of five inflorescences per evaluation temperature were used for statistical analyses.

Statistics. Whole plants were used as experimental units in completely randomized designs. Data were analyzed by analysis of variance and variance components were estimated using the Genstat 5 Statistical Package (Rothamsted, UK). Ratios of genotypic variance and total variance, defined as broad-sense

heritabilities, were used to compare the screening efficiency between treatments. Heritabilities were calculated as $H^2 = s_g^2/(s_g^2 + s_e^2/n)$, where s_g^2 denotes genotypic variance, s_e^2 denotes environmental variance, and n is the number of plants per genotype. All calculations were carried out for n=1, representing the early stage of selection (individual plant level). To compare the ranking of the genotypes between treatments, correlation coefficients (r) were calculated.

Results and Discussion

Bulb stock origin (Expt.1). Flowers of the same genotype obtained from bulbs produced by different growers did not significantly vary in individual flower longevity (Table 3.1). Variance in individual flower longevity between bulb origins of the same genotype was relatively small compared to genotypic variance (0.12 and 1.17 respectively).

Lily bulbs are propagated vegetatively and are, therefore, genetically identical within genotypes. Genetic variation between production lines of the same genotype could occur due to spontaneous mutations. This phenomenon is more likely to take place in long existing cultivars than in more recently released cultivars. Longevity differences within long existing cultivars like 'Harmony' and 'Connecticut King' (Leslie, 1982) were 1 day at most (found within 'Connecticut King') when different bulb origins were compared (Table 3.1).

Table 3.1 Flower longevity (days) of 10 Asiatic lily hybrids using bulb stocks originated from a varying number of commercial growers per genotype; n = 9 inflorescences, SED= 0.57.

Genotype (year of introduction)[z]	Bulb stock origin (grower)			
	1	2	3	4
Avignon (1984)	7.2	7.6		
Bright Beauty (1982)	4.4	4.9		
Connecticut King (1967)	7.6	7.7	8.2	8.5
Harmony (1950)	5.5	5.6		
Ladykiller (1974)	4.9	5.5		
Monte Rosa (1984)	6.5	7.3	7.5	
Montreux (1984)	5.5	6.4		
Pollyanna (1981)	6.1	6.2	6.3	
Red Night, syn. Roter Cardinal (1974)	5.2	5.4		
Sirocco (1984)	4.7	5.6		

[z] From Leslie (1982).

Variation for bulb growing conditions can cause physiological differences between lily bulbs of different production units (Van der Boon and Niers, 1986). These differences may influence plant quality in lily (Beattie and White, 1993; Miller, 1993) and could also lead to nongenetic variation in individual flower longevity. The data (Table 3.1) indicate that using bulbs of the same genotype produced by different growers will not greatly influence individual flower longevity. Bulb stock origin (grower) was a minor source of variation for individual flower longevity, and was not standardized in the experiments.

Inflorescence harvest stage (Expt. 2). Data on time to anthesis, flower longevity, percentage of buds that reached anthesis, and number of floral buds per stem of 16 genotypes are presented in Table 3.2.

Time to anthesis. Analyses of variance showed that the effect, of genotype and harvest stage on time to anthesis of the most mature floral bud were highly significant ($P<0.001$), but no significant interaction was found between genotype and harvest stage. Harvesting lily inflorescences based on color gradation (stages 1 and 2) resulted in a significant decrease in time from harvest to anthesis of the most mature floral bud if harvest was delayed (Table 3.2). Environmental variance (s_E^2) was decreased by delaying harvest, while the genotypic variance (s_G^2) hardly changed (Table 3.2).

In commercial situations and postharvest research, coloration of floral buds is a common method to define the harvest stage (e.g., Han, 1992; Spikman, 1989). However, in selection trials, the first goal should be to discriminate among a group of genotypes in a comparable way based on each selection character. Differences in developmental stage of the floral buds among genotypes resulted in different time intervals from harvest to anthesis when using a subjective harvest criterion, based on color gradation. These subjective criteria also influenced uniformity in developmental stage per genotype, causing high environmental variances. This undesirable nongenetic variation, was reduced by delaying harvest. Anthesis of the most mature floral bud (stage 3) would represent the most comparable harvest stage, among and within genotypes. This should be preferable in breeding trials.

Flower longevity. Analyses of variance showed that the effect of both genotype and harvest stage on individual flower longevity was highly significant ($P<0.001$), while the interaction between genotype and harvest stage was not significant. The effect of harvest stage on individual flower longevity was relatively small compared to the genotype effect. Heritability increased by delaying harvest. Heritability was greatest at harvest stage 3, since the reduction in genotypic variance compared to harvest stage 2 was compensated by the reduction in environmental variance (Table 3.2). In general, the average percentage of flowers that reached anthesis increased with the maturity of floral buds at the harvest time (Table 3.2). The percentage of open flowers per genotype varied from 28% to 99% for 'Ladykiller' to 100% for all three harvest stages within 'Bora' and 'Monte Rosa', and appeared to be independent of the mean number of buds per stem (Table 3.2).

Table 3.2 Days to anthesis, flower longevity (days), percentage of flowers that reach anthesis, and number of floral buds per stem of 16 Asiatic lily hybrids. Inflorescences were harvested at the time the most mature floral bud started to color (stage 1), was fully colored (stage 2), or showed anthesis (stage 3); n = 7 inflorescences.

Genotype	Days to anthesis		Flower longevity (d)			Open flowers (%)			Floral buds per stem
	Harvest stage		Harvest stage			Harvest stage			
	1	2	1	2	3	1	2	3	
Adelina	7.6	3.6	6.4	6.7	7.0	61	90	100	9.7
Apeldoorn (syn. Amsterdam)	8.0	3.9	6.0	5.9	5.9	30	46	85	9.9
Bora	8.2	3.9	6.6	7.6	7.6	100	100	100	5.4
Dreamland	8.0	3.3	6.2	6.4	6.4	64	89	99	10.1
Enchantment	9.0	3.6	5.6	5.9	5.9	87	96	96	7.8
Eurovision	8.4	2.9	6.3	6.5	6.7	70	92	99	9.0
Figaro	7.6	4.0	5.7	6.4	6.5	72	97	97	7.5
Gran Sasso	7.3	2.0	7.1	7.5	7.7	98	98	100	7.1
Ladykiller	8.7	4.4	5.9	5.6	6.0	28	72	99	10.5
Lavender Dream	7.5	2.7	5.5	5.9	5.9	56	91	100	7.8
Mont Blanc	7.0	2.1	7.4	8.1	7.9	96	86	100	6.6
Monte Negro	7.5	2.7	7.2	7.1	6.8	58	78	94	7.8
Monte Rosa	6.4	2.3	8.1	8.7	8.2	100	100	100	5.2
Napoli	6.7	2.9	6.2	6.8	7.1	96	99	100	9.9
Orange Mountain	7.6	3.7	5.3	6.0	6.8	93	100	100	11.7
Sahara	5.1	1.6	7.2	6.8	7.0	54	83	76	6.0
Mean	7.6	3.1	6.4	6.7	6.9	73	89	96	8.3
SED[z]	0.17		0.09			2.0			
s^2_g	0.58	0.55	0.47	0.68	0.52				
s^2_e	2.15	0.85	0.60	0.44	0.17				
H^2			0.44	0.61	0.75				

[z] Standard error of differences between harvest stages averaged over cultivars.

Longevity is often determined by harvest stage, due to the influence of the harvest stage on developmental factors like carbohydrate level and sensitivity to environmental stress (Halevy and Mayak, 1981; Spikman, 1989). Variation in inflorescence longevity of Asiatic hybrid lilies is known to be caused by harvest stage (Van der Meulen-Muisers et al., 1992) which influences the percentage of flowering buds. The observed effect of harvest stage on longevity of individual flowers was relatively small, despite the varying impact on percentage of open flowers between genotypes (Table 3.2). Screening efficiency, measured by heritability, improved with the maturity of floral buds at the harvest time. This improvement was mainly due to a decrease in environmental variance (Table 3.2).

The results indicate that undesirable variance caused by harvest stage can be reduced by harvesting lily inflorescences in a more mature stage. By delaying harvest, a larger uniformity with respect to stage of development can be expected and the ability to discriminate between genotypes with respect to individual flower longevity will be increased. Therefore, harvesting lilies at anthesis of the most mature floral bud will be used in the CPRO-DLO breeding program. In practice, newly bred cultivars will be harvested in an earlier stage of development. This may change the percentage of buds that reach anthesis, but only small differences in individual flower longevity can be expected. Different harvest stages have to be recommended per cultivar to obtain the optimum ornamental value, since it is a function of individual flower longevity and percentage of flowering buds.

Evaluation conditions (Expt. 3). The analyses of variance gave highly significant effects of genotype, evaluation criterion, and evaluation temperature on individual flower longevity. The two-factor interactions were also significant, but their contribution to the total variance was relatively small. The three-factor interaction was not significant.

Evaluation criterion. Loss of turgor of the tepals was significantly earlier in time than deformation of the flower for each temperature assessed (Table 3.3). Although the effect of genotype x criterion was significant, ranking of the genotypes based on longevity varied little between the two evaluation criteria. Correlation between longevity levels of the genotypes using two evaluation criteria was highly significant for all three evaluation temperatures ($r_{14°C}=0.86$, $r_{17°C}=0.87$, $r_{20°C}=0.89$). These high correlations indicated that the interaction between genotype and criterion was not a main factor determining flower longevity. For each temperature considered, heritability of flower longevity was the highest if flower deformation was used as the criterion for termination of flower longevity. This was mainly due to a larger genotypic variance in combination with a smaller environmental variance (Table 3.3).

Table 3.3 Flower longevity (days) of 35 Asiatic lily hybrids using two criteria to determine the end of flower life at three evaluation temperatures; n=5 inflorescences.

Genotype	Days to loss of turgor			Days to flower deformation		
	Evaluation temp (°C)			Evaluation temp (°C)		
	14	17	20	14	17	20
Bicolito	6.7	6.4	4.6	9.4	8.1	6.1
Bora	7.3	6.0	4.9	8.7	7.5	5.8
Bright Beauty	4.6	3.6	3.2	6.4	5.2	4.4
Connecticut King	6.7	5.4	4.6	9.7	7.7	6.2
Corsica	6.4	6.6	4.4	9.4	9.5	6.3
Dreamland	4.4	4.0	3.0	6.9	5.8	4.7
Enchantment	4.7	4.4	3.4	6.0	5.4	4.1
Ladykiller	4.4	3.7	2.8	6.6	5.1	4.0
Lavender Dream	5.9	5.0	4.0	7.2	6.3	5.0
Mont Blanc	6.5	5.9	5.0	9.0	8.3	6.4
Monte Rosa	6.3	5.8	5.0	9.2	8.6	6.6
Montreux	6.8	6.1	4.7	9.0	7.7	5.7
Napoli	7.1	5.6	4.0	9.2	7.1	5.5
Orange Aristo	5.6	4.9	3.8	7.8	6.3	5.0
Orlito	6.4	5.9	4.5	8.6	7.5	5.8
Pirate	6.2	5.6	4.1	7.4	7.2	5.0
Prominence	5.1	4.5	3.5	6.7	6.0	4.3
Red Night (syn. Roter Cardinal)	4.9	3.9	2.9	6.1	4.7	3.4
Rolito	7.1	5.8	4.8	8.6	7.7	6.3
Sarina	6.0	6.2	4.8	7.8	7.5	5.8
Sirocco	5.4	4.4	3.4	6.8	5.1	4.2
Snowstar	6.8	6.3	4.6	9.3	8.7	6.4
Whilito	5.0	4.8	3.2	6.6	6.3	4.3
Yellito	8.2	7.7	5.6	10.7	9.7	7.1
Yellow Blaze	6.6	6.2	4.5	8.6	7.0	5.0
CPRO-73139 *L. dauricum*	6.4	5.9	4.9	8.4	8.1	6.7
CPRO-77543.6 *L. concolor*	9.6	7.6	4.9	10.3	8.2	5.6
CPRO-85702.5 (4n)	5.2	4.3	4.0	7.5	6.1	5.1
CPRO-85707.1 (4n)	7.9	6.9	5.2	9.9	9.0	6.6
CPRO-85710.2 (4n)	7.1	6.1	4.1	8.8	7.5	5.5
CPRO-85774.2 (4n)	5.9	4.9	3.6	7.8	6.8	5.2
CPRO-85989 (4n)	6.0	5.6	4.6	8.0	7.6	6.0
CPRO-87205 (4n)	7.6	6.9	5.2	9.5	9.1	6.7
CPRO-901246 (4n)	6.5	4.4	4.0	8.4	6.0	5.1
CPRO-901264 (4n)	6.0	5.0	4.2	8.4	7.5	5.6
Mean (SED^z=0.06)	6.3	5.5	4.2	8.2	7.2	5.5
s_g^2	1.22	1.06	0.49	1.48	1.66	0.80
s_e^2	0.40	0.30	0.20	0.28	0.26	0.22
H^2	0.75	0.78	0.71	0.84	0.87	0.78

[z] Standard error of differences between treatment means.

In postharvest research, criteria used to define termination of longevity vary from fading of the petal color and abscission of the petals to several stages of petal wilting (Halevy and Mayak, 1979). In breeding evaluation criteria must be uniform and valid for a diverse group of genotypes (Van Eijk and Eikelboom, 1977). From preliminary experiments it was concluded that fading of the tepal color and abscission of the tepals could not be used as criteria to compare longevity levels in lily genotypes (data not shown). Color fading was difficult to score consistently, especially for the white and yellow colors. Tepal abscission occurred only in some genotypes or a long time after loss of ornamental value of the flowers. Loss of turgor of the tepals and deformation of the flowers occurred in all genotypes tested. The results show that flower deformation is preferable for discriminating between lilies than loss of tepal turgor, because of its higher heritability of flower longevity.

Evaluation temperature. Flower longevity significantly decreased with increasing temperatures (Table 3.3). In general, this decrease of longevity was more rapid at a temperature increase between 17 and 20°C than between 14 and 17°C. Longevity of 'Corsica' was constant at a temperature increment from 14 to 17°C, whereas flower longevity of *L. concolor* decreased more rapidly than the other genotypes (Table 3.3). Although the effect of genotype x temperature was significant, ranking of the genotypes based on their longevity varied very little among the temperature treatments. The correlations were high for loss of turgor of the tepals ($r_{14-17°C}=0.90$, $r_{14-20°C}=0.82$, $r_{17-20°C}=0.88$) and flower deformation ($r_{14-17°C}=0.90$, $r_{14-20°C}=0.87$, $r_{17-20°C}=0.94$). For both criteria, heritability of flower longevity was the greatest at 17°C and the smallest at 20°C. The latter was mainly due to a relatively large decrease in genotypic variance at 20°C compared to 14 and 17°C. Environmental variance slightly decreased with an increase of evaluation temperature and was relatively small compared to genotypic variance (Table 3.3).

Even though highly variable genetic material (cultivars, tetraploid clones, and species) was used, the response to the different evaluation temperatures, based on longevity, hardly varied between genotypes. The deviating decrease in flower longevity of *L. concolor* with an increase of screening temperature may be due to the subordinated role of this species in the parentage of the Asiatic hybrids (Marshall, 1981). An improvement of the screening efficiency can be expected by evaluating the cut flowers at 14 or 17°C compared to 20°C because of improved discrimination. Evaluation of flower longevity at 17°C is preferable for discriminating between genotypes in lily breeding research because of its higher heritability, particularly in combination with flower deformation as a criterion for evaluation. Evaluation of newly bred cultivars at 20°C, as proposed by Reid and Kofranek (1980), will change longevity levels, but with only small differences in the ranking of the genotypes.

In conclusion, our results imply that bulb stock origin and evaluation temperature do not determine nongenetic variation in individual flower longevity of Asiatic hybrid lilies. Undesirable nongenetic variation dependent on the developmental stage of lily

inflorescences at harvest time could be minimized by collecting inflorescences at the anthesis of the most mature floral bud. To locate genetic differences among longevity levels of Asiatic lily hybrids, an evaluation at 17°C was the most discriminative. An improved criterion for the termination of flower longevity was the deformation of the flower due to start of withering of the tepals. This was preferred instead of loss of turgor of the tepals. The harvest stage, the evaluation criterion and the evaluation temperature will be standardized in future experiments. This is done to improve the ability to discriminate between longevity levels of Asiatic hybrid lilies in breeding trials, directed towards the improvement of flower longevity.

Acknowledgements

Chapter 4

Genotypic variation in postharvest flower longevity of Asiatic hybrid lilies

J.J.M. van der Meulen-Muisers, J.C. van Oeveren and J.M. van Tuyl

Abstract. Genotypic variation in postharvest flower longevity was determined for 63 Asiatic lily hybrids (*Lilium* L.). The reliability of standardized test conditions for longevity screening was also examined. Improvement of lily flower longevity by breeding appears feasible. Considerable genotypic variation in individual flower longevity was obtained and estimates of the degree of genotypic determination were high. The rank order of the genotypes with respect to individual flower longevity was similar between years using standardized test conditions. Screening results for flowers forced in a growth chamber were similar to those obtained in a greenhouse. No plant traits suitable for indirect selection on flower longevity were detected.

Introduction

Asiatic hybrids represent a major part of the commercially important lilies for cut flower production in The Netherlands. These hybrids originate from interspecific crosses between species of the *Sinomartagon* section, one of the seven sections of the genus *Lilium* (Van Creij et al., 1993). Longevity of cut flowers is an important quality factor since it affects consumer satisfaction. To prolong the longevity of Asiatic hybrids, pretreatment with silver thiosulfate (STS) is obligatory at Dutch auctions. However, the extension of flower longevity is still restricted by genetic factors. Thus, breeding and selection techniques that improve lily flower longevity can eliminate the use of chemicals such as STS.

Flower longevity can be influenced by growing conditions, the developmental stage of the flowers at harvest and environmental conditions after harvest (Halevy and Mayak, 1979). To reduce undesirable nongenotypic variation, breeding of cultivars with long-lasting flowers requires the availability of a reliable screening test. Standardized conditions with sufficient sensitivity to discriminate between various longevity levels in Asiatic hybrid lilies have been developed (Van der Meulen-Muisers and Van Oeveren, 1997).

In The Netherlands, Asiatic hybrid lilies are forced year-round, and interactions between the environment and genotype longevity are expected (Swart, 1980). Therefore, in breeding experiments using growth chambers for forcing is preferred, since it eliminates the environmental variation encountered in greenhouse trials. To establish reliable results in a standardized screening test, the influence of forcing conditions on the cultivar ratings must be determined. Screening results obtained after forcing in a growth chamber using standard conditions must be similar to results obtained with greenhouse forcing.

To ascertain the possibility of improving lily flower longevity by breeding, knowledge of the genotypic variation within the Asiatic hybrids is required. Genotypic variation is expected, because the Asiatic hybrids possess complex parentage. They are derived from interspecific hybridization of at least 12 different *Lilium* species (Marshall, 1981).

The objectives of this research were to (1) evaluate the genotypic variation in flower longevity in Asiatic hybrid lilies and (2) test the reliability of the conditions used. With regard to the possibility of indirect screening and selection, it was also ascertained to which extent flower longevity is correlated with other plant characters.

Materials and Methods

Plant materials. Bulbs of Asiatic hybrid lilies, 12 to 16 cm in circumference, were obtained from commercial growers in The Netherlands and from the CPRO-DLO

(part of the current Plant Research International) lily collection in two successive years. Before planting, the bulbs were stored in moist peat at −2°C for about five months. Evaluated for individual flower longevity were 63 genotypes consisting of 56 cultivars, 2 *Lilium* species and 5 seedling clones (Table 4.1), that were selected on their broad genetic backgrounds; thus, differences in individual flower longevity could be expected. All cultivars, except 'Prominence', are listed in The International Lily Register (Leslie, 1982). All genotypes are diploid, except for 'Compass', which is triploid, and 'Avignon', 'Gran Paradiso', 'Tetra Aristo', 'Tetra Bicolito', 'Tetra Orlito', CPRO-85598.2, CPRO-85702.5, CPRO-85710.2, CPRO-85774.2 and CPRO-82297.2, which are tetraploid.

Three experiments were conducted in two successive years. In the first year (1991) all 63 lily genotypes were evaluated for individual flower longevity (Expt. 1). In the second year (1992) a subset of the genotypes consisting of 47 diploid cultivars was evaluated in two experiments using different forcing conditions (Expt. 2, 3). 'Ballade', 'Rolito' and 'Sylvester' were excluded from the 1992 experiments because no bulbs were obtained.

Forcing conditions. Bulbs were weighed and individually planted in 2.5-liter plastic pots using a prefertilized standard commercial potting medium. In Experiments 1 and 2, plants were forced in a growth chamber of the CPRO-DLO Selektron (Smeets, 1986), with a constant 17°C air temperature, 60% relative humidity (RH), and a 16-h photoperiod. Photosynthetically active radiation (PAR) (400 to 700 nm) at the top of the plants, was kept at a photosynthetic photon flux density (PPFD) of about 112 $\mu mol.m^{-2}.s^{-1}$, using high-pressure metal halide lamps (HPI-T 400W, Philips). In Experiment 3, plants were forced in a greenhouse at CPRO-DLO during spring 1992 (March-May) using natural light conditions and minimum day/night temperatures of about 18/14°C.

Harvesting conditions. Inflorescences were harvested at anthesis of the most mature floral bud by cutting the stems at soil level. This occurred within four hours after the onset of the light period (Expt. 1, 2) or from 0800h to 1100h (Expt. 3). The harvest dates of the genotypes fell within the period of six weeks. The data recorded were: (1) the number of flower buds, (2) stem weight, (3) stem length (the distance between the cut stem base and the pedicel base of the basal flower bud), and (4) the inflorescence length (the distance between the pedicel base of the basal and apical flower bud). The leaves on the basal 15 cm were removed and individual inflorescences were placed in 1-liter glass flasks, containing about 500 ml tap water.

Postharvest conditions. Cut inflorescences were held at a constant air temperature of 17°C, 60% RH, and a 12-h photoperiod. PAR (400 to 700 nm) at the top of the inflorescences was kept at a PPFD of about 14 $\mu mol.m^{-2}.s^{-1}$ using fluorescent lamps (TL-D84 36W, Philips). Each individual flower was observed daily and within four to six hours after the onset of the photoperiod. Flower longevity was recorded as the time between bud anthesis and deformation of the flower, the visual

withering of the tepals. Individual flower longevity, calculated as the mean flower longevity per stem, was used as a parameter for screening (Van der Meulen-Muisers and Van Oeveren, 1996).

Indirect selection. To examine the suitability of other plant characteristics for indirect selection on individual flower longevity, bulb weight at planting time, stem length, inflorescence length, stem weight and the number of buds at harvest, and the number of flowers were used. Forcing period (the time between planting and harvest), inflorescence longevity (the time between anthesis of the first floral bud and deformation of the last flower), and the percentage of floral buds that reached anthesis were also examined for their suitability for indirect selection.

Statistics. Experiments consisted of three blocks with three plants of each genotype per block. For practical reasons, the genotypes evaluated in 1991 (Expt. 1) were divided into two groups that were planted 11 weeks apart. Both groups contained the genotypes 'Orange Aristo', 'Connecticut King', 'Enchantment', 'Harmony', 'Monte Rosa' and 'Orlito', which were used as a control to determine the influence of the planting date on flower longevity. In 1992, bulbs were planted with a time interval of 11 weeks between both experiments (Expt. 2 and 3).

Data were analyzed by analysis of variance, and variance components were estimated using the Genstat 5 statistical package (Rothamsted, U.K.). Ratios of genotypic variance and phenotypic variance were calculated to measure the degree of genotypic determination (R^2). Predicted estimates of R^2 were obtained to determine the accuracy gained by repetition of measurements. The degree of genotypic determination was calculated as $R^2 = s^2_g/(s^2_g + s^2_e)$, where s^2_g and s^2_e denote genotypic variance and environmental variance, respectively. Environmental variances were calculated as $s^2_e = (s^2_i + (s^2_f/n_f))/n_i$, with s^2_i denoting the variance between inflorescences, s^2_f denoting the variance between flowers within inflorescences, n_f being the numbers of flowers, and n_i being the number of inflorescences.

To compare the ranking of the genotypes between experiments, correlation coefficients (r) were calculated. To examine the suitability of other plant characteristics for indirect selection on individual flower longevity regression coefficients were calculated and multiple regression was carried out.

Results

Genotypic variation. The analysis of variance gave highly significant differences ($P < 0.001$) between genotypes in all experiments. Effects of blocks and interactions between genotypes and blocks were also significant but small compared to genotypic effects.

When forced under standardized conditions in a growth chamber, variation in

longevity levels among genotypes ranged from 4.8 to 9.2 days per individual flower in Experiment 1 and from 4.0 to 8.9 days in Experiment 2. After being forced in a greenhouse during spring (Expt. 3), longevity levels ranged from 5.0 to 11.0 days per flower (Table 4.1). In Experiment 1, longevity values of the six genotypes used as a control did not significantly differ between the two planting dates (data not shown). Thus, longevity data of genotypes from different planting dates were considered comparable. Of the 63 genotypes, 46 were evaluated in all three experiments (Table 4.1). In Experiment 1, longevity data of 'Crescendo' were discarded from the analyses. The quality of the inflorescences was poor, since flowering was strongly reduced by bud abortion.

When forced in a greenhouse (Expt. 3), open flowers at harvest differed in their longevity between inflorescences of the same genotype depending on the date of harvest (anthesis of the most mature floral bud). Differences could be due to different temperatures from anthesis to harvest between dates. Therefore, in Experiment 3 open flowers at the time of harvest were excluded from the analysis.

In all three experiments, over 90 percent of the floral buds reached anthesis except for 'Bright Beauty', 'Harmony', 'Montreux', and 'Sahara'. They had an average flowering percentage ranging from 67% ('Harmony') to 89% ('Bright Beauty'). In general, the percentage of floral buds that reached anthesis and flower size were improved by greenhouse forcing compared to growth chamber forcing.

Comparisons of diploid and tetraploid genotypes (Expt. 1) showed no specific influence of ploidy level on the level of flower longevity (Table 4.1). Between the diploid genotypes 'Bicolito', 'Orange Aristo', 'Orlito' and their corresponding mitotic tetraploid genotypes 'Tetra Bicolito', 'Tetra Aristo' and 'Tetra Orlito', differences in longevity level did not exceed 0.8 days, either in favor of the diploid genotype in the case of 'Orange Aristo', or the tetraploid genotype in the case of 'Bicolito'. The two species *L. concolor* Salisb. and *L. dauricum* Ker-Gawl. tested in Experiment 1 showed only an average longevity compared to the cultivars and seedling clones tested (Table 4.1). Because of their moderate longevity, those species and polyploid genotypes were excluded from the second year experiments.

Degree of genotypic determination. Estimates of environmental variances (s^2_e) were small compared to genotypic variances (s^2_g). This resulted in large estimates of degree of genotypic determination (R^2) for flower longevity in all three experiments (Table 4.1).

Predicted estimates of R^2 were calculated for 1, 2, 4, 6, 8 and 9 inflorescences per genotype in combination with 1, 2, 4, 6 and 8 flowers per inflorescence using the mean genotypic variance ($s^2_g = 1.42$) and the mean environmental variances ($s^2_i = 0.20$, $s^2_f = 0.58$) over the three experiments (Fig. 4.1). Increasing the number of measurements per genotype reduced the amount of environmental variance that contributed to the total phenotypic variance, which improved the degree of genotypic determination. Increasing the number of inflorescences per genotype appeared to be

more effective in reducing environmental variance than increasing the number of flowers per inflorescence. The degree of genotypic determination based on a single measurement per genotype was moderately high ($R^2 = 0.64$).

Table 4.1 Individual flower longevity (days) of Asiatic hybrid lilies after growth chamber forcing in 1991 (Expt. 1) and 1992 (Expt. 2) and after greenhouse forcing in March-May in 1992 (Expt. 3), using standardized harvest and postharvest conditions; n = 9 inflorescences. Genotypes are sorted towards increasing flower longevity (Expt. 2).

Genotype	Flower longevity		
	Expt.1	Expt.2	Expt.3
Red Night	5.2	4.0	5.0
Prominence	5.3	4.9	5.3
Corina	5.8	4.9	5.3
Bright Beauty	5.2	5.0	6.1
Harmony	4.9	5.1	5.0
Ladykiller	5.6	5.2	6.1
Sirocco	5.6	5.3	5.1
Enchantment	5.6	5.3	6.0
Sterling Star	4.8	5.6	6.6
Lavender Dream	5.8	5.6	5.9
Whilito	6.2	5.8	6.7
Yellow Blaze	6.0	5.8	7.0
Jolanda	7.0	5.8	7.0
Apeldoorn	6.0	6.0	6.7
Monte Negro	6.3	6.1	7.0
Concorde	6.9	6.2	6.4
Pollyanna	6.2	6.3	8.3
Roma	6.4	6.3	9.2
Dreamland	6.5	6.4	7.4
Figaro	5.5	6.6	7.1
Montreux	5.6	6.6	7.4
Mona	6.2	6.6	8.0
Sarina	6.7	6.6	8.1
Eurovision	6.4	6.7	7.0
Orange Mountain	6.9	6.8	7.0
Adelina	7.2	7.0	7.8
Sahara	5.6	7.1	6.8
Orange Aristo	6.9	7.1	7.4
Napoli	7.0	7.2	8.1
Connecticut King	7.4	7.4	9.1
Bora	7.8	7.4	7.7
Fair	8.1	7.4	8.9
Orange Aristo	6.9	7.1	7.4

Table 4.1 (Continued)

Genotype	Flower longevity		
	Expt.1	Expt.2	Expt.3
Napoli	7.0	7.2	8.1
Connecticut King	7.4	7.4	9.1
Bora	7.8	7.4	7.7
Fair	8.1	7.4	8.9
Crescendo	.[z]	7.6	8.9
Revival	8.6	7.6	9.1
Orlito	6.7	7.7	7.3
Gran Sasso	7.9	7.7	8.3
Pirate	6.9	7.9	7.0
Snowstar	7.5	7.9	8.7
Yellito	8.2	7.9	9.8
Mont Blanc	7.5	8.0	8.4
Monte Rosa	7.3	8.2	8.2
Bicolito	6.8	8.3	9.0
Fashion	7.6	8.3	8.4
Corsica	8.8	8.4	11.0
Crete	7.9	8.5	9.9
Fuego	8.2	8.7	9.7
Commodore	7.9	8.9	9.1
Sylvester	6.6	.	.
Ballade	8.4	.	.
Rolito	8.8	.	.
Compass (3n)	7.4	.	.
Gran Paradiso (4n)	6.0	.	.
Tetra Aristo (4n)	6.3	.	.
CPRO-85774.2 (4n)	6.3	.	.
CPRO-85702.5 (4n)	6.4	.	.
Tetra Orlito (4n)	6.8	.	.
CPRO-82297.2 (4n)	7.1	.	.
Avignon (4n)	7.2	.	.
Tetra Bicolito(4n)	7.6	.	.
CPRO-85598.2 (4n)	8.6	.	.
CPRO-85710.2 (4n)	9.2	.	.
CPRO-77643.6 *L. concolor*	7.1	.	.
CPRO-73139 *L. dauricum*	7.5	.	.
Mean	6.7	6.8	7.6
SED[y]	0.26	0.26	0.27
n_f[x]	6.9	7.8	8.9
R^2[w]	0.97	0.98	0.98

[z]Missing values
[y]Standard error of differences between genotype means
[x]Mean number of flowers per inflorescence
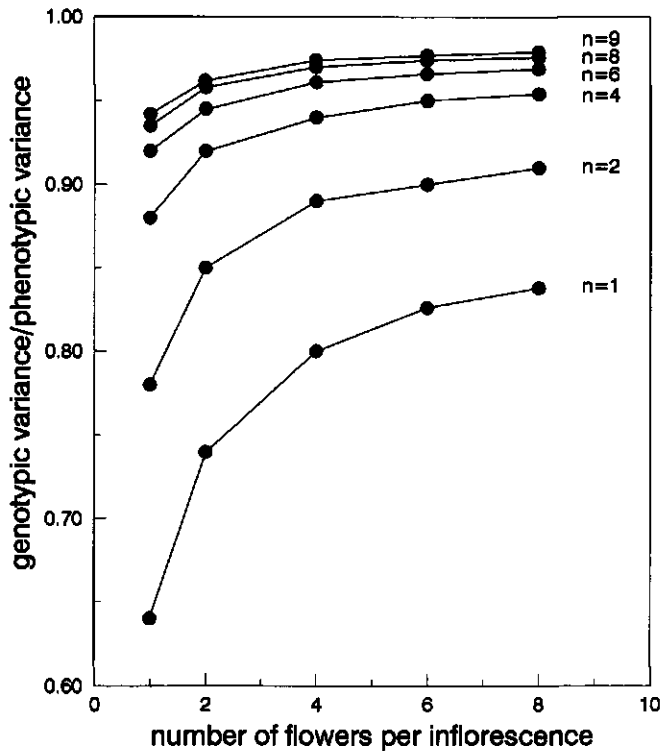[w]Degree of genotypic determination (genotypic variation/phenotypic variation)

Fig. 4.1 Relation between the degree of genotypic determination (genotypic variation/phenotypic variation), the number of inflorescences (n) and the number of flowers per inflorescence in Asiatic hybrid lilies.

Reliability of test conditions. High correlations (r = 0.83) were found between longevity values of 46 diploid cultivars tested in two successive years after forcing under controlled conditions in a growth chamber (Fig. 4.2). The average longevity levels of 47 diploid cultivars forced under standardized conditions in a growth chamber was improved by about 1 day when forced in a greenhouse during spring in the same year (Fig. 4.3). High correlations (r = 0.84) were found between longevity values of those 47 diploid cultivars comparing different forcing conditions, although some deviating genotypes ('Corsica', 'Pirate', 'Roma') were present (Table 4.1).

Indirect selection. Only small correlation coefficients up to r = 0.42 were found between flower longevity and other plant characteristics studied (data not shown). Bulb weight, stem length, stem weight, inflorescence longevity, and forcing period had little association with individual flower longevity. Significant, but small associations of number of floral buds and flowers per inflorescence and percentage of floral buds that reached anthesis with individual flower longevity were detected in all three experiments (Table 4.2). These three plant characteristics accounted for

only 15% to 21% of the total variance in individual flower longevity using multiple regression. The strongest association with flower longevity was found for the number of floral buds. An increase of one additional bud per inflorescence led to a decrease in the average longevity of approximately 0.2 days in all three experiments (Table 4.2).
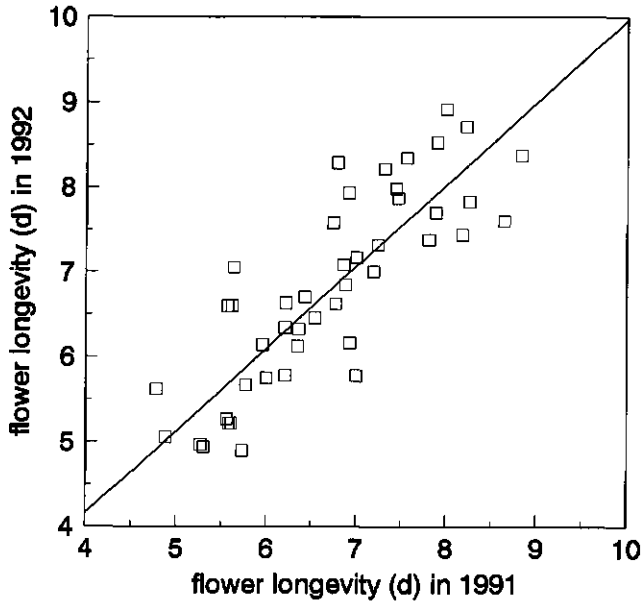

Fig. 4.2 Correlation between longevity levels (days) of individual flowers of 46 lily genotypes evaluated in 1991 and 1992 using standardized forcing, harvest and postharvest conditions; $r = 0.83$.

Table 4.2 Regression coefficients and corresponding standard errors (SE) for the relation between changes in individual flower longevity (days) per additional floral bud, per additional open flower, and per additional percent of floral buds that reached anthesis of Asiatic hybrid lilies. Lilies were forced in a growth chamber in 1991 (Expt. 1) and 1992 (Expt. 2) and in a greenhouse in 1992 (Expt. 3) using standardized harvest and postharvest conditions.

Plant characteristic	Expt.1	Expt.2	Expt.3
Floral bud	-0.177[**]	-0.228[**]	-0.193[*]
SE	0.054	0.073	0.076
Open flower	-0.165[**]	-0.192[*]	-0.162[*]
SE	0.059	0.081	0.081
Anthesis (%)	0.088[**]	0.071[**]	0.087[*]
SE	0.030	0.025	0.036
Degrees of freedom	60	45	45

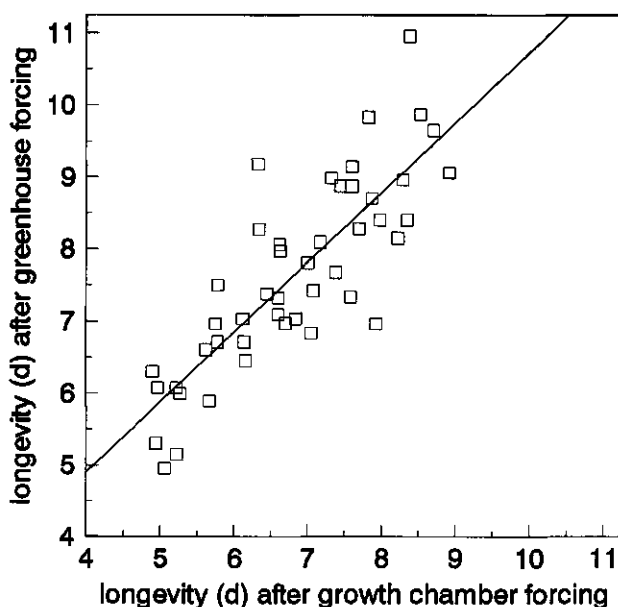[*], [**] Significant at $P = 0.05$ and 0.01, respectively.

Fig. 4.3 Correlation between longevity levels (days) of individual flowers of 47 lily genotypes either forced in a growth chamber using standardized conditions or in a greenhouse during spring. Standardized harvest and postharvest conditions were used; $r = 0.84$.

Discussion

Genotypic variation. The genotypic variation in flower longevity (Table 4.1), in combination with a large R^2 for this character indicates that flower longevity in Asiatic hybrid lilies can be improved by breeding and selection. Knowledge of this variation and reliable test conditions are beneficial to breeding programs directed on the improvement of lily flower longevity.

Comparisons of diploid and tetraploid cultivars showed no influence of ploidy level on the level of flower longevity. Chromosome doubling (polyploidy) is known to be effective in the improvement of quality characteristics in lily, such as flower size and morphology and stem firmness (Schenk, 1987; Van Tuyl, 1989), and in the improvement of flower longevity in *Tulipa* (Van Eijk and Eikelboom, 1986). The polyploid genotypes evaluated (Table 4.1) did not provide an additional genetic source for the improvement of flower longevity in lily.

Degree of genotypic determination. Quantitative traits, such as flower longevity, are expected to be influenced by environmental effects to a large extent. Our environmental variances, however, accounted for only 3 percent (Expt.1) or 2 percent (Expt. 2 and 3) of the total variance in this study. While the controlled environment in the growth chamber can explain the low estimates of environmental

variance in the Experiments 1 and 2, we also found a low estimate of environmental variance in the greenhouse experiment (Expt. 3). Although environmental variances increased, when the number of measurements per genotype decreased, the estimation of the degree of genotypic determination was moderately high only when one flower per genotype was used in the analysis (Fig. 4.1). These results suggest that flower longevity is controlled to a large extent by genetic effects.

From the predicted estimates of R^2 (Fig. 4.1), it could be concluded that the gain in accuracy for determining longevity levels of genotypes by their phenotypes was all the higher when the number of inflorescences used for evaluation was increased, rather than by increasing the number of flowers per inflorescence. However, the production of a large number of flowering bulbs per genotype involves vegetative propagation followed by cultivation over at least two years (Beattie and White, 1993). In breeding trials it would be preferable to carry out initial selection at the individual plant level to speed up the breeding progress. Since the estimation of R^2 based on a single measurement per genotype was moderately high ($R^2 = 0.64$), selection based on one plant per genotype is possible. In the case of selection at individual plant level, it would be advisable to evaluate as many flowers as possible to get a maximal reduction of the environmental variance.

Reliability of test conditions. Replications carried out under controlled forcing conditions in two successive years had similar results, indicating that test results were reproducible under standardized conditions. Some variation between the replications was, however, detected. This could be due to differences in bulb age or bulb quality. Differences due to bulb age were small for the six cultivars used as replicates in time (Expt. 1). Bulbs of the same genotype used in different years originated from different bulb growers and could, therefore, be of different quality (Van der Boon and Niers, 1986). Although bulb origin has been reported to be only a minor source of nongenotypic variation in individual flower longevity of Asiatic hybrid lilies, differences up to 1.0 day within a genotype have been found (Van der Meulen-Muisers and Van Oeveren, 1997). This could account for part of the variation between the different years (Expt. 1 and 2).

Although flower longevity generally increased after greenhouse forcing in spring, compared to growth-chamber forcing under standardized conditions, longevity data of experiments carried out under different forcing conditions were highly correlated. Therefore, the ranking of the genotypes based on longevity is reliable even after greenhouse forcing. Some deviations, however, were detected. Variation between experiments due to quality and age of the bulbs was unlikely. Bulbs used in both 1992 experiments originated from the same bulb grower per genotype and the 11 weeks difference in planting dates produced no significant differences in longevity as stated before (Expt. 1). Therefore, deviations between Experiments 2 and 3 were probably due to differences in genotype response to varying forcing conditions.

The controlled conditions used as a standard for forcing were generally

suboptimal for flower longevity. Longevity increased with about one day on average after greenhouse forcing in spring, the optimal season for forcing of Asiatic hybrids in The Netherlands (Fig. 4.3, Table 4.1). In practice, screening and selection will probably take place after forcing under non-standardized conditions. To make sure results are comparable year-round, some cultivars might be used as a control to correct for general seasonal effects. It should be taken into account that specific genotype x environmental interactions can occur. Thus, selections must be tested for year-round performance using a range of forcing environments.

Due to time differences in flowering dates, the condition of open flowers at the time of harvest could be influenced by temperature fluctuations during forcing under greenhouse conditions. Such fluctuations are likely to account for longevity differences between open flowers at the time of harvest in Experiment 3. It is known (Van der Meulen-Muisers and Van Oeveren, 1997) that lily flower life is highly influenced by temperature after anthesis. In practice, evaluation of lily flower longevity should be carried out using controlled postharvest conditions. Also, open flowers at the time of harvest should be excluded from the analyses. This will avoid large environmental variances within inflorescences due to temperature fluctuations before harvest. Discarding one or a few flowers per stem will not influence the accuracy of the longevity level determined per genotype (Fig. 4.1).

Indirect selection. Since only small correlation coefficients between plant characteristics and flower life were detected, none seemed suitable for indirect selection on flower longevity. The small effects found could be due to the influence of bulb weight on other plant characters (Van der Meulen-Muisers and Van Oeveren, 1996). However, only a relatively small variation in bulb weight was introduced because of the limited range of bulb sizes used. Another explanation could lie in the cultivars used in our experiments. Within cultivars, many traits are combined which were selected to obtain the desired lily. This preselection could disturb the natural correlation between flower longevity and other plant characters. Useful associations are more likely to appear when seedling populations are used.

Because of the relatively narrow range of bulb sizes used, the number of floral buds did not vary per genotype, and differences in number of buds between genotypes could be ascribed to genetic differences in the ability to produce buds. Within genotypes, no significant influence of number of flower buds on individual flower longevity has been found (Van der Meulen-Muisers and Van Oeveren, 1996). In the present study, an increase in number of flower buds between genotypes caused a small but significant reduction in the individual flower longevity independently of the forcing conditions used. These results indicate that two desirable characteristics - the potential for the formation of a large number of floral buds per inflorescence and a long individual flower longevity - are negatively correlated.

Acknowledgements

Chapter 5

Genetic analysis of postharvest flower longevity in Asiatic hybrid lilies

J.J.M. van der Meulen-Muisers, J.C. van Oeveren, J. Jansen and J.M. van Tuyl

Abstract. To investigate the genetic regulation of postharvest flower longevity in Asiatic hybrid lilies (*Lilium* L.), 10 cultivars and 45 progenies were forced, harvested and evaluated under standardized conditions in growth chambers. Analysis of variance for individual flower longevity indicated highly significant ($P < 0.001$) variation among parents, among progenies and among descendants within progenies. High broad-sense heritability (0.79) calculated at the individual plant level indicated that selection for long individual flower longevity can be expected to be very effective. General combining ability (GCA) effects were highly significant ($P < 0.001$), and the estimated narrow-sense heritability was high (0.74). Therefore, individual flower longevity of a genotype can be used as an indication for its breeding value. Although deviating results can be expected as specific combining ability (SCA) effects were also significant ($P = 0.046$). Small, but significant correlations between individual flower longevity and other plant characters were found. The impact of these correlations on the selection efficiency for improved postharvest performance of lily inflorescences is discussed.

Introduction

In The Netherlands *Lilium* is economically the second bulb-flower crop after *Tulipa* for cut flower production. In 1996 about 36 percent of the turnover of the lily cut flowers at the Dutch auctions is accounted for by the Asiatic hybrids, which have been developed from interspecific hybridization within the *Sinomartagon* section, one of the seven sections of the genus *Lilium* (Van Creij et al., 1993).

Postharvest treatments with silver thiosulphate (STS) are commonly used to enhance the vase life of Asiatic hybrid lilies (e.g. Nowak and Mynett, 1985; Swart, 1980) but the extent to which these treatments can improve flower longevity is limited and is dependent on the genotype. Developing cultivars with genetically improved postharvest longevity may provide the consumer with a more reliable expectation for postharvest quality. Therefore, research to evaluate the potential of plant breeding as a method for genetically improving longevity in lily is important.

Postharvest inflorescence longevity of lilies is complex because it is a function of the number of buds per inflorescence, of the expansion and opening of the buds and of the life-span of the individual flowers. Large environmental variances due to forcing conditions, harvest stage and postharvest conditions can be expected (Halevy and Mayak, 1979; Swart, 1980). By using standardized conditions for screening as developed by Van der Meulen-Muisers and Van Oeveren (1997), that variation is strongly reduced.

Individual flower longevity has been found to be a stable parameter for screening (Van der Meulen-Muisers and Van Oeveren, 1996). Improving individual flower longevity would give the potential for an increment of the number of flowering buds at the same time, it would extend the longevity of the whole inflorescence and, therefore, improve the postharvest performance of the inflorescence. Knowledge about the inheritance of individual flower longevity is a prerequisite for the successful use of this trait as a selection criterion.

Genotypic variation in individual flower longevity has been found to be present (Van der Meulen-Muisers et al., 1998). When using standardized conditions during forcing, harvest and postharvest evaluation the potential life of individual flowers on an inflorescence placed in water is about 4 to 9 days depending on the genotype. Individual flower longevity has been reported to have a high broad-sense heritability which should ensure effective selection in this vegetatively propagated crop (Van der Meulen-Muisers et al., 1998). Information on its narrow-sense heritability is lacking and the main objective of this study was to learn more about the inheritance of individual flower longevity.

Other important characters of lily postharvest performance are the number of buds per inflorescence and the percentage of flowering buds. Only small phenotypic associations between individual flower longevity and those two characters have been found (Van der Meulen-Muisers et al., 1998). However, this study was based on observations of vegetatively propagated material, mainly commercially grown

cultivars. In the study reported here, unselected seedling progenies were screened to determine if there are favourable associations between individual flower longevity and other desirable plant characters. Such associations can lead to an improvement of the selection efficiency for postharvest performance in Asiatic hybrid lilies.

Initial studies to improve lily flower longevity by cross breeding suggested an indirect linkage between the occurrence of male sterility and the improvement of flower longevity (Van der Meulen-Muisers et al., 1995b). Such an association could possibly be mediated by the absence of an ethylene peak which seems to occur in male fertile flowers at the end of the pollen meiosis as discussed by Van Tuyl et al. (1985). In the present study the association between male sterility and flower longevity was investigated in more detail.

Materials and methods

Plant materials. In spring 1992, crosses were made using 10 Asiatic hybrid lilies (*Lilium* L.) (Table 5.1). Parents were chosen on the basis of differences in individual flower longevity. The parental combinations which gave the progenies were largely dictated by practical considerations; 'Yellito' could mainly be used as a female parent, 'Revival' could mainly be used as a male parent and, most important, some crosses gave few or no seed. Nevertheless, the progenies which were studied can reasonably be considered as a representative sample with regard to the individual flower longevity character.

Table 5.1 Parentage of 45 progenies studied from crosses between ten Asiatic hybrid lily cultivars. Parental cultivars are arranged in decreasing order of individual flower longevity as determined in the experiment (Table 5.2).

Cultivar ♀/♂	FA	YE	RE	OR	CO	MO	HA	PR	BR	ST
Fashion (FA)		*	*	*		*	*			
Yellito (YE)						*	*	*	*	*
Revival (RE)				*			*			
Orlito (OR)	*					*			*	*
Montreux (MO)		*		*			*	*		
Connecticut King (CO)	*					*		*	*	*
Harmony (HA)			*	*		*		*		
Prominence (PR)	*		*	*	*	*				
Bright Beauty (BR)	*		*	*	*	*				*
Sterling Star (ST)	*	*	*		*				*	

In December 1992, seeds of 45 populations (Table 5.1), including 12 pairs of progenies from reciprocal crosses, were sawn in flat trays with peat. For each population 125-250 seeds were used. Trays were placed in a greenhouse at ±17/15°C (16h day/8h night). At the same time commercial bulbs of the 10 parents were vegetatively propagated by scaling. Scales were placed in perforated plastic bags with moist vermiculite at 26°C for 8 weeks to induce scale bulblets. This was followed by 4 weeks at 17°C and 8 weeks at 5°C. In May 1993, scale bulblets and seedling bulblets were planted simultaneously outdoors, using aphid-free facilities to prevent virus spread. Progenies and parental bulblets were cultivated for 2 years to obtain adult bulbs with the potential to flower. Bulbs harvested in autumn 1994 were rated and disinfected in captan (Captan Flow; 1.0%) and prochloraz (Sportak; 0.2%). Bulbs were stored at −2°C in plastic bags with moist peat for about 4 months until planted. To ensure flowering only bulbs ≥ 12 cm in circumference were used.

Experimental conditions. Cultivars and progenies were forced, harvested and evaluated for individual flower longevity utilising standardized conditions outlined by Van der Meulen-Muisers and Van Oeveren (1997). Plants were forced in a growth chamber at 17°C, 60% relative humidity (RH), 112 $\mu mol.m^{-2}.s^{-1}$ using high-pressure metal halide lamps (HPI-T 400W, Philips) during 16-h per day. Inflorescences were harvested at anthesis of the most mature floral bud. Cut inflorescences were placed in tap water without additives in a climate room at 17°C, 60% RH, 14 $\mu mol.m^{-2}.s^{-1}$ using fluorescent lamps (TL-D84 36W, Philips) during 12-h per day.

Flower longevity. Individual flower longevity was defined as the time between bud anthesis and visual wilting (start of deformation) of the flower. Plant means were determined from data collected on all flowers evaluated per plant. Evaluation of flower longevity was carried out at clonal level for the parental genotypes. Progeny means were determined for each cross from individual plant means.

Indirect selection. To examine the suitability of other plant characters for indirect selection on individual flower longevity, bulb weight at planting time, inflorescence length, stem weight, tepal length at anthesis, number of buds and number of flowers were determined. The forcing period (the time between planting and harvest), inflorescence longevity (the time between anthesis of the first floral bud and deformation of the last flower), percentage of floral buds that reached anthesis, and male sterility were also examined for their suitability for indirect selection. Male sterility (the complete absence of pollen production) was scored as present or absent.

Experimental design and statistics. Twenty-one descendants per cross and 24 inflorescences per parental clone were tested in 1995. They were forced in 3 blocks planted 3 weeks apart. Data were analysed by analysis of variance using the Genstat 5 statistical package (Rothamsted, U.K.). General combining ability (GCA) effects, specific combining ability (SCA) effects and reciprocal effects were calculated. Conclusions concerning GCA, SCA and reciprocal effects were assessed by analysis of variance.

Broad-sense heritability (coefficient of genotypic determination) of individual flower longevity was estimated per progeny and as a composite estimate calculated over 45 progenies. In addition, broad-sense heritability of the parental clones was estimated (based on a single plant level). Broad sense heritability (H^2) estimates were calculated from estimated variance components as $H^2 = s^2_g/(s^2_g + s^2_e/n)$, where s^2_g denotes genotypic variance, s^2_e denotes environmental variance (determined on the basis of variation between replicate plots of vegetatively propagated parental cultivars), and n is the number of plants per genotype. All calculations were carried out for n = 1. The heritability in narrow-sense (h^2), as an estimation of the part of the phenotypic variance resulting from additive gene effects, was calculated as the regression coefficient of the offspring on mid-parent.

Correlation coefficients (r) were calculated between parents and offspring. To examine the suitability of other plant characters for indirect selection on individual flower longevity phenotypic correlation coefficients (r_p) based on progeny means and genetic correlation coefficients (r_g) based on parental GCA were calculated and multiple regression was carried out.

Results

Within the parental clones 3 significantly different groups were distinguished with a long (L), moderate (M) and short (S) individual flower longevity respectively (Table 5.2). Broad-sense heritability (H^2) for individual flower longevity of the parents was estimated to be 0.88 based on a single plant level.

The analysis of variance showed highly significant differences ($P < 0.001$) in individual flower longevity among crosses and among plants within crosses. Individual flower longevity did not segregate into discrete classes within the progenies. Transgressive segregation for long individual flower longevity occurred in all progenies except for MO x PR (data not shown).

In Figure 5.1 the mean longevity values of the parents per cross (mid-parent values) were plotted against the longevity levels of the progenies. In general, longevity levels of the progenies increased with an increase of the longevity levels of their parents ($r = 0.87$). All progenies obtained from two parents with a long (L) flower longevity had a longer flower life than progenies obtained from one L parent and one parent with a short (S) flower longevity, and the latter progenies had a longer flower life than progenies obtained from two S parents (Fig. 5.1).

The composite broad-sense heritability for individual flower longevity was estimated to be 0.79 (coefficient of genetic variance based on progenies was 1.08, and coefficient of environmental variance calculated from parental clones was 0.29).

Table 5.2 Longevity levels of the ten parental clones (L = long, M = moderate, S = short), clonal flower longevity given as deviation from the grand mean across the ten parental clones, and parental GCA estimates given as deviation from the grand mean across the ten parents; parents are arranged in the order of decreasing flower longevity.

| Cultivar	Longevity level	Flower longevity	GCA
Fashion	L	1.8	0.5
Yellito	L	1.6	0.7
Revival	L	1.2	0.5
Orlito	L	1.0	0.5
Montreux	M	0.2	0.1
Connecticut King	M	0.1	0.0
Harmony	S	-1.2	-0.6
Prominence	S	-1.4	-0.7
Bright Beauty	S	-1.5	-0.5
Sterling Star	S	-1.8	-0.5
Grand mean		6.0	5.7
SED^z		0.17	0.14

^{z} Standard error of differences between parental means.
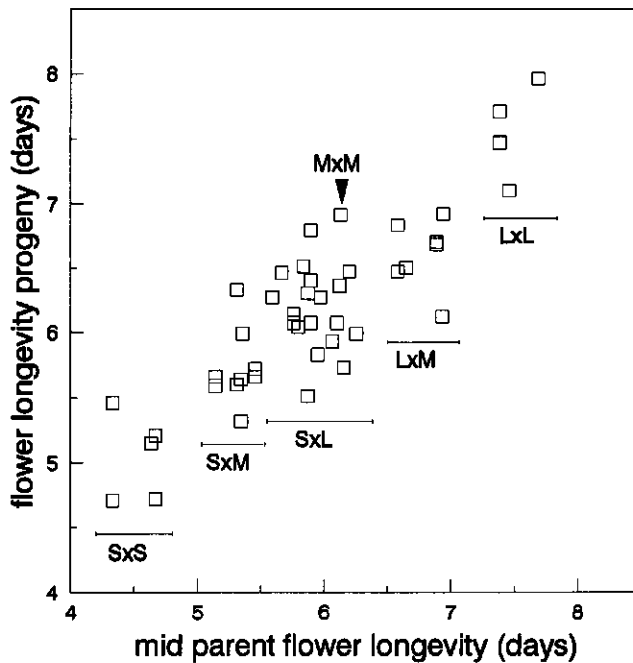
Fig. 5.1 Association between longevity values obtained after testing 45 Asiatic hybrid lily progenies and their corresponding mid-parent longevities (r = 0.87). The six different longevity cross combinations are given. Longevity levels: L = long, M = moderate, and S = short.

In separate analyses, broad-sense heritabilities were calculated for each progeny. Broad-sense heritabilities appeared to be little influenced by the degree of similarity of the parents. Estimated heritabilities ranged from 0.31 to 0.89, reflecting varying levels of genetic variance within the individual progenies (Fig. 5.2). The values obtained were mainly in the intermediate to high range although two deviating progenies (STxBR, PRxHA) with relatively low heritabilities were present (Fig. 5.2).
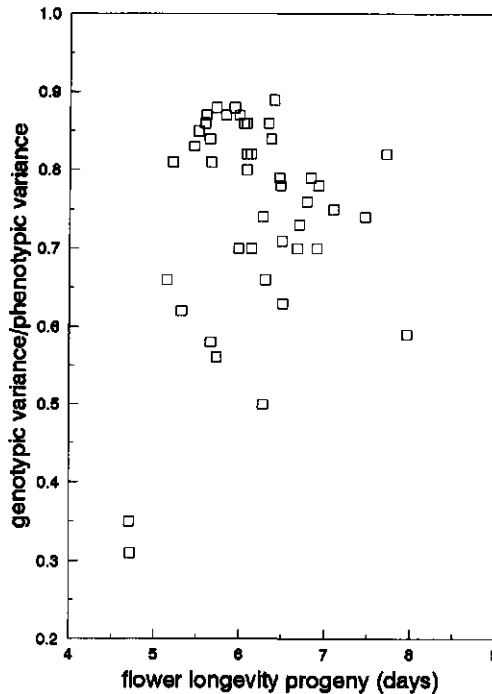
Fig. 5.2 Association between longevity values obtained after testing 45 Asiatic hybrid lily progenies and their corresponding broad-sense heritabilities (genotypic variance/phenotypic variance).

Analysis of variance showed GCA effects to be highly significant ($P < 0.001$), 81% of the variation in individual flower longevity between progeny means could be attributed to parental effects. However, the effects of specific combining ability (SCA), although of smaller importance, were also significant ($P = 0.046$); whereas reciprocal effects were not significant ($P = 0.161$). Breeding values (GCA) of the parental clones are presented in Table 5.2. Longevity values and breeding values were highly correlated ($r = 0.98$).

Narrow-sense heritability for individual flower longevity was estimated to be 0.74, indicating a marked influence of additive genetic variance. In Figure 5.3 the longevity values of the progenies were plotted against the longevity value per progeny

calculated with GCA effects only. Vertical distances between each point and the line x=y represent deviations from additivity. Although some deviations from the additive model were significant, the additive model explained almost all variation between crosses.
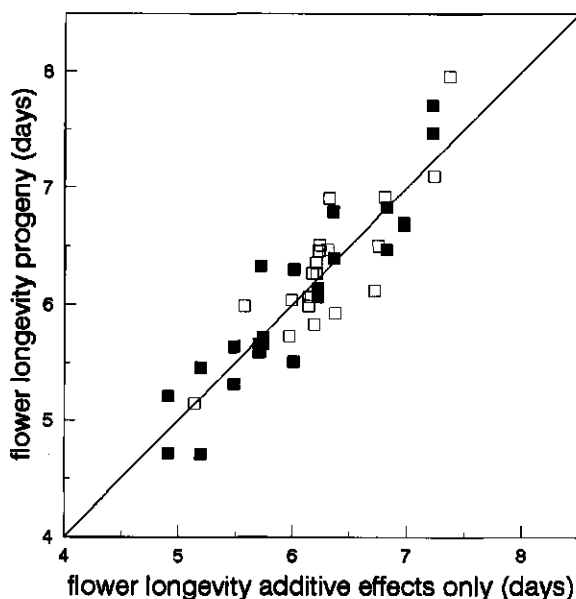

Fig. 5.3 Association between longevity values obtained after testing 45 Asiatic hybrid lily progenies and the longevity values calculated with only additive (GCA) effects ($r = 0.90$). Vertical distances between each point and the given line x=y represent variances from additivity. Solid squares indicate reciprocal crosses.

Longevity values of the descendants evaluated at individual plant level ranged from 2.0 to 10.8 days (Fig. 5.4). Overall 11 percent of the seedlings had an improved longevity (better than 'Fashion' i.e., >7.8 days, Table 5.2). Most of the descendants with an improved individual flower longevity were obtained in the L x L cross combinations, while in S x S combinations although descendants with a long (L) flower longevity were observed (between the longevity level of 'Orlito' and 'Fashion' i.e., 7.0 to 7.8 days, Table 5.2), no descendants with an improved flower longevity (>7.8 days) were found (Fig. 5.5).

In 33 of the progenies tested, male sterility occurred corresponding with 23% of all descendants tested. Many of the male sterile plants occurred in offsprings from 4 female cultivars: 'Bright Beauty', 'Connecticut King', 'Harmony', 'Yellito', whereas the breeding value for the occurrence of male sterility of those cultivars was considerably less when used as a male parent (data not shown).
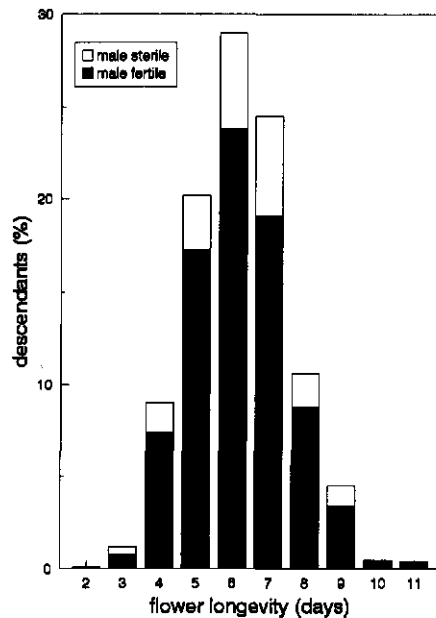
Fig. 5.4 Segregation of individual flower longevity of male sterile and male fertile inflorescences within the descendants of 45 lily progenies evaluated at individual plant level.

A large segregation in flower longevity of male sterile descendants existed (Fig. 5.4). In 21 populations the average individual flower longevity of the male sterile plants exceeded the average individual flower longevity of the male fertile plants of the same population. Overall, male sterile descendants and male fertile descendants did not significantly differ for individual flower longevity; whereas the tepal length at anthesis, the percentage of flowers that reached anthesis, the stem weight, and the number of buds per stem within male sterile descendants were significantly (P = 0.05) reduced compared to male fertile descendants (data not shown).

Within the male sterile descendants roughly two plant types could be identified. The first plant type was mainly comparable with the male fertile plants, except for the production of pollen; whereas the second plant type showed more or less phenotypic aberrations (e.g., reduced plant weight, relatively small flowers, deviating flower shape), which did not occur in the male fertile plants. Within the latter plant type flower longevity tended to be reduced compared to flower longevity of inflorescences of the first plant type and compared to the longevity of male fertile flowers of the same population.

Significant but small phenotypic associations were found between progeny means of individual flower longevity and inflorescence longevity, forcing time, percentage of floral buds that reached anthesis and number of floral buds (Table
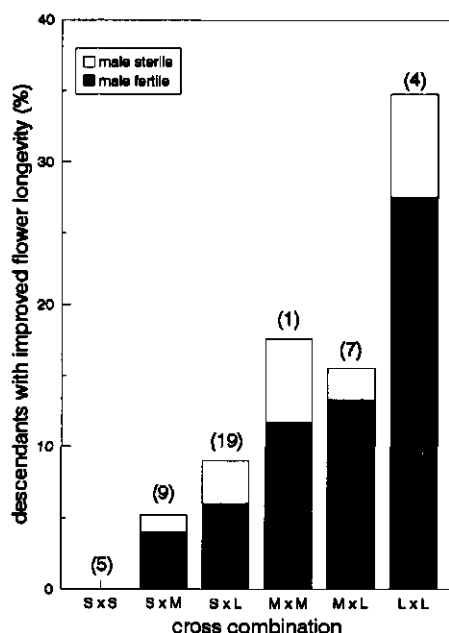
Fig. 5.5 Distribution of improved longevity levels (better than 'Fashion', i.e., > 7.8 days, Table 5.2) of both male sterile and male fertile descendants between six different longevity cross combinations. Longevity levels: L = long, M = moderate, and S = short; number of crosses per cross combination in parentheses.

5.3). Inflorescence longevity, forcing time and number of floral buds accounted for about 64 percent of the total variance in individual flower longevity using multiple regression. Because percentage of floral buds that reached anthesis was correlated with inflorescence longevity ($r = 0.50$) the former was excluded from the multiple regression calculation. Except for number of floral buds all correlation coefficients were positive. Overall tepal length at anthesis, stem weight, bulb weight and number of flowers had little association with individual flower longevity. Genetic associations based on parental GCA values appeared to be comparable with their corresponding phenotypic associations. However, only the genetic association between individual flower longevity and inflorescence longevity was significant (Table 5.3).

Among progeny means based on male sterile plants only, significant positive phenotypic correlation coefficients (r_p) were found between flower longevity and inflorescence longevity, forcing time, tepal length at anthesis and stem weight (Table 5.3). Among overall progeny means (both male sterile and fertile plants) no significant associations between flower longevity and stem weight and between flower longevity and tepal length at anthesis were found (Table 5.3).

Table 5.3 Phenotypic correlation coefficients (r_p) based on overall progeny means and genetic correlation coefficients (r_g) based on GCA of associations between individual flower longevity and six other plant characters. In addition, r_p based on progeny means calculated for sterile descendants only.

Character	Individual flower longevity (d)		
	Overall progeny means		Sterile descendants only
	r_p	r_g	r_p
Inflorescence longevity (d)	+0.62**	+0.81**	+0.62*
Forcing time (d)	+0.54**	+0.53	+0.34*
Flowering buds (%)	+0.31*	+0.61	+0.26
Number of floral buds	-0.29*	-0.38	-0.00
Tepal length at anthesis (mm)	+0.25	+0.41	+0.55**
Stem weight (g)	+0.23	+0.30	+0.45**
Degrees of freedom	43	8	31

*,** Significant at 5 and 1 percent level, respectively.

Discussion

The high value ($H^2 = 0.79$) for the broad-sense heritability of individual flower longevity, based on 45 progenies at individual plant level, confirms earlier estimations based on clonal material (Van der Meulen-Muisers et al., 1998). Phenotypic selection for long individual flower longevity in trials with one representative plant of each genotype ought, in consequence, to be very effective (Aikman and Langton, 1983).

The correspondence between the breeding value of the parents with their phenotypic performance as a clone was very close despite physiological differences in bulb material (seedling bulbs versus scale propagated bulbs). These results confirm earlier work comparing longevity data obtained from an individual plant test (seedling bulbs) with a clonal test (scale propagated bulbs) of the same progeny (Van der Meulen-Muisers et al., 1995b). This suggests an equal expression of flower longevity in inflorescences obtained from seedling bulbs and in inflorescences obtained from scale propagated bulbs. Therefore, initial selection for improved flower longevity can be carried out using seedling bulbs.

Knowledge of the way in which individual flower longevity is sexually inherited is important to the breeder. Individual progeny H^2 estimations show that similarities between parents in individual flower longevity do not necessarily indicate genetic homogeneity. This, together with the absence of discontinuous variation and the domination of transgressive segregation, suggests that individual flower longevity is inherited as a polygenic character. However, only by using genetic markers linked with loci involved in the encoding of flower longevity can a more explicit statement be

given upon the course of the inheritance of flower longevity in lily. The relatively low H^2 found within two progenies, PR x HA and ST x BR, could indicate that per cross both parents are mainly homozygous for the longevity genes.

The highly significant GCA component and high narrow-sense heritability estimate indicate the importance of additive genetic variance in the transmission of parental individual flower longevity to the progeny. Therefore, the individual flower longevity of the genotype can be used as an indication for its breeding value in practical breeding.

Because of the importance of additive genetic variance in the inheritance of individual flower longevity, genotypes with a short individual flower longevity should, whenever possible, be excluded as parents in *Lilium* breeding programmes. However, since both SCA and transgressive segregation also play a role in the inheritance of individual flower longevity, high heritability in broad sense can occur even when both parents have a short individual flower longevity. So, even if two S parents are used, some descendants with a long individual flower longevity could still be obtained.

In a standardized screening test containing 63 Asiatic hybrids a variation in individual flower longevity of about 4 to 9 days was found (Van der Meulen-Muisers et al., 1998). Hybrids with a long individual flower longevity could be useful as parents in breeding programmes to produce cultivars with an improved individual flower longevity. Within the populations tested individual descendants occurred with longevity levels which exceeded the highest longevity level found within the Asiatic hybrids of the screening test. Because of the effective selection due to high broad-sense heritability and because of the way of inheritance which has been discussed before, genetic improvement for individual flower longevity in this vegetatively propagated crop can be expected to be relatively rapid.

Although significant correlation coefficients between individual flower longevity and other plant characters were found, they were only moderately high. Because of the absence of strong correlations none of the plant characters tested was found to be suitable for indirect selection on individual flower longevity. Ideally, indirect selection should be carried out by using genetic markers linked with flower longevity genes. The high GCA effects together with the large segregation of flower longevity found in our progenies provide good prospects for a successful use of genetic markers in the search for loci involved in the encoding of lily flower longevity.

On the other hand the associations found between flower longevity and other plant characters will have some impact on the selection efficiency. The association of a long individual flower longevity with a long inflorescence longevity and a high percentage flowering buds ought to simplify selection because these characters will all improve the postharvest performance of lily inflorescences. Although the underlying cause of these associations is not known, it is possible that these three characters might, in some manner, be regulated by the available amount of carbohydrates within the inflorescence. It has been suggested that failure of bud

opening in lily may be caused by depletion of carbohydrates (Roh, 1990a), leading to a reduction in inflorescence longevity. Furthermore, in preliminary research tepal carbohydrate level was found to be associated with individual flower longevity in lily (Van der Meulen-Muisers et al., 1995a).

The negative correlation between individual flower longevity and number of buds per inflorescence might also be regulated by the available amount of carbohydrates within the inflorescence as developing lily flower buds are known to have a large sink strength (Wang and Breen, 1986b, 1987) and bud development has been reported to be at the expense of the longevity of accompanying flowers within the inflorescence (Van der Meulen-Muisers et al., 1995a).

Like in *Tulipa* (Van Eijk and Eikelboom, 1976) flower longevity was positively and significantly correlated with the forcing period. This association could cause some problems in selection, as a long forcing period is considered an undesirable character due to an increase in production costs as a consequence. Large numbers of plants must be available for selection on a long individual flower longevity combined with a short forcing period.

The association of male sterility with a longer flower longevity within about 64% of the progenies containing male sterile descendants, could be due to the possible absence of an ethylene peak, which occurs in male fertile flowers (Durieux et al, 1983; Van Meeteren and De Proft, 1982), and seems to coincide with the end of the pollen meiosis. Furthermore, in male sterile flowers a reduction of the ethylene precursor ACC, which is found in ripening pollen of many species (Spikman, 1987; Whitehead et al., 1983), might be expected. Influence of ethylene on longevity of Asiatic lily flowers has been found by Elgar et al. (1999) and Van der Meulen-Muisers and Van Oeveren (1993). Also the beneficial effect of the ethylene retardant silver thiosulphate (STS) in Asiatic hybrid lilies is known (Nowak and Mynett, 1985; Swart, 1980).

The absence of a significant overall association between male fertility and individual flower longevity is likely caused by the appearance of two plant types within male sterile descendants. This might be due to the fact that male sterility and flower distortion often seem to be associated in lily breeding (Wadekamper, 1977). Also other factors concerning inflorescence and flower development might be involved in determining flower life in male sterile flowers. This is supported by the significant positive correlation between individual flower longevity and both stem weight and tepal length at anthesis within male sterile descendants.

The results of this study indicate that there are good prospects for the genetic improvement of individual flower longevity in Asiatic hybrid lilies. Because of the associations of individual flower longevity with other desired characters of postharvest performance of lily inflorescences, genetic improvement within this vegetatively propagated crop can be expected to be effective.

Acknowledgements

Chapter 6

Postharvest flower development in Asiatic hybrid lilies as related to tepal carbohydrate status

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Abstract. For three Asiatic hybrid lily cultivars (Bright Beauty, Fashion, Orlito) the potential postharvest performance of floral buds in terms of growth, anthesis and longevity was studied in relation to tepal carbohydrate status. To determine the importance of carbohydrate redistribution, the postharvest performance of several inflorescence-attached buds and inflorescence-detached buds was compared at the time of anthesis of the most mature floral bud of the inflorescence. Detachment of buds increased failure of opening in small buds, whereas in the largest buds tepal size at anthesis and longevity were improved. In lily inflorescences apparently postharvest translocation of substrate from the basal to the upper buds takes place. Five bud classes have been characterised, comparing the postharvest performance of attached and detached buds. Classes were based on differences in tepal growth rate, absolute growth and the potential to reach anthesis. A bud length of about 60 mm at harvest, appeared to be critical for reaching anthesis of detached buds. Comparable bud development and flower longevity of attached and detached floral buds was determined in buds of 70-75 mm. At this bud length the total carbohydrate content (fructose, glucose, glycerol glucoside, starch, sucrose) covered about three-fourths of the total tepal carbohydrate content found in the largest bud stage just prior to anthesis. Per cultivar, postharvest flower longevity after anthesis of detached buds was well correlated with total carbohydrate content of the tepals at harvest. Longevity of attached flowers remained constant within the inflorescence, likely due to postharvest redistribution of tepal carbohydrate. These findings indicate an important role for tepal carbohydrate content in postharvest bud development and flower longevity of Asiatic hybrid lilies. Carbohydrate redistribution is suggested to play a major role in the postharvest performance of Asiatic lily inflorescences.

Introduction

Unlike most other horticultural crops, cut flowers are usually harvested before full development. Particularly in case of inflorescence-type flowers, which consist of several buds differing in stage of development, a large part of the floral buds is usually still in a premature developmental stage at harvest.

The formation of a mature flower depends on carbohydrate supply. Before harvest, inflorescences are supplied with carbohydrates by photosynthesis, which occurs in the green organs of the plant. After reaching the inflorescence these assimilates are distributed among the various floral buds. The proportion of assimilate uptake in each bud is correlated with the organ sink strength, which largely depends on the rate of utilization of imported assimilates in the sink tissue (Ho, 1988).

Postharvest light intensity is usually low and, therefore, the production of carbohydrates by photosynthesis of cut flowers generally is negligible. Because the amount of carbohydrate in cut flowers is limited, competition for carbohydrate among developing buds within the inflorescence may occur. A deficiency in carbohydrate reserve in the inflorescence may result in failure of bud opening, starting in the smallest bud stages as is hypothesized to take place in inflorescence-type bulbous species like *Freesia* (Spikman, 1989) and *Gladiolus* (Serek et al., 1994). Therefore, understanding carbohydrate metabolism in the flower requires that the development of the surrounding floral buds in the inflorescence is taken into account in terms of competition for carbohydrate import.

In *Lilium*, flower buds express a high sink strength throughout their development until anthesis (Wang and Breen, 1986b), probably correlated with the increase in growth of floral tissue, occurring after the anthers have reached the sporal mitosis stage (Clément et al., 1996). Variation in individual flower longevity within the lily inflorescence is relatively small despite large differences in developmental stage of the floral buds at harvest (Van der Meulen-Muisers and Van Oeveren, 1996; Van der Meulen-Muisers et al., 1998). In a previous paper, was demonstrated that preharvest floral bud reduction increases individual flower longevity in *Lilium* after harvest (Van der Meulen-Muisers et al., 1995a). The effects of preharvest floral bud reduction on flower longevity were probably mainly due to a reduction of the number of competitive sinks within the inflorescence after harvest. The role of carbohydrate redistribution in postharvest development of lily flowers might, therefore, be important.

The purpose of the present paper is to study flower carbohydrate metabolism during postharvest floral bud development by quantifying the development of the tepals and to correlate the results with their carbohydrate content. First the potential postharvest floral bud development was studied, and next, soluble and insoluble tepal sugar contents were assayed at several stages of bud development. Ultimately,

we attempted to determine the importance of carbohydrate redistribution in relation to flower development by comparing the postharvest performance of inflorescence-attached buds and inflorescence-detached buds. To affirm our hypothesis about the importance of carbohydrate redistribution in inflorescence-type cut flowers, we focussed on the postharvest flower development of three lily cultivars differing in individual flower longevity.

Materials and Methods

Bulbs of Asiatic lily hybrids (*Lilium* L.), 12-16 cm in circumference, were obtained from commercial growers in The Netherlands and from the CPRO-DLO (part of the current Plant Research International) lily collection. Three cultivars, Bright Beauty, Fashion, and Orlito were used. The choice of the cultivars is based on known differences in individual flower longevity (Van der Meulen-Muisers et al., 1998). Before planting, the bulbs were stored in moist peat at -2°C for about 8 months.

Standardized conditions were used during forcing, harvest and postharvest (Van der Meulen-Muisers and Van Oeveren, 1997). Plants were forced in a growth chamber at 17°C, 60% relative humidity (RH), and a 16h photoperiod. Photosynthetically active radiation (PAR) (400-700 nm) at the top of the plants, was kept at a photosynthetic photon flux density (PPFD) of about 112 $\mu mol.m^{-2}.s^{-1}$ using high-pressure metal halide lamps (HPI-T 400W, Philips).

Inflorescences were harvested at anthesis of the most mature floral bud by cutting the stems at the soil level within four hours after onset of the photoperiod. Tepal length was measured and subsequently, the buds were divided into groups differing by 5 mm increments in length. In addition, open flowers were included. When tepals were to be studied, they were excised from the inner whorl of the flower head at harvest.

Cut inflorescences and cut individual buds were both placed in glass flasks containing tap water, and were held at 17°C, 60% RH, and a 12h photoperiod. PAR (400-700 nm) was kept at a PPFD of about 14 $\mu mol.m^{-2}.s^{-1}$ using fluorescent lamps (TL-D84 36 W, Philips).

Tepal length of both inflorescence-attached and inflorescence-detached floral buds was recorded until anthesis, as a parameter for tepal growth. From each available developmental stage at harvest eight flowers were studied. Flower longevity was recorded as the time between anthesis and visual withering of the tepals. Each individual floral bud and flower were observed daily within 4 to 6 hours after onset of the photoperiod.

From each available developmental stage at harvest, twelve flowers were sampled. One tepal (inner whorl) of each flower was weighed to determine tepal

fresh weight, immersed in liquid nitrogen, freeze-dried and reweighed to determine tepal dry weight. Fresh weight/dry weight ratios were calculated.

Of the twelve flowers the freeze-dried tepals of three different flowers were pooled before grinding to provide four replicate samples of three tepals each. Using 10 mg of the powder, sugars were extracted in 80% methanol (76°C) for 15 min. Before extraction raffinose was added to the 80% methanol as the internal standard. After centrifugation the pellet was stored for starch analysis. The supernatant was vacuum-evaporated and its residue was taken up in 1 ml purified water (Milli-Q purification system, Millipore, Molsheim, France). After proper dilution the samples were injected in a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA). The HPLC was equipped with a CarboPac PA1 column and a pulsed-amperometric detection system with an Au working electrode and an Ag/AgCl reference electrode. Peaks were identified by comparing their retention times with the retention times of a mixture of standard sugars (De Bruijn et al., 1997). A peak that eluted before glucose has been identified as glycerol glucoside, and could be quantified by using the response factor of glucose after dividing it by 1.5 (U. Van Meeteren and A.C. Van de Peppel, personal communication). Total soluble carbohydrate was calculated by summing glucose, fructose, sucrose and glycerol glucoside.

Preliminary analysis ascertained that Asiatic lily tepal tissue did not contain any fructans, and that the storage carbohydrate in the developing floral buds was starch. Starch determination was performed on the tissue pellet that remained after soluble carbohydrate extraction, using a commercial starch determination kit (Boehringer, Mannheim, Germany) according to the protocol of the supplier.

Completely randomized designs were used. Data were analyzed by analysis of variance, using the Genstat 5 statistical package (Rothamsted, U.K.). Correlation coefficients of linear regression (r) were calculated to look for associations between tepal carbohydrate content and flower longevity.

Results

Bud growth. Postharvest growth of inflorescence-attached and inflorescence-detached buds was similar for the three cultivars tested, and is demonstrated for 'Orlito' in Figure 6.1. In buds reaching anthesis postharvest bud growth proceeded nearly linear in time. In buds that failed to open, postharvest bud growth was halted prematurely. In both inflorescence-attached and inflorescence-detached buds, tepal length at anthesis slightly decreased with developmental stage at harvest. At anthesis, the tepal length exceeded 75 mm in all three cultivars tested.

At harvest (at the time of anthesis of the most mature floral bud), tepal length distribution within the inflorescences varied per cultivar, with a minimal length of 30 mm ('Bright Beauty', 'Orlito') or 45 mm ('Fashion') and a maximal tepal length in the
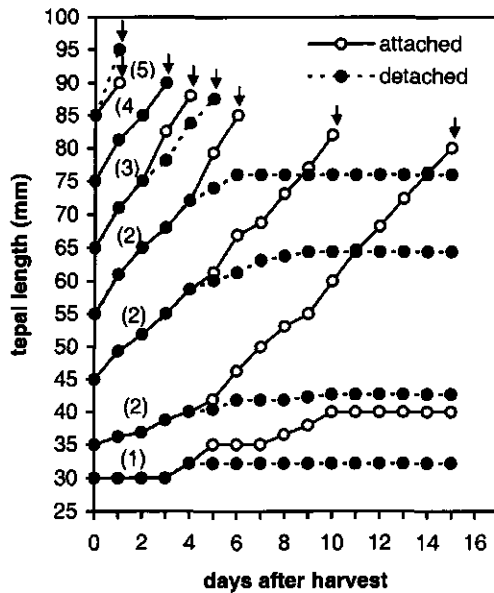
Fig. 6.1 Postharvest development of inflorescence-attached (open symbols) and inflorescence-detached (closed symbols) flower buds of the Asiatic hybrid cultivar Orlito, harvested at the time of anthesis of the most mature floral bud. Five bud classes were distinguished, comparing the postharvest performance of detached buds with attached buds of initially the same size. Class numbers are presented in parentheses: (1) Both attached and detached buds: little tepal growth, no anthesis; (2) Detached buds: lower tepal growth rate and smaller absolute growth than attached buds, no anthesis. Attached buds: anthesis; (3) Detached buds: lower tepal growth rate and equal absolute growth compared to attached buds. Both attached and detached buds: anthesis; (4) Both attached and detached buds: comparable tepal growth rate, anthesis, comparable absolute growth; (5) Detached buds: higher tepal growth rate and greater absolute growth than attached buds. Both attached and detached buds: anthesis. Arrows indicate open flowers; n = 8 flowers per treatment combination.

open flower of 85 mm ('Bright Beauty', 'Fashion') or 90 mm ('Orlito'). The study of the postharvest development of inflorescence-detached and inflorescence-attached flower buds made it possible to determine five classes. Classes were based on differences in tepal growth rate, absolute growth and the potential to reach anthesis (Fig. 6.1, Table 6.1).

All inflorescence-attached buds reached anthesis, except for buds of class 1. After detachment both buds of class 1 and 2 did not complete their development. Within buds of class 2 the growth rate of the tepals in detached buds was lower than the growth rate of attached buds, from three ('Bright Beauty') or four ('Fashion', 'Orlito') days after harvest. Of the detached buds reaching anthesis (class 3 to 5) only buds of class 4 completed their development in the same way as matching

inflorescence-attached buds. Within class 3 anthesis occurred later in time, whereas in class 5 the tepals of the resulting flowers were larger in size. In class 3 the growth rate of the tepals of detached buds was lower compared to the growth rate of the tepals of attached buds, from two days after harvest independent of the cultivar tested (Fig. 6.1. e.g. 'Orlito').

Anthesis. In 'Fashion' and 'Orlito' respectively 100 and 96 percent anthesis was obtained in inflorescence-attached flowers, whereas in inflorescence-attached flowers of 'Bright Beauty' 81 percent of the floral buds reached anthesis. After detachment, the percentage of floral buds that reached anthesis decreased 20 ('Fashion') to 48 percent ('Orlito'), (data not shown). For all three cultivars tested, a bud length of about 60 mm at harvest appeared to be critical for reaching anthesis for a bud detached from the inflorescence (Table 6.1).

Table 6.1 Distribution of five classes of flower buds (as defined in Figure 6.1) and open flowers at harvest (OF), within lily inflorescences of 'Bright Beauty', 'Fashion' and 'Orlito'. Classes were distinguished comparing the postharvest development of inflorescence-attached lily flower buds with inflorescence-detached buds of initially the same tepal length at harvest of the inflorescence.

Genotype	Class number												
	Tepal length of floral buds at harvest (mm)												
	30	35	40	45	50	55	60	65	70	75	80	85	90
Bright Beauty	1	1	1	2	2	2	2/3	3	4	4	5	OF	.*
Fashion	.	.	.	2	2	2	2/3	3	4	5	5	OF	.
Orlito	1	2	2	2	2	2	2/3	3	3	4	5	5	OF

* . = Tepal length not present at harvest of the inflorescence.

Flower longevity. Longevity of attached flowers remained nearly constant within the inflorescence independent of the developmental stage of the floral bud at harvest. After anthesis, longevity of inflorescence-detached flowers increased with the progression of the stage of development at harvest (Fig. 6.2). Longevities of the two largest bud stages and of the open flower were significantly improved ($P = 0.05$) in inflorescence-detached flowers compared with those of inflorescence-attached flowers in all three cultivars (Fig. 6.2). Detaching open flowers at harvest improved their longevity by about 14-18 percent depending on cultivar.

Tepal weight. Tepal fresh weight and dry weight increased almost proportionally with tepal length until the largest bud stage before anthesis. As a consequence, the tepal fresh weight/dry weight (fw/dw) ratio remained almost constant within the inflorescence (Fig. 6.3). Maximum fresh weight occurred in tepals of fully opened
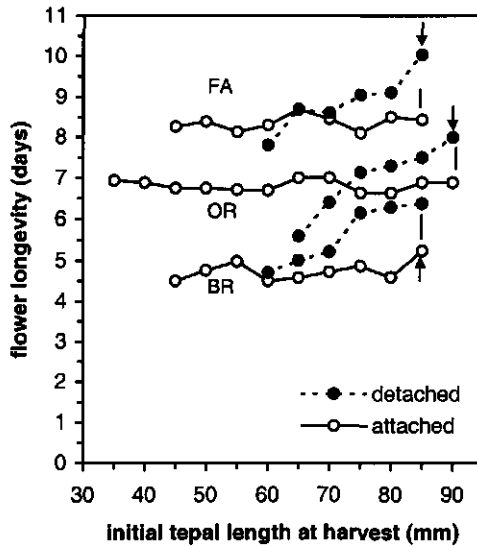
Fig. 6.2 Individual flower longevity (in days) of inflorescence-attached flowers (open symbols) and inflorescence-detached flowers (closed symbols) of 'Fashion' (FA), 'Orlito' (OR) and 'Bright Beauty' (BR), in relation to their initial tepal length (in mm) at harvest. Arrows indicate already open flowers at harvest per cultivar. Bars indicate least significant differences between means (LSD) at P = 0.05; n = 8 flowers per treatment combination.

flowers in all three cultivars tested. Maximum dry weight occurred at anthesis ('Orlito') or at the largest bud stage just before anthesis ('Bright Beauty', 'Fashion') (data not shown). Between the largest floral bud before anthesis and the open flower (a 24h time lapse in development) an increase in the fw/dw ratio occurred (Fig. 6.3). Tepal fw/dw ratios were similar for the three cultivars tested (Fig. 6.3).

Tepal carbohydrate. The total carbohydrate content (soluble carbohydrate and starch) of lily tepals gradually increased with the developmental stage of the floral buds (Fig. 6.4 e.g. 'Orlito'). Glycerol glucoside was the major tepal carbohydrate in the smaller bud stages up to about 55 mm; it remained constant or slightly decreased through all stages of bud development. From a bud length of about 55 mm, starch became the major tepal carbohydrate. Tepal starch content increased with bud development to a maximum value at the largest bud stage before anthesis and then declined to low levels at bud opening. Amounts of glucose and fructose were nearly identical through all stages of bud development, and gradually increased with the developmental stage of the floral buds. When starch content declined near anthesis, glucose and fructose content rapidly increased in 'Orlito' (Fig. 6.4, 6.5). In 'Bright Beauty' and 'Fashion', the increase in glucose and fructose with the decrease of
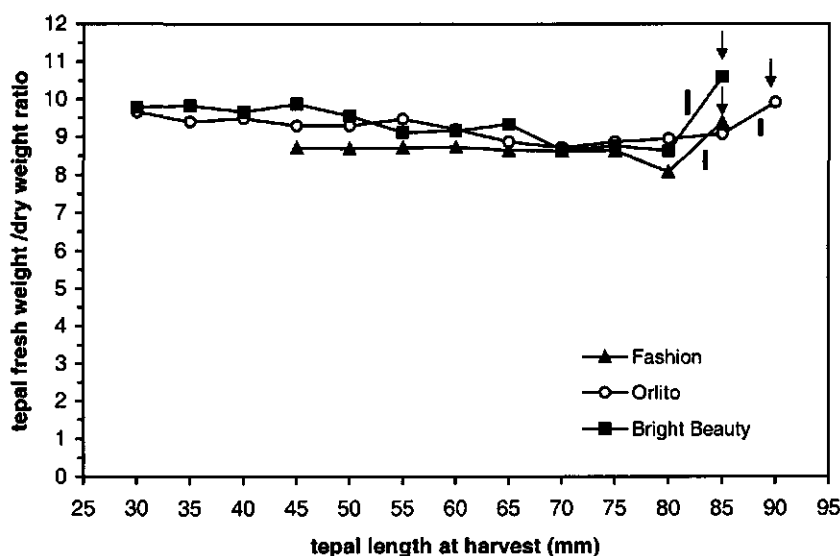
Fig. 6.3 Tepal fresh weight/dry weight ratio of flower buds of 'Fashion', 'Orlito' and 'Bright Beauty' at the stages of development present at the time of anthesis of the most mature flower bud. Developmental stages are defined by the tepal length of the flower buds (in mm). Arrows indicate open flowers. Bars indicate least significant differences between means (LSD) at P = 0.05; n = 12 flowers per treatment combination.

starch at bud opening was less profound, since total tepal carbohydrate content decreased (Fig. 6.5). Tepal sucrose content gradually increased with bud development, but remained at relatively low values (about 7% of the total tepal carbohydrate) for all the bud stages, with an increase to 12-15% in the open flower (Fig. 6.4, 6.5).

Flower development as related to tepal carbohydrate content. At a bud length of about 60 mm, the critical stage of detached buds to reach anthesis (Table 6.1), the total carbohydrate content (soluble sugar and starch) amounted to 12 mg ('Bright Beauty') to 14 mg ('Fashion') per tepal. At this bud length there was also a color change from greenish to the true flower color in each cultivar (data not shown). At a bud length of 70 mm ('Bright Beauty', 'Fashion') or 75 mm ('Orlito'), attached and detached buds showed a similar growth pattern towards anthesis and a comparable final tepal length (Table 6.1, group 4). After anthesis of those buds, longevities of inflorescence-attached and inflorescence-detached flowers were non-significantly different (Fig. 6.2). At this bud length total tepal carbohydrate content amounted to 21-25 mg, covering 68 ('Fashion') to 78 ('Bright Beauty') percent of the total tepal carbohydrate content found in the largest bud stage just prior to anthesis.
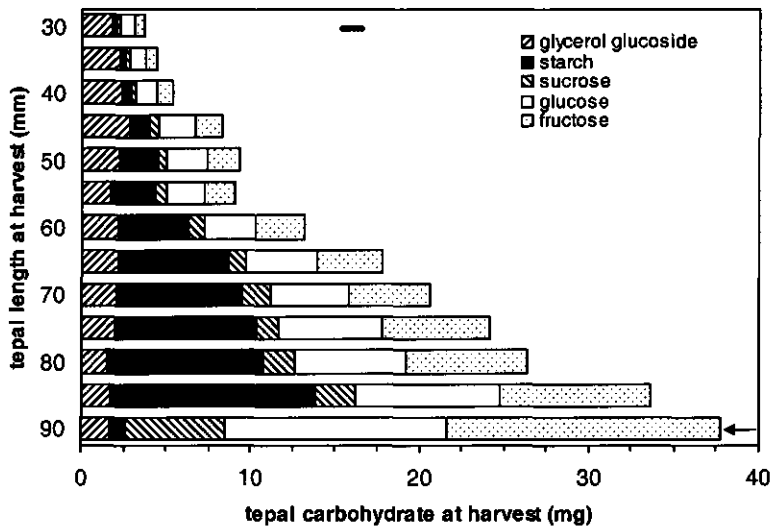
Fig. 6.4 Carbohydrate composition in tepals of 'Orlito' at the stages of development present at anthesis of the most mature flower bud. Developmental stages are defined by the tepal length of the flower buds (in mm) and are arranged in decreasing order, comparable with their location within the inflorescence. Arrow indicates open flower. Small bar indicates least significant difference between means (LSD) at $P = 0.05$; n = 4 samples per treatment combination.
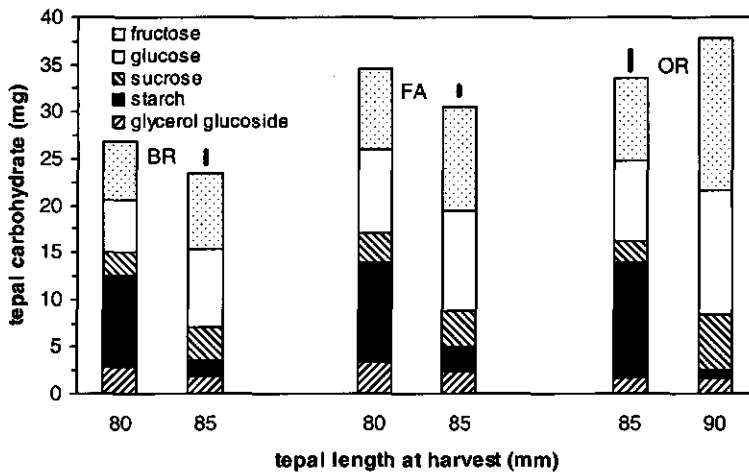

Fig. 6.5 Carbohydrate composition in tepals of the largest bud (left bar) and open flower (right bar) of 'Bright Beauty' (BR), 'Fashion' (FA) and 'Orlito' (OR). Small bars indicate least significant differences between means (LSD) at $P = 0.05$; n = 4 samples per treatment combination.
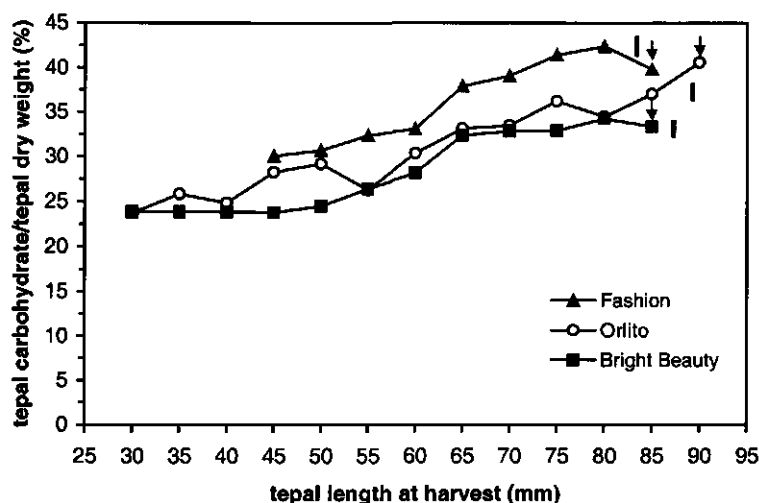
Fig. 6.6 Tepal carbohydrate status of lily flower buds of 'Bright Beauty', 'Fashion' and 'Orlito' at the stages of development present at the time of anthesis of the most mature flower bud. Developmental stages are defined by the tepal length of the flower buds (in mm). Arrows indicate open flowers. Bars indicate least significant differences between means (LSD) at $P = 0.05$; $n = 4$ samples per treatment combination.

Total tepal carbohydrate expressed as a percentage of the dry-weight increased with the stage of development of the floral buds (Fig. 6.6). Between the largest bud stage and the open flower the percentage of total tepal carbohydrate increased in 'Orlito', and slightly decreased in 'Bright Beauty' and 'Fashion' (Fig. 6.6). At anthesis, carbohydrates accounted for 33 to 41 percent of the tepal dry weight depending on the cultivar (Fig. 6.6).

Flower longevity of inflorescence-detached buds that reached anthesis was positively correlated with the total carbohydrate content of the tepals at detachment, for all three cultivars tested (Fig. 6.7). For all cultivars taken together, the association was moderately high ($r = 0.62$). No association was found between longevity of inflorescence-attached flowers and total carbohydrate content of the tepals at the stage the inflorescence was harvested, as flower longevity of attached flowers hardly changed within the inflorescence (Fig. 6.2).

Discussion

The postharvest development of inflorescence-attached lily floral buds follows a clearly defined pattern that hardly changes with the position of the bud on the stem increase in bud size (tepal length [Fig. 6.1], fresh weight and dry weight), and a
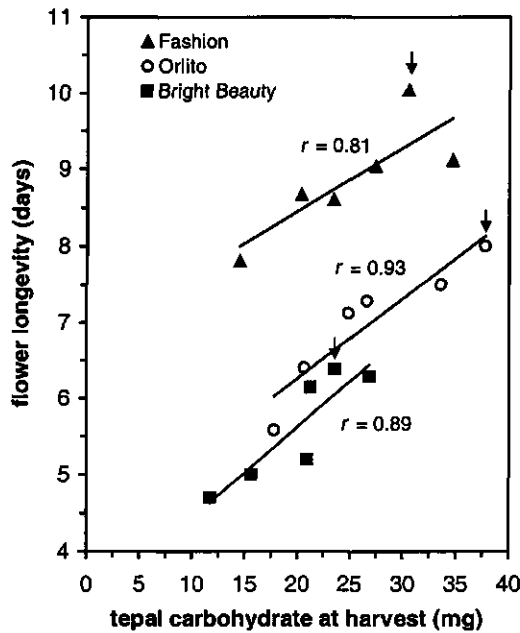
Fig. 6.7 Relation between longevity of 'Fashion', 'Orlito' and 'Bright Beauty' flowers and total tepal carbohydrate content at the time of detachment of the buds. Buds were detached from the inflorescence at several stages of development, present at the time of anthesis of the most mature flower bud. Arrows indicate open flowers at harvest; r = correlation coefficient of linear regression.

as illustrated for 'Orlito' in Figure 6.1. Lily flower maturation is characterized by an change in the pigmentation of the tepals (data not shown). Soluble carbohydrate levels and starch increase until anthesis (Fig. 6.4, e.g. 'Orlito'). At anthesis, tepal starch shows a decrease with a subsequent increase in the soluble carbohydrate levels (Fig. 6.5) and an increase in water content leading to an increase in fw/dw ratio (Fig. 6.3). Within inflorescences harvested at anthesis of the most mature floral bud nearly all buds reach anthesis, as reported before (Van der Meulen-Muisers and Van Oeveren, 1997). After anthesis, postharvest senescence of inflorescence-attached lily flowers hardly changes with the position of the flower on the stem resulting in a constant longevity level within the inflorescence (Fig. 6.2).

Data on inflorescence-detached floral buds showed that preventing translocation of metabolites within the inflorescence altered the pattern of postharvest maturation (Fig. 6.1, e.g. 'Orlito') and senescence (Fig. 6.2) in nearly all buds. Detachment increased failure of bud opening compared to inflorescence-attached buds from the smallest bud sizes up to a size of about 60 mm. At the same time, in the largest bud stages tepal size at anthesis (Fig. 6.1, e.g. 'Orlito') and longevity (Fig. 6.2) were

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improved in inflorescence-detached flowers compared to inflorescence-attached flowers. These results suggest that within cut lily inflorescences the developing buds up to about 60 mm in size are strong sinks throughout their growth. The demands of those sinks are, at least partially, accounted for by translocation of metabolites from the larger flower buds (and open flower) reducing their tepal length at anthesis and/or their longevity. However, the smallest buds sizes in 'Bright Beauty' (30-40 mm) and to a lesser extent in 'Orlito' (30 mm) appeared to be weak sinks. Their growth was minimal when attached to the inflorescence and they failed to open (Table 6.1, class 1). Their sink strength might be insufficient to compete with the other developing buds for substrate provided by the open flower and the largest bud stages within the inflorescence. In 'Fashion' no such small buds were present at harvest (Table 6.1), mainly due to cultivar differences in flower bud distribution as reported before (Van der Meulen-Muisers and Van Oeveren, 1996). For buds up to 60 mm, developmental differences between inflorescence-attached and inflorescence-detached flower buds became visible about three to four days after harvest (Fig. 6.1, e.g. 'Orlito'). Within the inflorescence, translocation of substrate from the basal to the upper buds apparently starts near this point of time.

The stage of maturity of the buds is marked by the content of carbohydrate of the tepals, more mature buds containing more tepal carbohydrate (Fig. 6.4, e.g. 'Orlito'). Failure of bud opening in lily could be due to lack of carbohydrate as discussed in other inflorescence-type bulbous species such as *Freesia* (Spikman, 1989) and *Gladiolus* (Serek et al., 1994). In lily, failure of bud opening is known to increase when inflorescences are harvested in a less mature stage (Van der Meulen-Muisers and Van Oeveren, 1997). From the data presented above can be concluded that harvesting inflorescences in a less mature stage will considerably reduce the carbohydrate content available for redistribution from the larger to the smaller buds.

In buds of 70 mm ('Bright Beauty', 'Fashion') or 75 mm ('Orlito'), with a tepal carbohydrate content of 21-25 mg, comparable bud development and flower longevity of inflorescence-attached and inflorescence-detached floral buds was determined (Table 6.1, Fig. 6.1, 6.2). Those carbohydrate amounts cover 68 ('Fashion') to 78 ('Bright Beauty') percent of the total tepal carbohydrate content found in the largest bud stage just prior to anthesis, indicating a carbohydrate surplus in tepals of the most mature floral bud stages and in tepals of the open flower. In inflorescence-attached floral buds at least part of the carbohydrate surplus will likely be available for redistribution to smaller buds. As discussed above, redistribution will lead to an increase in the number of buds that will reach anthesis (Table 6.1, Fig. 6.1 e.g. 'Orlito'), comparing inflorescence-attached and inflorescence-detached floral buds. At the same time, longevity of the flowers obtained from the largest bud sizes and of the open flower at harvest will decrease in inflorescence-attached flowers compared to inflorescence-detached flowers (Fig. 6.2). The relatively smallest carbohydrate surplus (22%) was found in tepals of the cultivar with the shortest

flower longevity ('Bright Beauty'), and the relatively largest carbohydrate surplus (32%) was found in tepals of the cultivar with the longest flower longevity ('Fashion'). This could be an important factor in the search for an explanation of the existence of cultivar differences in flower longevity.

The gradual increase of the total carbohydrate/dry weight ratio with developmental stage of the buds indicates carbohydrate filling of the tepals as they develop (Fig. 6.6). Near anthesis total tepal carbohydrate in percentage of dry-weight appeared to amount to 33-41 percent, comparable with the value that can be derived from data on *Gladiolus* flowers (Yamane et al., 1993). Anthesis after detachment of floral buds appeared to be dependent on the stage of maturity of the buds at harvest. To obtain anthesis, a bud length of about 60 mm, concomitant with 12-14 mg carbohydrate content per tepal, appeared to be critical (Table 6.1; Fig. 6.4, e.g. 'Orlito').

Reducing sugars appeared to form a large proportion of the carbohydrate pool in the tepals (Fig. 6.4, 6.5). This supports the view that the floral buds are active metabolic centers, since the translocation sugar is probably sucrose. The nearly identical amounts of fructose and glucose within lily tepals (Fig. 6.4, 6.5) suggest that sucrose is probably rapidly converted by invertase at its site of accumulation, a characteristic which is specific for sink organs (Avigad, 1982).

Anthesis appeared to be accompanied by hydrolysis of starch leading to an increase in sugar content (Fig. 6.5). Similar interactions between starch and sugar content have been reported during expansion of rose petals (Evans and Reid, 1988) and *Alstroemeria* petals (Collier, 1997). Besides supplying substrate for respiratory processes, starch hydrolysis especially provides osmotic solutes for water influx and cellular expansion. The tepal fresh weight/dry weight ratio, an indirect measure of the proportion of dry weight that consists of asthmatically active solutes (Evans and Reid, 1988), increased at anthesis (Fig. 6.3). This shows that part of the tepal expansion was due to an increased water uptake.

The increase in sugar content near anthesis did not quantitatively correspond with the disappearance of starch (Fig. 6.5). Within 'Orlito' total tepal carbohydrate content increased, whereas in 'Bright Beauty' and 'Fashion' it decreased at the time of starch hydrolysis. These data suggest that at anthesis both net-import and net-export of carbohydrates can take place in lily tepals, depending on the cultivar.

Tepals of developing lily buds contained, in addition to glucose, fructose, sucrose and starch, glycerol glucoside (Fig. 6.4, 6.5). Glycerol glucosides seem to be characteristic constituents of the genus *Lilium* and their structures have been reported to vary depending on the species (Kaneda et al., 1974; 1982; 1984). The glycerol glucoside detected in our study could be lilioside C, isolated from *Lilium tigrinum* (syn. *Lilium lancifolium*) (Kaneda et al., 1982) one of the ancestors of the Asiatic hybrids. Tepal glycerol glucoside was especially a dominant component at the youngest bud stages, while its content hardly changed with the developmental stage

of the floral buds. Glycerol glucoside is, therefore, not likely to play an important role in the carbohydrate metabolism of lily flowers.

Per cultivar no association was found between longevity of inflorescence-attached flowers and total tepal carbohydrate content at harvest. This was likely due to postharvest redistribution of tepal carbohydrate present at harvest, leading to constant flower longevity within the inflorescence. Despite its limited range flower longevity after detachment was well correlated with total carbohydrate content of the tepals per cultivar (Fig. 6.7). A moderate association ($r = 0.62$) between flower longevity and tepal carbohydrate was found using the unified data of all three cultivars. Apparently besides tepal carbohydrate content also other factors account for cultivar differences in lily flower longevity.

Asiatic lily inflorescences offer an interesting model system for studies on postharvest flower development, because individual flowers provide a graded series of stages of development in an identical genetic and environmental background. Furthermore, cultivars differing in flower longevity correspond in a similar way. A dominant role of tepal carbohydrate content in bud development and flower longevity of Asiatic lilies can be proposed from the findings of the present study. Further studies on factors like e.g. carbohydrate degradation and redistribution in lily flowers and inflorescences are needed for a better understanding of cultivar differences in the relationship between carbohydrates and longevity of Asiatic hybrid lilies.

Acknowledgements

Chapter 7

Postharvest flower senescence in Asiatic hybrid lilies as related to tepal carbohydrate status

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and J.M. van Tuyl

Abstract. In order to determine the importance of carbohydrate redistribution in the regulation of senescence in inflorescence-type flowers, the role of sugars during the senescence of *Lilium* flowers was characterised in three Asiatic hybrid cultivars differing in individual flower longevity. During the postharvest senescence of inflorescence-attached flowers a sharp fall occurred in tepal carbohydrate content of the bottom flower two days before visual withering, that coincided with the increase in sink strength in the developing buds of the same inflorescence. This change in sink strength might trigger the bottom flower to become a carbohydrate source, and so determine its life span. Preventing distribution of metabolites within the inflorescence by detaching flowers reduced tepal dry weight loss and tepal carbohydrate loss, whereas senescence was postponed for about two days compared to inflorescence-attached flowers. From these findings it was concluded that tepal carbohydrate content is a key factor in lily flower senescence. Although the presence of developing buds in the inflorescence considerably influenced flower longevity of inflorescence-attached flowers, it did not determine the existing differences in flower longevity between cultivars. Detachment increased flower longevity for each cultivar in a similar way, maintaining cultivar differences in flower longevity. It was concluded that redistribution of carbohydrates was not the only mechanism regulating flower senescence in *Lilium*.

Introduction

Generally, senescence and subsequent wilting of the petals determine flower longevity. One of the important factors for delaying petal senescence in flowers is the maintenance of their carbohydrate pool (Coorts, 1973). Various possible effects of carbohydrate in delaying petal senescence are reported. Nichols (1973) assumed that their effect on lowering of the osmotic potential or as a respiratory substrate was involved. Furthermore, carbohydrates can have a protective effect on membrane integrity, they may maintain protein synthesis (Halevy and Mayak, 1979) and regulate gene expression (Chevalier et al., 1996; Davies et al., 1996).

Especially in case of cut flowers the beneficial effect of additional sugar on flower longevity can be easily demonstrated as their carbohydrate pool is limited because photosynthesis is negligible (Coorts, 1975; Rogers, 1973). The size of this petal carbohydrate pool in senescing cut flowers is affected by the rate of hydrolysis of starch and other polysaccharides, translocation to the petals, respiration and translocation out of the flower to other plant parts (Halevy and Mayak, 1979). The latter is particularly the case in inflorescence type flowers that usually contain several flowers in different stages of development. In carnation also translocation within the flower from the petals to the ovary was reported (Nichols and Ho, 1975).

In *Lilium*, an inflorescence-type bulb flower, both flower bud development and flower senescence occur within the same inflorescence. Despite large differences in developmental stage of the buds at harvest, the development and senescence of flowers attached at different positions on an inflorescence are similar after harvest (Van der Meulen-Muisers et al., 1998), indicating an important role for carbohydrate redistribution in postharvest development of lily flowers (Chapter 6). Therefore, understanding the role of carbohydrate during the senescence of individual flowers requires that the development of the other floral buds in the inflorescence is taken into account in terms of competition for nutrients.

In this paper the role of carbohydrates in inflorescence-type flower senescence is analysed. Physiological and biochemical changes accompanying postharvest flower senescence in inflorescence-attached *Lilium* flowers are described and compared with changes occurring in inflorescence-detached flowers to reveal the importance of competition for nutrients within the inflorescence in individual flower senescence. To confirm our hypothesis about the importance of tepal carbohydrate redistribution in inflorescence-type flower senescence, we analyzed the postharvest flower senescence of three lily cultivars differing in individual flower longevity.
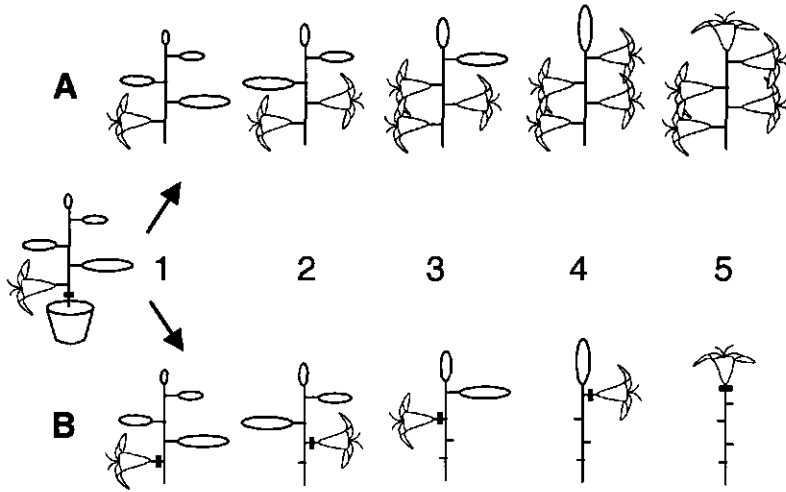
Fig. 7.1 Schematic representation of the treatment of inflorescence-attached (**A**) and inflorescence-detached (**B**) flowers in time: (1) Inflorescence at time of harvest, anthesis of bottom flower, other flowers in various stages of development. For the detached-flower analysis the bottom flower was detached at this stage; (2-5) Buds continue development at the harvested inflorescence. When the second (third, fourth, etc.) flower reaches anthesis, it is detached for the detached-flower analysis.

Materials and Methods

Bulbs of Asiatic lily hybrids (*Lilium* L.), 12-16 cm in circumference, were obtained from commercial growers in The Netherlands and from the CPRO-DLO (current Plant Research International) lily collection. Three cultivars, Bright Beauty, Fashion, and Orlito were used. The choice of the cultivars is based on known differences in individual flower longevity (Van der Meulen-Muisers et al., 1998). Before planting, the bulbs were stored in moist peat at $-2°C$ for about 8 months.

Standardised conditions were used during forcing, harvest and postharvest (Van der Meulen-Muisers and Van Oeveren, 1997). Plants were forced in a growth chamber at 17°C, 60% relative humidity (RH), and a 16h photoperiod. Photosynthetically active radiation (PAR) (400 to 700 nm) at the top of the plants, was kept at a photosynthetic photon flux density (PPFD) of about 112 $\mu mol.m^{-2}.s^{-1}$ using high pressure metal halide lamps (HPI-T 400W, Philips).

Inflorescences were harvested at anthesis of the bottom flower (most mature floral bud) by cutting the stems at the soil level within four hours after onset of the photoperiod. Tepal length of flowers and buds was measured at harvest. Open flowers at harvest and floral buds reaching anthesis after harvest either stayed attached to the inflorescence or were detached at anthesis (Fig. 7.1). Both inflorescence-attached and detached flowers were studied.

Cut inflorescences and cut individual flowers were both placed in glass flasks containing tap water, and were held at 17°C, 60% RH, and a 12h photoperiod. PAR (400 to 700 nm) was kept at a PPFD of about 14 μmol.m^{-2}.s^{-1} using fluorescent lamps (TL-D84 36 W, Philips). Flower longevity was recorded as the time between anthesis and visual deterioration of the tepals. Each individual flower was observed daily within 4 to 6 hours after onset of the photoperiod.

To study physiological changes after anthesis, open flowers were sampled by excising tepals from the flower head (inner whorl). From the bottom flower (open at harvest of the inflorescence) samples were taken at anthesis, either 2, 4 or 6 days after anthesis and at the end of flower life, which was marked by visual deterioration of the tepals. From the other flowers samples were taken at anthesis and at the first signs of deterioration. These excised tepals were grouped based on the developmental stage of the bud at harvest of the inflorescence, which was determined by tepal length. Per cultivar flowers of several initially different developmental stages were studied. The developmental stages which were sufficiently available per cultivar could be used. Besides flowers open at harvest of the inflorescence, in 'Bright Beauty' additional flowers were obtained from a bud size of 80, 55 and 45 mm at anthesis of the first flower; in 'Orlito' additional flowers were obtained from a bud size of 80, 60 and 40 mm; in 'Fashion' additional flowers were obtained from a bud size of 80, 55, 50, 45 and 40 mm. Per sampling time and per developmental stage tepals of nine different flowers were used per cultivar.

After excision, each tepal (inner whorl) was weighed to determine tepal fresh weight, immersed in liquid nitrogen, freeze-dried and reweighed to determine tepal dry weight. Fresh weight/dry weight ratios were calculated.

Of the nine flowers the freeze-dried tepals of three different flowers were pooled before grinding to provide three replicate samples of three tepals each. Using 10 mg of the powder, sugars were extracted by 80% methanol (76°C) for 15 min. Before extraction raffinose was added to the 80% methanol as the internal standard. After centrifugation the pellet was stored for starch analysis. The supernatant was vacuum-evaporated and its residue was taken up in 1 ml purified water (Milli-Q purification system, Millipore, Molsheim, France). After proper dilution the samples were injected in a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA). The HPLC was equipped with a CarboPac PA1 column and a pulsed-amperometric detection system with an Au working electrode and an Ag/AgCl reference electrode. Peaks were identified by comparing their retention times with the retention times of a mixture of standard sugars (De Bruijn et al., 1997). A peak that eluted before glucose has been identified as glycerol glucoside, and could be quantified by using the response factor of glucose after dividing it by 1.5 (U. Van Meeteren and A.C. Van de Peppel, personal communication).

Preliminary analysis ascertained that Asiatic lily tepal tissue did not contain any fructans, and that the storage carbohydrate in the developing floral buds was starch.

Starch determination was performed on the tissue pellet that remained after soluble carbohydrate extraction, using a commercial starch determination kit (Boehringer, Mannheim, Germany) according to the protocol of the supplier. Total tepal carbohydrate was calculated by summing glucose, fructose, sucrose, glycerol glucoside and starch.

Completely randomised designs were used. Data were analysed, using the Genstat 5 statistical package (Rothamsted, U.K.). Correlation coefficients of linear regression (r) were calculated to look for associations between tepal carbohydrate content and flower longevity.

Results

Longevity bottom flower. Detaching open flowers from the inflorescence at the time of harvest, caused a lag in their time course of senescence of about 2 days compared to inflorescence attached flowers (Fig. 7.2 arrows). Flower longevity differed between different cultivars and was the shortest in 'Bright Beauty' and the longest in 'Fashion'. Visual deterioration of the tepals was determined by withering in inflorescence attached flowers, whereas in inflorescence-detached flowers tepal tissue became water-soaked.

Tepal weight bottom flower. From anthesis to the first visual signs of deterioration of the tepals, tepal dry weight declined in both inflorescence attached and detached flowers, with an additional sharp decrease in attached flowers from about 2 days ('Bright Beauty'), 4 days ('Orlito') or 6 days ('Fashion') upon anthesis (Fig. 7.2). Depending on cultivar, total tepal dry weight loss during senescence amounted to about 30-40 mg in attached flowers, about 40% of the tepal dry weight present at anthesis. In detached flowers total tepal dry weight loss during senescence amounted to about 10-20 mg, circa 15-30% of the tepal dry weight present at anthesis depending on the cultivar.

In general, tepal fresh weight decreased with dry weight in inflorescence-attached flowers but it increased in detached flowers. As a consequence, the fresh weight/dry weight (fw/dw) ratios hardly changed in inflorescence-attached flowers, whereas they increased in detached flowers. Cultivar differences in fw/dw ratios were small except for a relatively smaller ratio in detached flowers of 'Fashion'.

Tepal carbohydrate bottom flower. Total tepal carbohydrate content at anthesis ranged from about 35-40% of tepal dry weight (Fig. 7.3), with about 80-95% coming from the three major soluble sugars fructose, glucose, sucrose (Fig. 7.4 e.g. 'Fashion') and with starch and glycerol glucoside covering the remaining percentages (data not shown).

In inflorescence-attached flowers, tepal carbohydrate content declined, with an additional sharp fall from about 2 days ('Bright Beauty'), 4 days ('Orlito') or 6 days
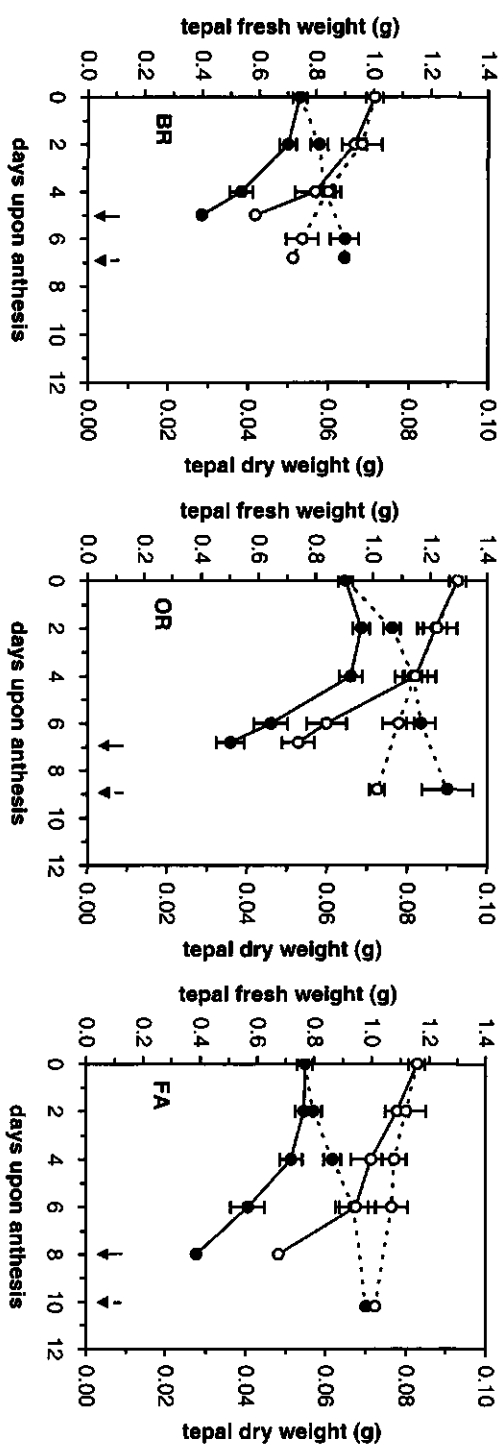
Fig. 7.2 Tepal fresh weight (closed symbols), and tepal dry weight (open symbols) of inflorescence-attached (solid lines) and inflorescence-detached (broken lines) bottom flowers of the Asiatic hybrid lily cultivars Bright Beauty (BR), Orlito (OR) and Fashion (FA), from anthesis until first signs of deterioration (arrows). Flowers had just reached anthesis at the time of harvest of the inflorescence; n=9, bars represent ± SE.
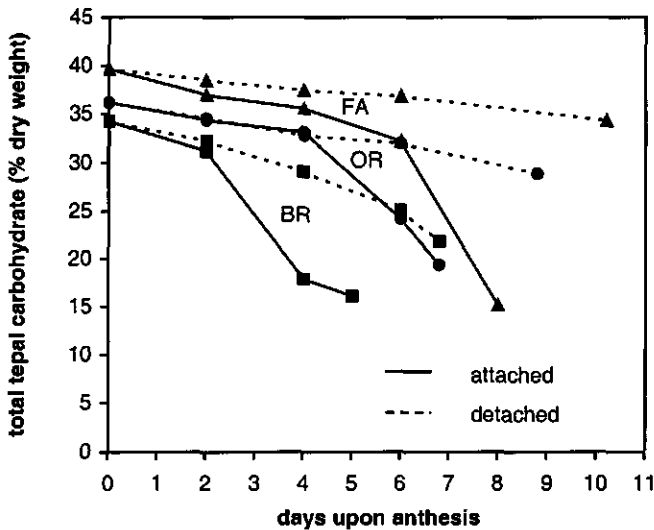
Fig. 7.3 Total tepal carbohydrate content (% dry weight) of inflorescence-attached and inflorescence-detached bottom flowers of the Asiatic hybrid lily cultivars Fashion (FA), Orlito (OR) and Bright Beauty (BR) from anthesis until first signs of deterioration. Flowers had just reached anthesis at the time of harvest of the inflorescence; n=3.

('Fashion') upon anthesis (Fig. 7.3). This sharp fall was mainly accounted for by a decrease in tepal fructose and glucose as demonstrated for 'Fashion' in Fig. 7.4. At the first signs of deterioration differences in total tepal carbohydrate were relatively small for the three cultivars, ranging from 15-19% of tepal dry weight in attached flowers (Fig. 7.3).

Total tepal carbohydrate content gradually declined in detached flowers during senescence, with relatively the largest decline in 'Bright Beauty' (Fig. 7.3). This decline was mainly accounted for by a decrease in tepal fructose and glucose which was partially compensated for by an slight increase in the relative amount of sucrose in tepal dry weight (Fig. 7.4 e.g. 'Fashion'). At the first signs of wilting total tepal carbohydrate content was the lowest in 'Bright Beauty' (22% of tepal dry weight) and the highest in 'Fashion' (35% of tepal dry weight), resulting in larger differences in tepal carbohydrate content between cultivars than were present at anthesis (Fig. 7.3).

Hardly any detectable sugar was recovered from the vase water samples taken from under the senescing detached flowers.

Effect of detaching flowers at anthesis on successive flowers in the inflorescence. Longevity of inflorescence-attached lily flowers hardly changed with their position on the inflorescence (Fig. 7.5A). Detaching successive flowers at their anthesis, as illustrated in Fig. 7.1, caused a gradual decrease in longevity with their position in the inflorescence at harvest (Fig. 7.5B). Longevity of successively detached
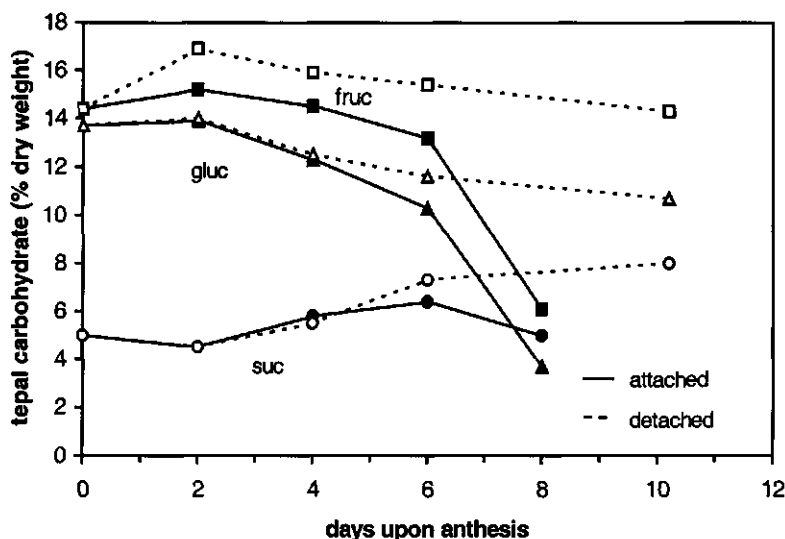
Fig. 7.4 Tepal fructose (fruc), glucose (gluc) and sucrose (suc) (% dry weight) of inflorescence-attached and inflorescence-detached bottom flowers of the Asiatic hybrid lily 'Fashion' from anthesis until first signs of deterioration. Flowers had just reached anthesis at the time of harvest of the inflorescence; n=3.

flowers was improved compared to attached flowers, but it slightly decreased towards the longevity of attached flowers with a higher position in the inflorescence (Fig. 7.5).

Tepal dry weight of flowers detached at anthesis declined from the bottom flower to the upper flower tested. In inflorescence-attached flowers tepal dry weight hardly changed with the position of the flowers on the inflorescence (data not shown).

When flowers were detached from the inflorescence at anthesis, tepal carbohydrate (% dry weight) decreased acropetally with the position of the flower on the inflorescence (Fig. 7.5B). At the first signs of deterioration a similar decrease in total carbohydrate was found, only at a lower level (data not shown). In inflorescence-attached flowers variation in tepal carbohydrate at anthesis was much smaller, and no clear association with flower position was determined (Fig. 7.5A). At the first signs of deterioration total tepal carbohydrate became nearly constant independent of the position of the flower within the inflorescence (data not shown).

Per cultivar, tepal carbohydrate (% dry weight) at anthesis was well correlated with longevity in flowers detached from the inflorescence at anthesis (Fig. 7.5B), whereas no correlation was found between tepal carbohydrate at anthesis and longevity in inflorescence-attached flowers (Fig. 7.5A).
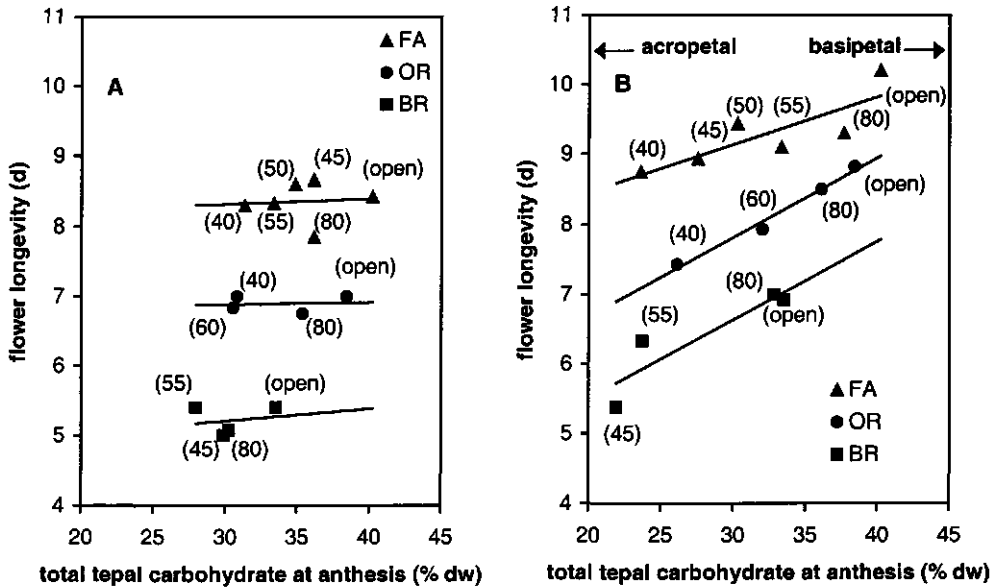
Fig. 7.5 Flower longevity as related to tepal total carbohydrate content at anthesis (% dry weight) of inflorescence-attached (**A**) and inflorescence-detached (**B**) flowers of the Asiatic hybrid lily cultivars Fashion (FA), Orlito (OR) and Bright Beauty (BR). Flowers were grouped based on their developmental stage at harvest of the inflorescence. Per cultivar each data point corresponds with another developmental stage at harvest (tepal length at harvest of the inflorescence between parentheses); n=3; **B**: coefficient of linear correlation (r) is 0.79 (FA), 0.99 (OR), 0.91 (BR).

Discussion

The postharvest senescence of inflorescence-attached lily flowers is characterised by a decrease in tepal fresh weight and tepal dry weight (Fig. 7.2), tepal carbohydrate (% dry weight) (Fig. 7.3) and a change in tepal carbohydrate composition (Fig. 7.4 e.g. 'Fashion'). Tepal dry weight and tepal carbohydrate showed a sharp fall about 2 to 3 days before occurrence of the first signs of deterioration (Fig. 7.2, Fig. 7.3), mainly due to a decrease in reducing sugars (Fig. 7.4 e.g. 'Fashion'). Tepal carbohydrate decreased from 35-40% at anthesis to 15-19% at the first signs of deterioration (expressed on a dry weight basis) (Fig. 7.3). This carbohydrate decrease is comparable with a loss of about 70-80% of the carbohydrate amount present in the tepals at anthesis (data not shown). Dry weight loss amounted to about 40% of the initial tepal dry weight (Fig. 7.2), with about 25 ('Fashion') to 60% ('Bright Beauty') of dry weight loss coming from non-carbohydrate components, e.g. protein (Woodson and Handa, 1987) and organic acids (Woodson, 1987).

Data on flowers detached from the inflorescence at anthesis showed that preventing distribution of metabolites within the inflorescence altered the pattern of postharvest flower senescence. Detachment of the bottom flower reduced its tepal dry weight loss and subsequent tepal carbohydrate loss considerably. Flower longevity increased about 2 days per cultivar. At the first signs of deterioration tepals of detached bottom flowers had lost about 15-30% of their initial tepal dry weight (Fig. 7.2). Their initial tepal carbohydrate decreased from 35-40% at anthesis to 22-35% at the first signs of deterioration (expressed on a dry weight basis) (Fig. 7.3). This decrease in carbohydrate is comparable with a loss of about 25% ('Fashion') to 55% ('Bright Beauty') of the carbohydrate amount present in the tepals at anthesis (data not shown). Losses in inflorescence-detached flowers were far less than those in inflorescence-attached flowers, indicating redistribution of metabolites from the attached bottom flower to other parts of the inflorescence.

The overall loss of tepal carbohydrate in detached flowers could be due to passive loss, redistribution within the flower and/or respiration. Hardly any detectable sugar was recovered from the vase water samples taken from under the senescing detached flowers. So, passive loss of carbohydrate in detached flowers can be considered negligible. Redistribution to the ovary, the most obvious flower part for carbohydrate redistribution within the flower (Nichols, 1976; Nichols and Ho, 1975), is known to increase after pollination. However, no visual signs of ovary swelling, an indicator for pollination, were detected. An environmental temperature of 17°C is known to be too low to obtain (self-)pollination in lily (personal communication J.M. van Tuyl) and no fertilisation was detected using microscopic techniques. Therefore, redistribution of carbohydrates within the flower was considered to be of minor importance. These findings indicate that carbohydrate loss in detached flowers is most likely due to respiration. Respiratory CO_2 production rates, calculated from the data on total carbohydrate loss in detached flowers, amounted to 0.34 ('Fashion'), 0.50 ('Orlito') and 0.95 ('Bright Beauty') μmol/kg/s. These values are in the same range of CO_2 production rates measured during postharvest senescence of several lily cultivars (0.5 to 1.5 μmol/kg/s at 20°C) as reported by Elgar et al. (1999).

Assuming that respiration in inflorescence-attached and detached bottom flowers is comparable and that in both attached and detached flowers redistribution within the flower does not take place, the extra loss of tepal carbohydrate in attached flowers is due to export via the phloem. From these data it can be calculated that phloem export amounts to 30, 40 and 60% of the initial tepal carbohydrate content in 'Bright Beauty', 'Orlito' and 'Fashion', respectively.

The time of the additional fall in tepal carbohydrate in attached bottom flowers, (between 2 to 6 days after anthesis), is consistent with data reported before (Chapter 6). At this time the demand for carbohydrate of developing buds (active sinks) within the inflorescence is increasing. The bottom flower is expected to become a

carbohydrate source, exporting sucrose towards the developing buds in the inflorescence.

The content of the reducing sugars glucose and fructose on a dry weight basis in lily tepals decreased during senescence, but that of sucrose hardly changed (in attached flowers) or slightly increased (in detached flowers) (Fig. 7.4 e.g. 'Fashion'). At the first visual signs of deterioration, tepals of detached flowers had a higher sucrose content than tepals of attached flowers, both on a dry weight basis (Fig. 7.3 e.g. 'Fashion') and on a per tepal basis (data not shown). Apparently, there is synthesis of sucrose in senescing lily tepals, although less clear than reported for daylily, where senesced tepals of detached flowers had about a 5 times higher sucrose concentration compared to senesced tepals of attached flowers (Bieleski, 1995). In inflorescence-attached flowers glucose and fructose are presumably transformed into the transport sugar sucrose which is likely to be exported immediately after synthesis, as its amount in tepal tissue of attached flowers remained nearly constant in time (Fig. 7.4 e.g. 'Fashion'). The synthesis of sucrose becomes clear when this export is blocked, by detaching the flower from the inflorescence, resulting in sucrose accumulation.

Rapid export of carbohydrate from tepals of attached bottom flowers to upper flowers in the inflorescence is accompanied by a decline in tepal fresh weight. The loss of carbohydrate as osmotic component may not only reduce water uptake by tepal tissue, but it may also be accompanied with water loss used to support the phloem stream. Therefore, export of carbohydrate is considered to be crucial for the wilting of the tepals of inflorescence-attached flowers. First visual signs of deterioration in these inflorescence-attached flowers were caused by withering of the tepals. In contrast, in inflorescence-detached flowers carbohydrate content of the tepals only slightly decreased. First visual signs of deterioration in these flowers occurred, when tepal tissue became water-soaked, likely due to the loss of membrane integrity and infiltration of the intracellular spaces of the tepals (Horie, 1961). Apparently carbohydrates play no major role in the protection of the membrane integrity of lily tepals, because a relative large amount of carbohydrate was still present when the first visual signs of tepal deterioration occurred.

Because the sharp fall in tepal carbohydrate in attached flowers coincides with an increase in sink strength of developing buds in the inflorescence (Chapter 6), this change in sink strength might trigger the bottom flower to become a carbohydrate source, and determine its life span. The absence of this putative trigger indeed expanded flower life by about 2 days. Preliminary results indicated that reducing the sink strength by preharvest floral bud reduction increased postharvest flower longevity in Asiatic hybrid lilies (Van der Meulen-Muisers et al., 1995a). As stated above, a sharp fall in carbohydrates may lead to tepal wilting. In case this fall occurred earlier in time, cultivar flower longevity appeared to be shorter in attached flowers (Fig. 7.3). Furthermore, cultivar differences in flower longevity appeared to coincide with

differences in tepal carbohydrate content at anthesis (Fig. 7.3) and with differences in respiratory loss of carbohydrates calculated from carbohydrate loss after detachment (varying from 0.34 $\mu mol.kg^{-1}.s^{-1}$ in 'Bright Beauty' to 0.95 $\mu mol.kg^{-1}s^{-1}$ in 'Fashion'. These data imply that tepal carbohydrate content is a key factor in lily flower senescence.

Detachment of the flowers upon anthesis caused an acropetal decrease within the inflorescence of both tepal carbohydrate content at anthesis and flower longevity, leading to a high correlation between both components per cultivar (Fig. 7.5B). In case flowers stayed attached to the inflorescence, tepal carbohydrate content at anthesis varied less per cultivar and had no clear relation with flower position, whereas flower longevity remained constant within the inflorescence (Fig. 7.5A). Over all three cultivars together the correlation between tepal carbohydrate content and flower longevity in attached flowers was moderate ($r = 0.66$). These results correspond with earlier data on the correlation between tepal carbohydrate content of floral buds and flower longevity upon anthesis (Chapter 6).

Striking differences in first signs of deterioration as well as in the accompanying tepal carbohydrate content between treatments were found. Start of visual wilting of the tepals in attached flowers occurred at a constant amount of tepal carbohydrate independent of the position of the flower in the inflorescence (data not shown), whereas tepal carbohydrate amounts hardly varied between cultivars at the end of flower life (Fig. 7.3). These data could imply a threshold-value of osmotic components at the visual start of wilting. In contrast, in detached flowers tepals became water soaked, whereas tepal carbohydrate content decreased acropetally within the inflorescence at the first signs of deterioration (data not shown). This might be associated with cultivar dependent loss of membrane integrity.

Our data underline the importance of tepal carbohydrates in lily flowers. They play a major role in postharvest flower senescence as discussed above. However, their possible contribution in explaining genetic differences in flower longevity appears to be of minor importance. Although the presence of developing buds (active sinks) in the inflorescence considerably influenced tepal carbohydrate content during flower senescence it did not affect the existing differences in flower longevity between cultivars. Absence of these carbohydrate sinks after detachment of the flowers from the inflorescence increased flower longevity per cultivar in a similar way, maintaining cultivar differences in flower longevity.

It seems that the reason for the existence of cultivar differences in lily flower longevity has to be found within the flower itself. Studies on redistribution from senescing flowers are limited (Borochov and Woodson, 1989). The accent usually lies on the role of ethylene, as in the study by Nichols and Ho (1975) in which ethylene enhanced carbohydrate redistribution from the senescing carnation petals to other flower parts and to other plant parts. In contrast, senescence of lily is hardly affected by ethylene (Woltering and Van Doorn, 1988; Elgar et al., 1999). Bieleski (1995)

suggested a programmed increase in phloem loading capability at the start of senescence in the ethylene-insensitive daylily flower that might be associated with the synthesis of a sucrose carrier protein and a change in hormone level (GA_3). Jones et al. (1994) concluded that petal wilting and senescence in certain bulb flowers (*Gladiolus*, *Iris*, and *Narcissus*) relies on *de novo* protein regulation. They implied that petal senescence in these ethylene-insensitive flowers is controlled by an ordered sequential gene expression. Eason et al. (1997) found that the quantities of specific proteins present during flower development and senescence in *Sandersonia aurantiaca*, an ethylene-insensitive liliaceous cut flower, were regulated by sugars.

Our data imply that lily flower longevity is at least partly determined by the redistribution of tepal carbohydrate within the lily inflorescence. Blocking of this redistribution by detachment of source flowers at anthesis can not be compensated by redistribution out of the leaves or stem. The underlying mechanisms determining flower senescence in *Lilium* are most likely not restricted to carbohydrate redistribution pathways as reported in this paper, but should be investigated further at a molecular level to enable the identification of other (co-)factors explaining cultivar differences in lily flower senescence.

Chapter 8

General discussion

Introduction

This thesis resulted from a study into genetic and physiological aspects of flower longevity in lily (*Lilium* L.) and the potentials to improve flower longevity by breeding. The main objectives in the present research were to obtain knowledge of (1) test methods for screening and selection, (2) available genotypic variation, (3) mode of inheritance and (4) physiological regulation of postharvest flower longevity in Asiatic hybrid lilies.

The first part of this general discussion deals mainly with the first three objectives in view of the results presented in Chapters 2 to 5. The second part discusses the possible physiological mechanisms causing the observed genotypic variation of flower longevity in lily (objective 4), in view of the results presented on tepal carbohydrate content (Chapter 6 and 7). In the last part suggestions for further research are made and practical implications are given.

Perspectives of improving lily flower longevity by cross breeding

In general, for a breeding program to be effective the following conditions are essential:
(1) a practical screening method
(2) adequate genotypic variation in the breeding material
(3) a reliable selection method
(4) knowledge of the mode of inheritance.

Screening

To study the possibilities for improving flower longevity in lily by breeding we started with the development of a standardized screening method to determine genotypic differences among longevity levels. For practical use such a screening method requires to be (1) discriminative, (2) repeatable and (3) reliable.

Discrimination. Discriminative conditions determine which genotypic differences of flower longevity can be measured. They can be divided into conditions controlling environmental variance and conditions influencing genotypic variance. To optimize the screening procedure sources creating environmental variance should be minimal and sources creating genotypic variance should be maximal. Therefore, the main sources of environmental variance (parameter for screening, harvest stage) were standardized. At the same time conditions improving the degree of variation among genotypes (evaluation conditions) were optimized (Chapter 2 and 3). The most significant step to improve discrimination among longevity levels was the decision to use the longevity of an individual flower as a parameter for screening

instead of the longevity of the inflorescence as a whole (Chapter 2). This decision was mainly due to the complexity of inflorescence longevity being a function of several components (e.g. total number of floral buds and the postharvest expansion and opening pattern of the buds) some of which are genetic components by themselves (Van Tuyl et al., 1985; Van der Meulen-Muisers et al., 1997). This complexity is known to be a common problem in defining longevity in inflorescence-type flowers leading to a large diversity in interpretations (e.g. Elgar et al., 1999; Serek et al., 1994), which hampers mutual comparison in postharvest research and is especially unwanted in breeding research.

Repeatability. A screening method is considered repeatable if screening results are consistent between experiments. Screening experiments at clonal level which were repeated under standardized conditions in different years showed similar results (Chapter 4), indicating agreement of results between experiments. Variation in bulb stock origin (grower, production location) can be responsible for the relatively small deviations found (Chapter 3), as bulb quality can, for instance, be influenced by the amount and time of application of nitrogen (Van der Boon and Niers, 1986).

Reliability. Test conditions are referred to as reliable if agreement exists between screening results obtained under standardized conditions and under forcing conditions in practice. As Asiatic hybrid lilies are most commonly forced under greenhouse conditions year-round, greenhouse experiments could provide information on the reliability of the standardized test conditions. Growing and harvest in a greenhouse in spring, the optimal season for forcing of Asiatic hybrids in The Netherlands, followed by evaluation under standardized conditions in a climate-controlled room gave similar screening results compared with screening after standardized forcing in a growth chamber. Because deviations between experiments were unlikely due to quality and age of the bulbs (Chapter 4), the small variation found was probably on account of differences in genotype response to varying forcing conditions.

The agreement between screening results using standardized forcing conditions and growers forcing conditions in practice, in combination with the small environmental variance found within experiments (Chapter 4), indicates that the expression of flower longevity does not depend to a large extent on environmental conditions in the forcing phase. This makes screening results highly reliable. However, it should be taken into account that in practice specific genotype x environment interactions can occur. To make sure that results under greenhouse conditions are comparable year-round, some cultivars not showing important genotype x environment interactions might be used as a control to correct for general seasonal effects and selections must be tested for year-round performance using a range of forcing environments.

For practical reasons testing for flower longevity leaving the inflorescence uncut could be preferred. Similar screening results were obtained comparing longevity

levels of cut and uncut inflorescences using standardized conditions in a climate-controlled room (unpublished results). However, with respect to flower evaluation of uncut inflorescences under greenhouse conditions, it should be noted that because of the considerable effect of the evaluation temperature on flower longevity (Chapter 3), an increase in environmental variance might be expected. Such an increase will reduce the discrimination among longevity levels under greenhouse conditions compared to testing under standardized conditions in a climate-controlled room (Chapter 4). It is therefore highly recommended to carry out the evaluation of flower longevity under controlled climate conditions.

Genotypic variation

Although relevant studies were lacking at the start of our study, it was commonly accepted that among modern lily cultivars differences could be observed for flower longevity (H.P. Pasterkamp and J.M. van Tuyl, personal communications). Indeed, great differences in individual flower longevity were found when screening a wide range of genetic material containing (old) cultivars, species, and seedling clones, ranging form about 4 to 9 days (Chapter 4). Both bulb forcers and plant breeders can exploit this variation in flower longevity.

However, the in potential reachable flower longevity under standardized conditions is only one (genetic) component determining the life span of a flower at the consumers (Chapter 1). In our study besides potential individual flower longevity several other components have been studied (e.g. stress tolerance, sensitivity to ethylene, ploidy level, carbohydrate content). The former three components accounted for only small additional genotypic variation in individual flower longevity, whereas the role of carbohydrate content in lily flower longevity appeared to be important but complex as discussed below.

Stress and ethylene. Common stress conditions that can be expected to occur during handling of cut flowers in practice reduce the in potential reachable flower longevity (unpublished results). Reduction of individual flower longevity was particularly caused by a short period (20 hours) of dry unpacked storage at 17°C (see Fig. 8.1A). This treatment reduced flower longevity with about 22% on average compared to the in potential reachable flower longevity. Exposure to several concentrations (up to 2 ppm) of the phytohormone and common air pollutant ethylene during the dry storage period caused only a relatively small reduction in individual flower longevity (Fig. 8.1B) compared to the longevity reduction in ethylene-sensitive flowers like e.g. carnation (Wu et al., 1991). Cultivars of carnation show already a tremendous decrease in flower longevity at ethylene levels of 0.01 ppm or even less. Storage at 2°C during 7 days after being packed in plastic bags, to simulate conditions occurring during handling of cut flowers, reduced individual flower
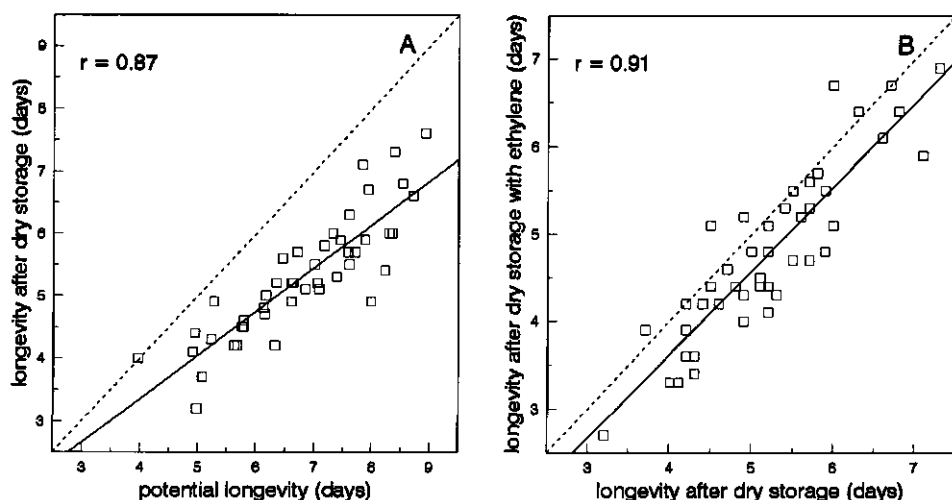
Fig. 8.1 Correlation between longevity with and without dry storage (20 hours at 17°C) **(A)**, and correlation between longevity after dry storage with or without ethylene (2 ppm) **(B)**, of individual flowers of 47 lily genotypes using standardized forcing, harvest and postharvest conditions (see Chapter 4).

longevity with about 14% on average (Fig. 8.2). Differences in sensitivity to several stress conditions were relatively small as correlations with flower longevity without exposure to stress were high (Fig. 8.1, Fig. 8.2). Because of the relatively small genotypic variation in sensitivity to several important postharvest stress conditions in lily, the in potential reachable individual flower longevity not preceded by stress is a reliable overall parameter for screening for genotypic differences in individual flower longevity after harvest.

Ploidy level. Influence of the ploidy level on individual flower longevity could not be studied in detail due to the limited number of polyploid genotypes investigated (Chapter 3 and 4). Although higher levels of ploidy are known to be effective in the improvement of quality characteristics in lily (Schenk, 1987; Van Tuyl, 1989), and in the improvement of flower longevity in *Tulipa* (Van Eijk and Eikelboom, 1986), the polyploid genotypes studied did not provide an additional genetic source for the improvement of lily flower longevity.

Carbohydrate. Carbohydrates are generally known to play an important role in flower longevity (Chapter 1). Therefore, the role of carbohydrates in lily flower longevity was studied more in detail. At anthesis genotypic variation in lily tepal carbohydrate content was found, testing the ten parental genotypes used for crossing in Chapter 5 (unpublished results). However, it was poorly associated with individual flower longevity. The association considerably improved by correlating individual flower longevity and the relative carbohydrate consumption per day, with a coefficient
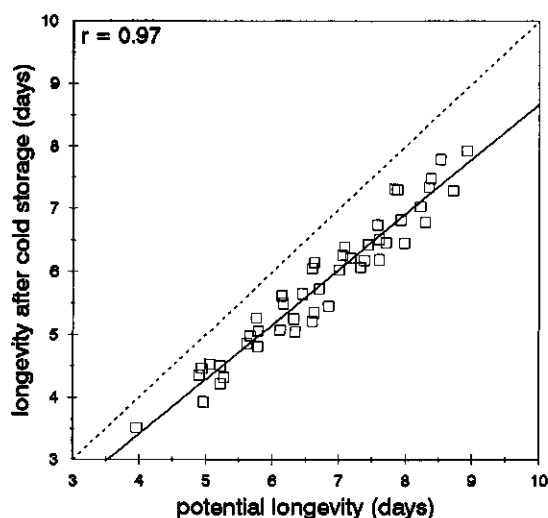
93

Fig. 8.2 Correlation between longevity with and without cold storage (7 days at 2°C packed in plastic bags) of individual flowers of 47 lily genotypes using standardized forcing, harvest and postharvest conditions (see Chapter 4).

of linear correlation (r) of -0.70 if carbohydrate content was expressed on a per tepal basis (unpublished results), indicating an important role of tepal carbohydrate in lily flower longevity. This was confirmed when tepal carbohydrate content during flower development and senescence of three genotypes was studied more in detail (Chapter 6 and 7). It was concluded that the role of tepal carbohydrate in lily flower longevity can be expected to be important but complex as discussed in the second section of this chapter.

Selection

Large variation in individual flower longevity within and among progenies was found (Chapter 5). Variation within progenies makes selection possible. Selection for flower longevity can take place according to the screening conditions described in the Chapters 2, 3 and 4 as discussed above. However, these conditions are based on the availability of a large number of flowering bulbs per genotype, which can only be obtained in Asiatic lily hybrids by vegetative propagation followed by cultivation over at least 2 years (Beattie and White, 1993). Although clone testing improves the discrimination among longevity levels (Chapter 4), in breeding trials it would be preferred to carry out initial selection at the individual plant level to speed up the breeding process and reduce costs.

The estimated degree of genotypic determination (R^2) based on clonal material appears already to be moderately high after evaluation of just one flower per

genotype (R^2 = 0.64, see Chapter 4). From this result it was concluded that selection based on one individual plant per genotype is possible, which was confirmed by the high value for broad-sense heritability of individual flower longevity (H^2 = 0.79), based on 45 progenies at individual plant level (Chapter 5). Furthermore, if cultivars which are clonally propagated in commerce are initially selected from progenies grown from seed, selection among seedlings only can be considered efficient in case equal expression of the trait selected for is present in both seedling bulbs and scale propagated bulbs (Langton, 1991). Results presented in Chapter 5 suggest that for individual flower longevity in lily this is the case, because the correspondence between the breeding value of the parents (conducted from seedling bulbs) with their phenotypic performance as a clone was very close. Therefore, initial selection for improved flower longevity in lily can take place using individual seedling bulbs. This was recently confirmed when promising initial selections at seedling level (Chapter 5) were tested again at clonal level (J.M. van Tuyl, personal communication).

Mode of Inheritance

For the improvement of flower longevity by cross breeding knowledge of test methods for screening and selection and insight in the available genotypic variation is essential. However, for an efficient selection of genotypes with improved flower longevity, additional knowledge is required on the inheritance of flower longevity.

In Chapter 5 it was shown that additive genetic variance is important in the inheritance of individual flower longevity due to a highly significant general combining ability (GCA) component in combination with a high narrow-sense heritability estimate (h^2). Consequently, progenies will have a mean value for flower longevity close to the mean of their parents and individual flower longevity of the genotype can be used as an indication for its breeding value in practical breeding. No important maternal effects were observed.

Due to the limited number of descendants of related populations (e.g. no back crosses, and no selfings), the inheritance of flower longevity in lily could not be studied in detail. However, test results of the genetic analysis of individual flower longevity (Chapter 5) lead to the conclusion that flower longevity is likely determined by more than one gene. This is based on the facts that (1) similarities between parents in individual flower longevity do not necessarily indicate genetic homogeneity, (2) discontinuous variation is absent and (3) transgressive segregation dominates (Chapter 5).

A more specific statement on the inheritance of flower longevity in lily can be given by indirect selection using genetic markers linked with loci involved in the encoding of flower longevity. Advantages of genetic markers as a tool for indirect selection are the possibility of determining flower longevity independently from

environmental variation, and the fact that selection can take place in an early stage of development, even before flowering (Gebhardt and Salamini, 1992).

Although no complete genetic map of lily is available, initial results obtained from genetic linkage studies using morphological markers and randomly amplified polymorphic DNAs (RAPDs) (Williams et al., 1990) with loci involved in flower longevity were promising (Van der Meulen-Muisers et al., 1995b). RAPD markers have proven to be useful in lily for linkage studies of both qualitative traits (flower color, petal spots, male sterility, resistance to tulip breaking virus [TBV]) and quantitative traits (*Fusarium* resistance), (Straathof et al., 1996; J.M. van Tuyl, personal communication). At the moment over 500 genetic markers are scored and about 300 are placed on a genetic map of lily (J.M. van Tuyl, personal communication).

In conclusion, large transgressive segregation in combination with effective selection due to high broad-sense heritability (Chapter 5) and the way of inheritance as discussed above makes the prospects for improving flower longevity in lily by breeding and selection promising. In addition, a high broad-sense heritability together with a large segregation of the desired character are of great benefit in linkage studies and future breeding programs based on indirect selection using genetic markers.

Physiology of genotypic variation for lily flower longevity

The second part of this chapter discusses physiological mechanisms possibly involved in the large genotypic variation observed for individual flower longevity of lily. Specific attention has been paid to the contribution of carbohydrates.

Preharvest

The carbohydrate status at harvest largely determines the postharvest performance of cut flowers, as carbohydrate production after harvest can be expected to be negligible due to low light conditions in the postharvest phase. The carbohydrates available in the flowers at harvest might be determined by (1) the photosynthetic activity of the plant and (2) distribution within the plant during cultivation. Genotypic differences in postharvest flower longevity of lily might result from differences in the availability of carbohydrate at the time of harvest.

Photosynthesis. The carbohydrates available in the flowers at harvest can be dependent on the photosynthetic activity of the plant during cultivation and, therefore, on light intensity and day-length. Plant quality and flower longevity of Asiatic lily hybrids are known to be sensitive to changes in light sum during forcing (Chapter 4; Van der Meulen-Muisers et al., 1992; Van Tuyl et al., 1985). Furthermore, a positive

association both phenotypic and genetic, was found between forcing time (light sum), and flower longevity of 45 lily progenies (Chapter 5).

Distribution. During lily forcing, most of the assimilates synthesized in the vegetative organs are destined for the stem apex during floral evocation, and then to the flower buds throughout their development (Wang and Breen, 1986ab). Genotypic differences in flower bud number at harvest appeared to be associated with postharvest flower longevity. Using a large number of genotypes at clonal level it was calculated that each additional bud caused a reduction in flower life of about 0.2 days (Chapter 4). Within progenies both a phenotypic and genetic negative correlation was found between number of buds at harvest and postharvest flower longevity (Chapter 5). Reduction of the number of buds during forcing either due to preharvest bud abortion (unpublished results) or artificially disbudded (Van der Meulen-Muisers et al., 1995a), significantly improved tepal carbohydrate content at harvest and subsequent postharvest flower longevity in lily indicating competition for carbohydrate within inflorescences in the preharvest phase. Based on these results it was concluded that genotypic differences in flower bud number (sink strength), might introduce genotypic differences in carbohydrate availability at harvest.

Before harvest genotypic variation in assimilate production and/or distribution within the intact inflorescence can be expected, as tepal carbohydrate content at harvest varied among genotypes (Chapter 6 and 7; Van der Meulen-Muisers et al., 1995a). However, no direct relationship was found between tepal carbohydrate content at harvest and postharvest flower longevity among genotypes (Chapter 6; unpublished results).

Postharvest

A moderate association ($r = -0.70$) was found between flower longevity and the relative tepal carbohydrate consumption per day in inflorescence-attached bottom flowers (unpublished results), for the ten parental genotypes used for cross breeding in Chapter 5. These data suggest that genotypic differences in flower longevity might at least partially be due to genotypic differences in postharvest carbohydrate utilization. The carbohydrates available in the flowers at harvest can be used for (1) redistribution, (2) maintenance of the osmotic potential and (3) maintenance respiration.

Redistribution. The various flowers and buds within an inflorescence will compete for the carbohydrates that are available at harvest. Small buds will use a relatively large amount of the carbohydrates for the synthesis of various structural cell components and as vacuolar osmoticum to maintain tugor pressure during cell expansion. Larger or more developed flowers and buds will have a higher maintenance respiration compared to small or less developed flowers and buds as maintenance respiration is assumed to be related to the amount of tissue present.

Carbohydrate redistribution is often correlated with invertase activity (Borochov and Woodson, 1989). During flower development in *Lilium longiflorum* an increase in invertase activity has been detected, highly correlated with dry weight gain in most of the flower organs (Ranwalla and Miller, 1998). Loss in invertase activity during petal senescence, linked to the *de novo* synthesis of an invertase inhibitor, is revealed by an increase in the ratio of sucrose to reducing sugars as senescence precedes (Borochov and Woodson, 1989). The presence of such an inhibitor may prevent the conversion of sucrose and thus makes it available for redistribution.

In our study the importance of postharvest carbohydrate redistribution is revealed after blocking carbohydrate export out of the flower by detaching flowers from the inflorescence at anthesis, as sucrose accumulated at the first visual signs of deterioration (Chapter 7). Detachment of the buds at several stages of development increased flower size and longevity in the larger bud sizes and reduced flower longevity or even halted anthesis and bud growth in the smaller bud sizes. In contrast, development and longevity hardly varied within the inflorescence if flowers and buds stayed attached to the inflorescence after harvest (Chapter 6).

The actual need for carbohydrates within inflorescences will be different, because flowers and buds vary in size, number and in their developmental stage, characteristics that are partially determined by the genotype (Chapter 2).

Osmoticum. During bud growth, anthesis and flower senescence carbohydrate has an important function as a vacuolar osmoticum to maintain turgor pressure. From research on carbohydrate supply to cut flowers it is known that it accumulates in the flower, increasing the osmotic capacity, and improving the ability of the flower to absorb water and maintain its turgidity (Halevy and Mayak, 1979). Maintenance of improved water status seems to be the most important aspect in extending flower longevity. It therefore seems that one of the main effects of applied carbohydrate on flower longevity results from its contribution to the osmotic adjustment of the flowers.

In lily, tepal carbohydrate was responsible for only a part of the osmotic potential (Van Meeteren et al., 2000) and it was assumed that organic acids were the other important osmotic components. The osmotic potential was found to be nearly constant at various developmental bud stages, in spite of fluctuations in the carbohydrate level, which indicates the non-specificity of carbohydrate as osmoticum.

Another possibility to maintain osmotic potential is uptake of less water resulting in smaller flower tepals and lower fresh weights. Shortage of carbohydrate will first lead to a reduction in tepal size; at a more severe carbohydrate decrease flower longevity decreases. Genotypic differences in adjusting floral bud size and, therefore, flower size at anthesis were found if bud numbers were varied during forcing by disbudding (Van der Meulen-Muisers et al., 1995a). Therefore, the potential for 'self-correction' of the flower by adjusting its tepal size to the available carbohydrate amount might be dependent on the genotype.

Respiration. In general, to maintain normal functioning, plant tissue will use carbohydrate by respiration to generate energy for protein-turnover and maintenance of ion gradients. The contribution of this 'maintenance respiration' to the carbohydrate balance is very pronounced during vase life of cut flowers. Although respiration was not studied in the experiments described in this thesis estimations can be made based on disappearance of tepal carbohydrate during the vase life of detached flowers. Carbohydrate loss in detached open flowers can be expected to be due solely to respiration because in these flowers carbohydrate loss, caused by redistribution, is blocked and carbohydrate loss due to structural growth is likely negligible because open flowers have reached their final size (Chapter 7). Estimated respiration rates showed a large variation among genotypes but appeared to fall in the same range as the rates measured by Elgar et al. (1999) within a large number of lily genotypes. The large genotypic differences in the estimated respiration rates were associated with differences in flower longevity (Chapter 7). However, considerable amounts of carbohydrate were still present in lily tepals when flower deformation occurred, as has been reported for other flowers like carnation (Halevy and Mayak, 1979). This suggests that carbohydrate exhaustion is not the cause for the onset of senescence. On the other hand, tepal carbohydrate content of the flowers of the ten parental genotypes used for cross breeding in Chapter 5, was about the same at the time flower deformation occurred, despite the rather large differences at harvest (unpublished results). This could indicate that at the time flower deformation occurs, in lily a threshold value of carbohydrate is reached.

In addition, an interesting aspect of detaching lily flowers at anthesis was the increase in flower longevity with about two days independent of the genotype (Chapter 7). This indicates that genotypic variation in flower longevity is at least partially located within the flower itself.

In conclusion, as a result of the before-mentioned processes, the carbohydrate balance of a cut lily inflorescence behaves very dynamic. The final result will depend, amongst others, on genotype, amount of carbohydrate present at harvest, number of floral buds and their individual developmental stages. The importance of carbohydrate in lily postharvest performance has been proven, and genotypic differences related to carbohydrate availability clearly exist. However, because of the complexity of the carbohydrate balance within lily inflorescences more research is needed to understand its association with genotypic differences in flower longevity. Due to the possible gene-regulating role of sugars during petal wilting and flower senescence (Eason et al., 1997), further investigations should include studies on molecular genetic level.

Recommendations and practical implications

The results presented in this thesis are part of the research performed within the project 'Breeding research on flower longevity of lily and tulip', one of the 13 projects within the framework of the Urgency Programme for Research on Diseases and Breeding of Flower Bulbs (1989 – 1996). This programme was started to eliminate the lack of (fundamental) knowledge, compared to other horticultural crops, in order to face the major problems within the flower bulb sector. It was financed by the Dutch government and the flower bulb industry.

The use of chemical solutions to ensure a satisfactory postharvest performance of cut flowers must be reduced to prevent further environmental pollution. This in combination with an increase in competition due to the growing international supply of ornamentals is the reason that the often limited longevity of bulb flowers is expected to become a serious problem in maintaining their position at the international market. Improvement of flower longevity by breeding could be an effective environmentally friendly way to ensure economic benefit of bulb flower production.

Development of new cultivars. The variation in lily flower longevity found in this study (Chapter 4 and 5) and the test methods described for screening and selection (Chapter 2 and 3) make it possible to obtain new lily cultivars with an improved flower longevity by the breeding companies. Already existing cultivars and newly released cultivars (over a hundred a year in case of lily) can be tested for flower longevity to look for enhancement of the exploitable genetic variation. Using Asiatic hybrids with a long individual flower longevity (about 8 days) in breeding trials will immediately increase the opportunity to develop cultivars with an improved longevity, due to the way of inheritance of individual flower longevity. A number of Asiatic hybrid genotypes with the potential to introduce a superior flower longevity are detected, as selection performed in this study resulted in genotypes with a flower longevity up to 11 days (Chapter 5).

Effective selection can take place at individual plant level to speed up the breeding process. The time necessary for the development of new cultivars with improved flower longevity is partly dependent on associations between longevity and other important characteristics like plant performance, bulb production and resistance to pathogens. Examination of favorable associations between flower longevity and other important crop characteristics has been taken place at a limited scale within the research presented (Chapter 4 and 5). Selection is expected to be simplified by the associations between long individual flower longevity with long inflorescence longevity and a high percentage flowering buds because they all will improve the postharvest performance of lily. Especially the significant correlation (both phenotypic and genetic) between individual flower longevity and inflorescence longevity can be

applied in breeding programmes. In contrast, the association between a long flower longevity and a long forcing period is not in all cases favorable.

Broader application. Development of test methods to determine genotypic variation for flower longevity in other lily groups can take place conform to the methods as described for Asiatic hybrid lilies. Likely the available test methods are, with only minor adjustments, suitable to evaluate those groups that have not been investigated yet. Knowledge on the available genotypic variation within all lily groups in combination with tissue culture techniques developed to overcome crossing barriers (Van Creij et al., 1993; Van Tuyl et al., 1991) will even increase the possibilities for improving flower longevity in *Lilium*. The developed test methods might (with minor adjustments) also be suitable for other bulb crops as can be concluded from the results obtained for *Tulipa* (Van der Meulen-Muisers et al., 1997).

Genetic markers. To improve the future breeding efficiency the construction of genetic maps is important especially in crops with a long juvenile phase and/or slow propagation rate like commonly found in flower bulbs. More genetic markers linked with genes involved in the expression of several useful traits such as longevity of flowers are needed that can be used for indirect selection in progenies after cross breeding. By using such techniques several important traits can be selected at the same time, independently from environmental variation and in an early stage of development.

Carbohydrate. In view of the importance of tepal carbohydrate for the postharvest performance of lily (e.g. longevity, percentage flowering buds) (Chapter 6 and 7) and other inflorescence-type cut flowers it is recommended to continue the investigations of the relationship between (tepal) carbohydrate and flower longevity. It is important to obtain more knowledge of the mode of carbohydrate redistribution within the inflorescence (e.g. by using labeling techniques), and the way carbohydrate is used in maintenance respiration. Although the construction of a carbohydrate balance is very labor intensive, it will be helpful in understanding physiological mechanisms involved in cultivar differences in flower longevity and postharvest performance of lily and other inflorescence-type flowers. This knowledge is needed to support both breeding research and physiological research directed on the improvement of postharvest quality of flowers.

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Summary

In The Netherlands *Lilium* is economically the fourth overall flower crop for cut flower production. Longevity is a main quality characteristic for cut flowers. During postharvest handling of Asiatic hybrid lilies pretreatment with chemical solutions containing silver is carried out to ensure a satisfactory longevity at the consumers'. However, the extent to which such a treatment can delay senescence is limited and is dependent on the genotype. Developing cultivars with genetically improved longevity may provide the consumer with a more reliable expectation for postharvest quality in a less environment polluting way. Flower longevity is a particularly difficult genetic characteristic to assess, since it is markedly affected by growing conditions prior to harvest, stage of flowering at harvest and environmental conditions during distribution and after sale. Additionally, longevity of lily is complex because of its inflorescence-type flower. Longevity is determined by two conflicting processes: (1) promotion of flower bud growth and anthesis; (2) retardation of metabolic processes leading to senescence. Therefore, besides knowledge of genetic aspects of flower longevity also insight in the physiological regulation is needed for its improvement. The objectives of the experiments described in this thesis were to obtain knowledge of (1) methods for longevity determination, (2) available genotypic variation, (3) mode of inheritance and (4) physiological regulation of postharvest flower longevity in Asiatic hybrid lilies.

The development of a standardized method for the determination of flower longevity in lily is described. To optimize the screening procedure, the main sources of environmental variance (parameter for screening, harvest stage) were located and standardized, whereas conditions improving the degree of variation among genotypes (evaluation temperature) were determined and optimized (Chapter 2 and 3). By using standardized conditions during forcing, harvest and postharvest evaluation measurements became discriminative and repeatable. Standardization of the forcing method is usually not possible in practice. The ranking of the genotypes based on their longevity levels using standardized conditions during forcing was comparable with the ranking found when forcing conditions in practice were used (Chapter 4). This makes screening results highly reliable for practical use. Because of the large influence of temperature on flower longevity postharvest evaluation should preferably be carried out under controlled temperature conditions.

For breeding purposes adequate genotypic variation in the breeding material is necessary. Large differences in individual flower longevity were found screening a wide range of genetic material of Asiatic hybrid origin (Chapter 4). Interspecific hybridization research showed that crosses between genotypes from different taxonomic sections is possible (Chapter 1 and 8). So, enhancement of the exploitable genetic variation may be obtained by screening also other lily groups. The in potential reachable individual flower longevity, not preceded by stress,

appeared to be a reliable overall parameter for screening as postharvest stress conditions (storage, ethylene) introduced no large differences in the ranking of the genotypes based on their longevity levels (Chapter 8).

Segregation after crossbreeding makes selection possible. Large variation in flower longevity within and among progenies was found. Selection at seedling level appeared to be possible because of a high broad-sense heritability based on one individual plant per genotype and because of an equal expression of individual flower longevity in plants obtained from both seedling bulbs and scale propagated bulbs (Chapter 5). This can considerably speed up the breeding process.

For an efficient selection of genotypes with improved flower longevity, knowledge is required of the inheritance of flower longevity. Genetic analysis of the results of the individual plant test showed that additive genetic variance is important in the inheritance of individual flower longevity as the general combining ability (GCA) was the most important genetic component and the estimate for narrow-sense heritability was high. Consequently, individual flower longevity of the genotype can be used as an indication for its breeding value in practical breeding (Chapter 5). A more specific statement on the inheritance of flower longevity can be obtained by indirect selection using genetic markers as discussed in Chapter 8.

The physiological regulation of postharvest longevity was investigated by studying the role of tepal carbohydrate content (Chapter 6 and 7). The importance of tepal carbohydrate content and distribution in lily postharvest performance (bud-growth, anthesis, longevity) was revealed by comparing the development of inflorescence-attached and detached buds and flowers. Genotypic differences in the availability and use of carbohydrate were found. Per genotype tepal carbohydrate and individual flower longevity of detached flowers were well correlated. Blocking carbohydrate export by detaching the flowers from the inflorescence increased their longevity compared to the longevity of inflorescence-attached flowers. The complexity of the carbohydrate economy within lily inflorescences hampered the detection of a causal relationship among genotypes. However, the data presented suggest that genotypic differences in flower longevity might at least partially be due to genotypic differences in postharvest carbohydrate utilization (Chapter 7).

In conclusion, the prospects for improving flower longevity in lily by breeding and selection are promising. The methods described for screening and selection, the genotypic variation in flower longevity found in this study and the way of inheritance as discussed in Chapter 5 make it possible to obtain new lily cultivars with an improved flower longevity in a relatively short period of time. Furthermore, a high broad-sense heritability in combination with a large segregation of individual flower longevity are of great benefit in linkage studies and future breeding programs based on indirect selection using genetic markers. The physiological regulation of lily flower longevity and the important but partially unknown role of carbohydrates in this complex process needs still further research.

Samenvatting

In Nederland neemt lelie economisch gezien de vierde plaats in bij de snijbloemen. Houdbaarheid is een hoofdkenmerk van de kwaliteit van snijbloemen. Om als consument verzekerd te zijn van een bevredigende houdbaarheid worden Aziatische lelie hybriden in de na-oogst fase voorbehandeld met chemische oplossingen die zilver bevatten. De mate waarin zo'n behandeling kan leiden tot een vertraging van de veroudering van de bloem is echter beperkt en is mede genotype-afhankelijk. De ontwikkeling van cultivars met een genetisch verbeterde houdbaarheid kunnen op een meer betrouwbare manier aan het verwachtingspatroon van de consument voldoen en zijn tevens minder vervuilend voor het milieu. De houdbaarheid van bloemen is een moeilijke genetische eigenschap om te bepalen. Dit komt omdat houdbaarheid in grote mate beinvloed wordt door zowel teeltcondities, het ontwikkelingsstadium op het moment van oogsten en de condities tijdens distributie en na aankoop. Daarnaast is de houdbaarheid van lelie bloemen met hun samengestelde bloeiwijze complex omdat deze wordt bepaald door twee elkaar tegenwerkende processen: (1) Bevordering van de bloemknopgroei en bloemopening; (2) Vertraging van de stofwisselingsprocessen die leiden tot veroudering. Daarom is voor de verbetering van de houdbaarheid van de bloem naast kennis van de genetische aspecten van houdbaarheid tevens inzicht nodig in de fysiologsiche regulering. De in dit proefschrift beschreven experimenten hadden tot doel kennis te verwerven betreffende (1) toetsmethoden, (2) de beschikbare genetische variatie, (3) de wijze van overerving en (4) de fysiologische regulering van de houdbaarheid van Aziatische lelie hybriden na de oogst.

De ontwikkeling van een gestandaardiseerde toetsmethode voor het bepalen van de houdbaarheid van leliebloemen is beschreven. Om de procedure te optimaliseren werden de factoren die hoofdzakelijk verantwoordelijk waren voor milieuvariatie vastgesteld (parameter voor screening, oogststadium) en gestandaardiseerd, terwijl condities die de mate van genetische variatie tussen genotypen verbeterden werden bepaald (temperatuur tijdens het vaasleven) en geoptimaliseerd (Hoofdstuk 2 en 3). Het gebruik van gestandaardiseerde condities tijdens het forceren, oogsten en tijdens het vaasleven maakte het mogelijk om de waarnemingen beter te onderscheiden en bevorderde herhaalbaarheid van de resultaten. Het op deze wijze standaardiseren van het in bloei trekken is in de praktijk normaal gesproken niet mogelijk. De rangorde van de genotypen gebaseerd op hun houdbaarheidsnivo bleek echter vergelijkbaar indien het in bloei trekken plaatsvond onder gestandaardiseerde omstandigheden danwel onder praktijk-omstandigheden (Hoofdstuk 4). Dit vergroot de betrouwbaarheid van de verkregen resultaten voor gebruik in de praktijk. Gezien de grote invloed van de temperatuur op de houdbaarheid van de bloemen is het echter wel aan te bevelen om het

vaststellen van de houdbaarheid plaats te laten vinden onder gecontroleerde temperatuur-condities.

Voor veredelingsdoeleinden is voldoende genetische variatie in het uitgangsmateriaal noodzakelijk. Grote verschillen in de houdbaarheid van individuele bloemen werden gevonden na het screenen van materiaal met een brede genetische achtergrond (Hoofdstuk 4). Uit soortkruisingsonderzoek is gebleken dat kruisingen tussen genotypen van verschillende taxonomische secties mogelijk zijn (Hoofdstuk 1 en 8). Door ook andere lelie groepen erbij te betrekken zou de genetische variatie nog vergroot kunnen worden. Blootstelling aan stress na het oogsten (bewaring, ethyleen) veroorzaakte geen grote verschuivingen in de rangorde van de genotypen gebaseerd op hun houdbaarheidsnivo. Hieruit volgt dat de potentieel haalbare houdbaarheid van de individuele bloem, zonder voorafgaande stressbehandeling, een betrouwbare parameter is voor het bepalen van de houdbaarheid (Hoofdstuk 8).

Uitsplitsing na kruisingsveredeling maakt selectie mogelijk. Er werd een grote variatie gevonden in houdbaarheid van de individuele bloem binnen en tussen nakomelingschappen. Selectie op zaailingnivo bleek mogelijk door (1) een hoge waarde van de erfelijkheidgraad (in brede zin) gebaseerd op één individuele plant per genotype en door (2) een overeenkomstige expressie van de houdbaarheid in planten verkregen uit zaailingbollen danwel uit bollen vermeerderd via schub (Hoofdstuk 5). Dit kan het veredelingsproces aanzienlijk versnellen.

Om genotypen met een verbeterde houdbaarheid van de bloem op een efficiënte wijze te kunnen selecteren, is kennis van de overerving van houdbaarheid vereist. Genetische analyse van de resultaten van de individuele plant test liet zien dat additiviteit belangrijk is in de overerving van de houdbaarheid. Dit bleek uit het feit dat de algemene combinatie geschiktheid (ACG) de belangrijkste genetische component was in combinatie met een hoge waarde voor de schatting van de erfelijkheidsgraad (in enge zin). Hierdoor kan de houdbaarheid van de individuele bloem gebruikt worden ter indicatie van de geniteurswaarde in de praktische veredeling (Hoofdstuk 5). Een gerichtere uitspraak over de overerving van houdbaarheid kan gedaan worden na indirecte selectie met gebruikmaking van genetische merkers (Hoofdstuk 8).

De fysiologische regulatie van houdbaarheid na de oogst werd onderzocht door het bestuderen van de rol van de hoeveelheid koolhydraten in de bloembladen (Hoofdstuk 6 en 7). Het belang van de koolhydraathoeveelheid in bloembladen van lelie en van de herverdeling van koolhydraten voor de naoogst kwaliteit (knopgroei, bloemknop-opening, houdbaarheid) werd aangetoond door de ontwikkeling van losse knoppen en bloemen te vergelijken met de ontwikkeling van knoppen en bloemen aan de tak. Er werden genotypische verschillen in de beschikbaarheid en het verbruik van koolhydraten gevonden. Per genotype werd een duidelijke correlatie gevonden tussen de koolhydraten in bloembladen en de houdbaarheid

van individuele afgesneden bloemen. Het blokkeren van de export van suikers uit de bloem door deze af te snijden van de tak gaf een verlenging van de houdbaarheid te zien ten opzichte van bloemen die aan de tak bleven zitten. De koolhydraathuishouding in de bloeiwijze van lelies bleek complex, en bemoeilijkte het vinden van een causaal verband tussen genotypen. De gevonden resultaten duiden erop dat genotypische verschillen in houdbaarheid van de bloem op zijn minst gedeeltelijk toe te schrijven zijn aan genotypische verschillen in het verbruik van koolhydraten na de oogst (Hoofdstuk 7).

Dit onderzoek toont aan dat de vooruitzichten voor de verbetering van houdbaarheid in lelie door middel van veredeling en selectie veelbelovend zijn. Het verkrijgen van nieuwe lelie cultivars met een verbeterde houdbaarheid is relatief snel mogelijk via de ontwikkelde toetsmethoden, door de in dit onderzoek gevonden genotypische variatie in houdbaarheid en door de manier van overerving zoals bediscussiëerd in Hoofdstuk 5. De fysiologische regulering van de houdbaarheid van leliebloemen en de belangrijke maar nog deels onbekende rol van koolhydraten in dit complexe proces verdient nog verder onderzoek.

Account

Parts of this thesis have been or will be presented elsewhere:

Chapter 2:
Van der Meulen-Muisers, J.J.M. and J.C. Van Oeveren, 1996. Influence of variation in plant characteristics caused by bulb weight on cut flower longevity in Asiatic hybrid lilies. Journal of the American Society for Horticultural Science 121: 33-36.

Chapter 3:
Van der Meulen-Muisers, J.J.M. and J.C. Van Oeveren, 1997. Influence of bulb stock origin, inflorescence harvest stage and postharvest evaluation conditions on cut flower longevity of Asiatic hybrid lilies. Journal of the American Society for Horticultural Sciences 122: 368-372.

Chapter 4:
Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren and J.M. Van Tuyl, 1998. Genotypic variation in postharvest flower longevity of Asiatic hybrid lilies. Journal of the American Society for Horticultural Science 123: 283-287.

Chapter 5:
Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, J. Jansen and J.M. Van Tuyl, 1999. Genetic analysis of postharvest flower longevity in Asiatic hybrid lilies. Euphytica 107: 149-157.

Chapter 6:
Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, L.H.W. Van der Plas and J.M. Van Tuyl. Postharvest flower development in Asiatic hybrid lilies as related to tepal carbohydrate status. Postharvest Biology and Technology. (accepted).

Chapter 7:
Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, L.H.W. Van der Plas and J.M. Van Tuyl. Postharvest flower senescence in Asiatic hybrid lilies as related to tepal carbohydrate status. (submitted).

Related publications

Ki-Byung Lim, J.J.M. van der Meulen-Muisers and J.M. van Tuyl. Breeding for flower longevity enhancement of Asiatic hybrids. (submitted).
Van der Meulen-Muisers, J.J.M. and J.C. van Oeveren, 1993. Preliminary examination of some factors causing variation in flower longevity of *Lilium* cut flowers. The Lily Yearbook of the North American Lily Society 1990, 43: 61-66.
Van der Meulen-Muisers, J.J.M. and J.C. van Oeveren, 1993. Genetic variation in longevity of cut lily and tulip flowers. In: Creating genetic variation in ornamentals (T. Schiva and A Mercuri, eds.). Proceedings of the XVIIth Symposium of Eucarpia, section Ornamentals, Sanremo, Italy, March 1-5 1993, pp. 191-198.

Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, J.M. Van Tuyl, and H.M.C. Van Holsteijn, 1992. Examination of conditions for the selection of flower longevity in lily breeding. Acta Horticulturae 325: 637-642.

Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, B.B. Meijkamp and F.H.M. Derks, 1995a. Effect of floral bud reduction on flower longevity in Asiatic hybrid lilies. Acta Horticulturae 405: 46-57.

Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren, J.M. Sandbrink and J.M. Van Tuyl, 1995b. Molecular markers as a tool for breeding for flower longevity in Asiatic hybrid lilies. Acta Horticulturae 420: 68-71.

Van der Meulen-Muisers, J.J.M., J.C. Van Oeveren and J.M. Van Tuyl, 1997. Breeding as a tool for improving postharvest quality characters of lily and tulip flowers. Acta Horticulturae 430: 569-575.

Nawoord

Hoewel een proefschrift een 'proeve van bekwaamheid tot het *zelfstandig* uitoefenen van de wetenschap' is, zouden er eigenlijk net als boven een artikel meerdere namen op moeten staan.

Joop van Oeveren, je kunt er helaas niet meer bij aanwezig zijn, maar in feite promoveer jij ook. Ik ben je heel erg dankbaar voor je warme kameraadschap, je enthousiaste hulp en je nooit aflatende inzet, zelfs nadat die vervelende ziekte in 1994 de kop opstak. Als gelijkwaardige partner heb je steeds meegedacht en meegewerkt. Je bent dan ook niet voor niets mede-auteur van alle artikelen waarop dit proefschrift gebaseerd is. Zonder jou was het nooit gelukt.

Jaap van Tuyl, mijn co-promotor, je gaf me de vrijheid om een eigen invulling te geven aan dit onderzoek wat ik als zeer prettig heb ervaren. Toen we een aardig eind op dreef waren heb je mede de aanzet gegeven om dit onderzoek "om te buigen" tot een promotie onderzoek met dit proefschrift als eindresultaat.

Linus van der Plas, mijn promotor, je deur stond altijd open, ik kon bij jou terecht voor een luisterend oor of een goed advies. Onze vele besprekingen op Plantenfysiologie, waarbij vaak ook Folkert Hoekstra aanwezig was, leidden altijd tot waardevolle discussies waarmee ik weer verder kon. Jullie inbreng heeft interessante en leuke extra dismensies aan dit onderzoek toegevoegd.

Hein van Holsteijn, meerdere petten had je op, naast afdelingshoofd was je tevens programmaleider van het Urgentieprogramma en was je lid van de projectgroep Houdbaarheid. Deze korte lijnen maakten het voor mij alleen maar gemakkelijker. Je hebt alle versies van de "veredelingsartikelen" mede becommentarieerd. Ook de andere nog niet genoemde leden van de projectgroep Houdbaarheid (Frans Bonnier, Wim Eikelboom, Bertus Meier) hebben meegedacht over de richting van het onderzoek. Wim, er ligt genoeg materiaal voor nog zo'n boekje maar dan over tulp. Jouw bijdrage aan dit (hier onbesproken) deel van het onderzoek was hierbij onmisbaar.

Gerrit Terwoert, medebewoner van het Fytotron, jij zorgde voor een continue kwaliteit van de planten in het Selektron en voor het spoelen van de vele flessen voor de houdbaarheidsproeven. Door jou stond ook bij mij thuis altijd wel wat te bloeien. Ad van Dijken, als vaste rots in de regelmatig wisselende bezetting van de Leliekas zorgde jij met je medewerkers altijd voor de juiste bollen en voor de opkweek van onze kruisingsprodukten. Steef de Bruijn (vakgroep Plantenfysiologie), jij was de garantie voor het blijven draaien van de vaak nukkige Dionex en je hebt me wegwijs gemaakt in de wereld van de koolhydraten. Barbara Meijkamp, als stagiaire heb jij de eerste moeizame schreden gezet in de richting van het onderzoek naar koolhydraten. Anton Grim, Joke Mouris en Tiny Verhaegh jullie sprongen bij tijdens vakanties en tijdens de ziekte van Joop.

Hans Jansen, Ritsert Jansen en Paul Keizer (wandelende Genstat manual) mede door jullie kon de complexe bloeiwijze van lelie ook statistisch bedwongen worden.

Last but not least het thuisfront. Pap en Mam, jullie hebben mij de kans gegeven om te gaan studeren en mij altijd hierin gestimuleeerd. Ondanks de afstanden rukten de hulptroepen (Oma, Opa, Beppe, Pake) geregeld uit, vooral in de afrondingsfase. Anouk, je hebt heel wat geduld moeten hebben maar het boekje is eindelijk klaar en de computer beneden weer vrij; ik vrees alleen dat concurrentie van Luuk in aantocht is. Klaas, het is zover, mijn werkzaamheden voor Verbelco BV kunnen uitgebreid worden.

Bennekom,
10 augustus 2000 José

Curriculum vitae

Johanna Josephina Maria (José) Muisers werd geboren op 8 maart 1965 te Sittard. In 1983 behaalde zij haar Gymnasium-B diploma aan het Bisschoppelijk College Broekhin te Roermond. Om deze B nog meer uit te breiden volgde zij aansluitend in deeltijd onderwijs in de vakken natuur- en scheikunde waarin zij in 1984 met succes staatsexamen deed. Vervolgens werd in 1984 begonnen aan de studie Tuinbouw aan de toenmalige Landbouwhogeschool (nu Wageningen Universiteit) te Wageningen. Zij deed afstudeervakken bij de vakgroepen Plantenfysiologie, Tuinbouwplantenteelt, Plantencytologie en –morfologie, Erfelijkheidsleer en Fytopathologie. Praktijkervaring gedurende de studie werd opgedaan aan het Department of Environmental Horticulture, University of California in Davis. In augustus 1990 breidde ze haar achternaam uit tot Van der Meulen-Muisers en studeerde zij af. Van september 1990 tot februari 1991 doceerde zij in deeltijd aan de Agrarische Hogeschool Delft de vakken Tuinbouwplantenteelt en Plantenfysiologie. In november 1990 startte ze daarnaast op het DLO-Centrum voor Plantenveredelings- en Reproductieonderzoek (tegenwoordig onderdeel van Plant Research International) als onderzoeker aan het vijfjarig project Veredelingsonderzoek naar de houdbaarheid van lelie en tulp in het kader van het door de overheid en bloembollenbedrijfsleven gefinancierde Urgentieprogramma Bollenziekte- en Veredelingsonderzoek. Een gedeelte van dit onderzoek staat beschreven in dit proefschrift.