Doctoral Thesis

The temporal dynamics of kernel set in tropical sweet maize (Zea mays L.) determined by visual markers

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The temporal dynamics of kernel set in tropical sweet maize
(Zea mays L.) determined by visual markers

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presented by

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Abstract

Maize (*Zea mays* L.) is one of the most important crops for human and animal consumption. The crop is grown in a wide range of climates, in both temperate and tropical regions. The grain yield of maize per area has largely increased through improved field management and the development of high-yielding varieties with greater stress tolerance. The grain yield per plant, however, has only slightly improved. It depends on the number of ears per plant and the number of kernels per ear, as well as individual kernel weight. The number of kernels per ear is determined at and shortly after flowering.

Today, modern sweet maize hybrids can be grown on a large scale and in a broad range of growing areas, because the vigor of these hybrids is comparable to that of non-sweet maize (e.g. flint or dent maize). The kernels of sweet maize look different from kernels of flint maize due to a mutation that alters the carbohydrate content in the endosperm. Class 1 of sweet maize mutants produces shrunken kernel, which can easily be distinguished from non-sweet kernels, even if both types are present on the same ear.

All experiments were conducted at the National Corn and Sorghum Research Center (Suwan Farm) in Thailand. The research was carried out including one or two of the Thai tropical sweet maize hybrids Hibrix10, Sugar73 and Insee2 in 2004, 2005, 2007 and 2008.

The first part of this study focused on a visual marker system as a mean to investigate non-destructively the temporal dynamics of silk emergence and its impact on kernel set on maize ears. The system relies on sweet maize as pollen receptor, whose silks are pollinated with pollen of sweet maize on six days and with pollen of flint maize on one day during the week following the emergence of the first silks. The day, on which flint maize pollen was supplied, varied from one to seven (i.e. seven pollination treatments). The position of flint-type kernels on the ears of sweet maize allowed modeling the temporal dynamics of kernel set. The distribution of kernel set on the first day of silking corresponded to a bell-shaped curve whose peak was located at the tenth kernel position counted from the bottom of the ear. The distribution of kernels that were set on the following days followed a doubled bell-shaped curve, whose major peak moved towards to the tip of the ear and whose minor peak was located at the bottom of the ear and decreased over time. The highest number of kernels was
set on the first day of silking. More than 90% of silks were exposed to pollen within three or five days in the two experiments.

The second part of this study focused on genotype- and year-specific differences in the temporal dynamics of kernel set. The pattern of daily kernel set and the distribution of kernels along the ear was characteristic for the hybrids used: Sugar73 had a faster rate of kernel set than Hibrix10. Nevertheless, the highest number of kernels always resulted from pollinations done on the second day of silking and both hybrids produced approximately the same total number of kernels per ear. The duration of the period, in which more than 90% of the final number of kernels per ear was initiated, was stable across years for Hibrix10 but not for Sugar73. The year-specific differences had a modulating effect on the percentage of daily kernel set in both genotypes.

The third part of the study focused on effect of drought stress on the temporal dynamics of kernel set. Drought stress is an important abiotic constraint to rainfed agriculture worldwide and is expected to become an even more severe problem. The deleterious effect of drought stress on maize yields is particularly large at flowering when the kernel number is determined. While the role and regulation of the anthesis-to-silking interval has been studied thoroughly, the importance of the temporal dynamics of silk emergence and kernel has been considered less in breeding. Since only mild drought stress was applied in the present study, the drought-induced reductions in kernel number per ear were low. Significant effects of drought stress were observed in only two of the four experiments. Drought stress modulated the temporal dynamics of kernel set in all genotypes, although to unequal extents, and deceased number of kernels per ear, reflecting a reduction in the number of exposed silks. These reductions resulted from a reduction in the number of kernel-bearing position along the ear and in the number of kernels per position on each pollination day.

In conclusion, the temporal dynamics of kernel set and the distribution of kernels along the ears were determined by genotypic and environmental effects. Although the highest number of kernels always resulted from pollinations done on the first two days of silking, the percentage of daily kernels differed among hybrids. These genotype-specific differences remained relatively constant across years. The percentage of kernels on first two days of silking increased in years with high relative air humidity at flowering. In years with rather low relative air humidity, high wind and high water evaporation from the soil surface, in contrast, silk
emergence on first four days of silking was slowed down and kernel set delayed. Drought stress reduced the number of kernels per ear and the duration of silk emergence, depending on the genotype. All in all, the visual marker system was efficacious to trace the dynamics of silk emergence and its impact on kernel set under varying environmental conditions.
Zusammenfassung


Im dritten Teil der Studie wurde der Einfluss von Trockenstress vor und während der Maisblüte auf die zeitliche Dynamik des Kornansatzes untersucht. Trockenstress stellt weltweit ein immer grösseres Problem für die Landwirtschaft dar. Während der Blütezeit ist Mais besonders anfällig auf Trockenstress. Der Ertragsausfall (Kornetrag) pro Tag Trockenstress ist zur Blüte deutlich grösser als in andern Entwicklungsstadien. Die Bedeutung einer kurzen zeitlichen Verzögerung der weiblichen (Seidenscheibe) gegenüber der männlichen Blüte (Pollenfreisetzung) wurde vielfach untersucht und ist bestens bekannt. Die Frage, synchrones oder asynchrones Seidenwachstum (bzw. Bestäubung) den Kornetrag unter Trockenstress beeinflusst, wurde in der Züchtung hingegen weniger stark berücksichtigt. Da in den

Chapter 1

General introduction

Maize (Zea mays L.) is one of the most important cereals for both human and animal consumption. About 822 million tons of grains are produced on about 161 million hectares (FAOSTAT, 2008). The crop is grown in a wide range of climates in temperate and tropical regions where the daily mean temperature are above 15°C during the cropping season. Moreover, at least 500 to 800 mm water are required for successful maize cultivation (FAOWATER, 2008).

Maize in Thailand

In Thailand, for example, maize is grown on about 33% of the upland area. However, this area declined from nearly 1.3 million ha in 1998 to about 0.9 million ha in 2007. This decline in maize cultivation was due mainly to an increase in the cultivation of alternative crops, such as cassava, sunflower and sugarcane, to produce biofuel. Especially under drought stress, the yield of these crops is more stable than that of maize. Farmers therefore favor these alternative crops when the price of maize is low. While the maize area declined over the last decade, the average yield per area increased from 3,343 in 1998 to 3,950 kg/ha in 2007, which reflects the higher yield potential of improved varieties, most of which are single-cross hybrids (Office of Agricultural Economics, 2007). A higher yield potential and new varieties, which are more tolerant to biotic (e.g. rust diseases) and abiotic (e.g. drought and low soil fertility) stress, explain the increases in grain yield (Ekasingh et al., 2004). Despite its susceptibility to water-limited conditions, in particular at flowering, maize remains a major crop in Thailand, not only because of the higher yield potential of new varieties, but also because maize can be grown in two seasons per year or in one season in rotation with other crops such as mungbean, groundnut or sunflower. Given that the demand for maize and the maize price will increase in the near future, it is assumed that maize cultivation in Thailand will expand further. This is based on the prediction that the global demand for maize will outstrip the demand for wheat and rice within the next 10 to 15 years, and that the demand for maize will be highest in sub-Saharan Africa, followed by South and Southeast Asia.
Drought stress and drought resistance

Drought is considered to be the single most common cause of severe food shortage in developing countries (FAO). Climate change will further aggravate the situation. Many regions, particularly in Africa and in South and Southeast Asia, will experience higher temperatures and lower rainfall. From 1993 to 2002, 0.73 billion people were affected by drought (Sivakumar, 2005). These constraints to crop production as well as the greater demand for maize pose tremendous challenges to agriculture in these regions and globally. The requirement for water for agriculture was predicted to increase from 1,700 km$^3$ in 1999 to 2,300 km$^3$ in 2025 (UNESCO, 2003). To keep the demand for water as low as possible and to make the best use of available water resources, there is an urgent need to increase water use efficiency and water productivity.

There are numerous ways to achieve higher water productivity, among which is the development and cultivation of more drought-resistant crop varieties, which requires a sound understanding of the morphology and physiology of the crop. Drought resistance is based on all the plant characteristics that contribute to drought escape, drought avoidance and drought tolerance. For example, plants with a short life cycle, during which water supply does not limit growth, escape drought. Thus, the risk of yield losses due to terminal drought stress is lower for early flowering genotypes. However, drought escape through early flowering is often associated with a low yield potential.

Drought avoidance has a significant impact on yield, because it helps plants to maintain their water status and enables continuous photosynthesis, growth and development (Mullet, 2009). The basic mechanism of drought avoidance is the maintenance of tissue hydration. This is achieved by maintaining water uptake or reducing water loss (e.g. high root/shoot ratio, deep roots, thick leaves, rolling leaves). Maize roots can reach a depth of 2 to 3 m in to reach and take up water. The efficient exchange of water for CO$_2$ in photosynthesis also influences drought tolerance. For example, C$_4$ grasses, such as sorghum, millet and maize, are better adapted to hot dry environments compared to C$_3$ grasses such as rice due to their ability to concentrate and fix CO$_2$ in bundle sheath cells, thus reducing stomatal conductance and water loss. Maize leaves control gas exchange in such a way that the daytime leaf water status is unaffected by soil water deficit and, thus, must control stomatal conductance by “signals” from the root (abscisic acid, ABA). Dry soil enhances the production of ABA in the roots and leaves.
(Lambers et al., 2008), which leads to a decline in stomatal conductance before water shortage can have an adverse effect on the leaves.

Tolerance mechanisms allow plants to withstand dehydration through the accumulation of specific proteins and osmolytes in the cells (dehydrins (hydrophilins), heat shock proteins and a wide range of compatible solutes (e.g. polyols, glycine, betaine, proline, inositol)). Plants also increase the level and activity of enzymes and pathways that protect tissues from potentially damaging reactive oxygen species (ROS), which are generated during periods of water shortage and stomatal closure. Physiological responses that confer tolerance to dehydration are especially important in turf grasses, forages and biofuel crops, for which biomass accumulation is the main determinant of yield. The retention of leaf function ensures that plants continue to grow after periods of relatively severe water deficit.

In reality, drought resistance is often a combination of all three sub-classes of resistance. Simple drought resistance or drought tolerance does not exist optimal. Drought resistance is a complex network of morphological and physiological characteristics and processes, which depends on the timing, intensity and duration of the stress (Bruce et al., 2002).

*Morphology of maize inflorescences*

Maize is a monoecious plant with unisexual and physically separated male and female flowers. The tassel (staminate or male inflorescence) forms directly from the shoot apical meristem, whereas the ears (pistillate or female inflorescences) originate from axillary bud apices of branches arising from nodes above the soil surface. Usually only the upper one or two ears of modern maize varieties develop, with the others degenerating. The ear primordia and the tassels are initiated approximately at the 8- to 10-leaf stage. The male inflorescence is a panicle, consisting of a central spike (rachis) and around five to 10 lateral branches with many anthers that release a large amount of pollen.

The female inflorescence is a spike. It is covered with about eight to 14 modified leaves (husks), on the subtending ear stalk, and a prophyllum. The spikelets are arranged in pairs on the rachis or cob. Each individual spikelet consists of two florets, surrounded by an outer and an inner glume. Each floret includes one ovary, one silk, two lodicules, a lemma, a palea and a rudimentary stamen. Although both florets are initiated, only the upper one develops; the lower one aborts at an early stage of development. Thus, each spikelet usually produces only one
kernel. Since the spikelets are arranged in pairs, there is an even number of kernel rows on the ear (Bonnett, 1966; Kiesselbach, 1980; Cheng and Pareddy, 1994). At flowering, the silk of each ovary must elongate beyond the husk in order to be pollinated (Bonnett, 1966; Kiesselbach, 1980). The maize silk merges the function of both stigma and style. The exposed silks capture pollen grains and allow each pollen grain to penetrate the silk and produce a pollen tube. The latter grows through the silk to the ovary where the fertilization takes place.

Maize under drought

The morphology of the ear and tassel and its consequence for pollination explains in part why maize is very susceptible to drought stress at flowering (Salter and Goode, 1967; Tollenaar and Daynard, 1978; Andrade et al., 1999, Saini and Westgate, 2000). The effects of drought stress at flowering on maize plants are due to the limited uptake of nutrients from the soil and, more importantly, to reduced overall photosynthesis in response to tissue dehydration. The amount of assimilates available for plant growth and for ear and silk growth is limited under drought (Andrade et al., 1999). Plant growth rate at flowering is indicative of the capacity of the plant to set kernels. When the rate of plant growth is low due to drought stress, high plant density or other unfavorable environmental conditions the negative effects on the ear are greater than those on other parts of the plant (Andrade et al., 2000).

Compared to anthesis, the emergence of the silk is delayed, which causes the characteristic widening of the anthesis-silking interval (ASI). Moreover, the receptivity of silks declines as the water potential of the silk declines (Schopere et al. 1986). Many silks cannot be pollinated because of a lack of pollen when they appear or because they do not appear at all, and only few kernels are initiated (Edmeades et al. 2000). The most important consequence of drought stress at flowering is a reduction in ovary growth (dry matter accumulation) (Zinselmeier et al., 1995) and, finally, a reduction in the number of kernels per ear. In contrast, kernel weight is affected by drought stress after flowering (Bolaños and Edmeades, 1996).

Several studies shed light on the role and regulation of the anthesis-silking interval (e.g., Edmeades et al. 2000). Thus, it is known that a short ASI is an indicator of the tolerance of maize to drought (and other stress) at flowering. The ASI has been widely regarded as a secondary trait for improving the drought tolerance of tropical maize. However, the ASI, which
is recorded from the appearance of the first silks, does not provide information about the role of synchronous as opposed to asynchronous silk emergence (Campos et al. 2004).

**Growth and emergence of the silk**

In a first phase, the silks grow exponentially from the spikelet to the tip of the husk. Later, and especially after emergence from the husk, they elongate linearly (Cárcova et al., 2003). However, the rate of silk elongation is not constant. It follows a circadian rhythm and is highest early in the photoperiod and decreases gradually during the day (Westgate and Boyer, 1985). The extension dynamics of individual silks is similar at all ovary positions (Cárcova et al., 2003). According to Bassetti and Westgate (1993a), the first silks to emerge from the husks are those from the ovaries on the lower third of the ear (i.e. from positions 6 to 15 on ear primordia with approximately a total of 45 positions on the ovary), while the appearance of silks from the basal flower position is delayed (Bonnett, 1966; Kiesselbach, 1980). Thereafter, silk emergence follows an acropetal sequence of floret differentiation along the ear. The silks of ovaries located at the base of the ear are longer than those of ovaries at the tip, which emerge last (Cárcova et al., 2003).

The silks emerge rapidly on the first and second day of silking (defined as the period during which the silks emerge from the husks). Depending on the variety, all the silks emerge by the fifth to tenth day of silking (Anderson et al., 2004; Bassetti and Westgate, 1993a; Lonnquist and Jugenheimer, 1943; Sadras et al., 1985). Similarly, the rate of silk elongation was most rapid on the first day of silking and decreased gradually thereafter (Anderson et al., 2004; Bassetti and Westgate, 1993a). The seasonal dynamics of silk emergence in the field, which is based on the above-mentioned dynamics of silk emergence of individual plants, is described by a double Gauss model. The model represents the duration of silking, which lasts about 34 days (Lizaso et al., 2003), and is used to predict the daily total number of emerged silks as well as the grain yield (total number of kernels) per area.

The dynamics of silk emergence is affected characteristics of the ear, such as ear length and size of the husk. Cárcova et al. (2003) observed that silk growth of a long-eared hybrid (with a high number of spikelets per ear) was delayed compared to a short-eared hybrid with silks which grew more slowly. The delayed appearance of silks from the basal ovaries may be due to the long distance to the tip of the husk. The shorter the ear, the less time needed to complete
silk emergence (Bassetti and Westgate, 1993a; Sadras et al., 1985). Silk emergence is slower in ears with large husks compared to ears with smaller husks (Anderson et al., 2004). Thus, the onset of silking, as estimated by the appearance of the first silks, actually reflects the age of the flowers (at similar position) of the different plants (Bassetti and Westgate, 1993a).

Silk receptivity
As well as the dynamics of silk elongation, the time, during which the silks are receptive for pollination influences kernel set and grain yield. Peterson (1942) found that when maize silks were prevented from being pollinated, they remained receptive for more than 20 days. Lonnquist and Jugenheimer (1943) reported somewhat shorter periods: 12 and 14 days for an inbred line and a single cross hybrid, respectively. Bassetti and Westgate (1993b) observed the appearance of silks under the microscope and found that the silks senesce (collapse) after seven days after emergence. Lizaso et al. (2003), Cárcova et al. (2003) and Anderson et al. (2004) concluded that exerted silks stay receptive for at least six days, depending on the variety. Considering the effect of the position of the ovary on the dynamics of silk emergence, older silks at the base of the ears lose their receptiveness before the younger silks at the tip of the ear (Andrew, 1952). As a result, senescing silks collapse and become dehydrated, growing pollen tubes, may not reach the ovaries and pollen grains in contact with the surface of the silk cannot germinate and penetrate the silks because the permeability of the cuticle and/or its epidermis changes (Bassetti and Westgate, 1993b).

Pollen viability
The percentage of kernel set decreased sharply when the rate of pollen shedding was low ($\leq 100$ grains cm$^{-2}$ d$^{-1}$) (Bassetti and Westgate, 1994). It was estimated that at least two pollen grains per exposed silk is the minimum required to reach 95% kernel set (Uribelarrea et al., 2002). Pollen viability is quite resistant to drought stress but not heat stress (Hall et al., 1982; Schoper et al., 1986).
Kernel development

The maximum number of grains set at ample pollen supply on one day was usually attained three to five days after emergence of the first silks (Kaeser et al., 2003). Cárcova and Otegui (2007) found that, without being pollinated, the ovaries at the base and in the middle of the ears were always the heaviest and those at the tip were always the lightest. In contrast, the pollinated ovaries showed an exponential increase in weight at all floret positions of open-pollinated plants. Otegui and Melon (1997) suggest that the partitioning of assimilates to the ear during silking is critical to kernel set and is related to the rate of ear growth (length) around silking. Even upon successful pollination and fertilization of ovaries, the plants may be unable to sustain the growth of already fertilized ovaries, which increases the frequency of kernel abortion (Bassetti and Westgate, 1993c; Zinselmeier et al., 1995). Seed set and kernel abortion are required to maintain a balance between sink demand and sink capacity for photosynthates. To maintain ovary growth, a steady flux of carbon is required (Zinselmeier et al., 2000). Moreover, Cárcova et al. (2000) proved that early fertilization of ovaries at the base of the ear promote kernel abortion of late-fertilized ovaries at the tip.

Sweet corn

Many of the methods described in the above-mentioned studies are destructive, which makes it impossible to relate flowering dynamics to the final kernel number of the same plants. Kernels of flint maize on ears of sweet maize can serve as visual markers to overcome this limitation. Maize plants with the sweet-kernel phenotype have a mutation at a particular gene that alters the carbohydrate content in the endosperm. Two classes of mutants are known: class 1 mutants, such as brittle1 (bt1), brittle2 (bt2) and shrunken2 (sh2), accumulate sugars at the expense of starch and have a much lower content of total carbohydrates at maturity. Class 2 mutants, such as amylose extender1 (ae1), dull1 (du1), sugary1 (su1) and waxy1 (wx1), alter the type and amount of polysaccharides (Tracy, 2001). For both classes, the alleles associated with the sweet corn phenotype are recessive. Therefore, pollination of sweet corn ears with pollen of other maize results in tough, starchy kernels, which, at maturity, can be clearly distinguished from the shriveled sweet kernels.
Objectives

It is the main hypothesis of the study that the temporal grain set patterns in maize can be studied by a visual marker system based on sweet maize.

The subsequent objectives are the following:

1. To develop a reliable marker system by implementing the marker system on the basis of vigorous new sweet maize hybrids.
2. Test adequate statistical methods that allow a meaningful analysis of the actual grain set of single ears into a broadly adapted model.
3. To describe on the basis of these innovative tools the principles of grain set patterns.
4. To verify if there are genotypic impacts on grain set patterns.
5. To investigate the influence of mild pre-anthesis drought on grain set patterns.
Chapter 2

The temporal dynamics of kernel set in tropical sweet maize (*Zea mays* L.) determined by visual markers

* A publication based on this chapter has been submitted.

Abstract

The initiation of kernels along the maize ear depends on the temporal dynamics of silk emergence and pollen shedding. We conducted a non-destructive examination of the dynamics of silk emergence of tropical sweet maize; flint-type grains were the visual markers. The silks were pollinated on consecutive days with pollen of sweet maize (recessive allele) on six days and with pollen of flint-type maize (dominant allele) on one day (one pollination treatment for each of the seven possible days). The resulting hard kernels could be distinguished from the shriveled sweet kernels. The time of pollination had a strong effect on kernel set. The highest percentage of daily kernel set was observed on the first day of silking (day 1). It accounted for 31% (2007) and 42% (2008) of the total kernels per ear. The distribution of these kernels followed a bell-shaped curve with a peak at around the position of the tenth kernel from the bottom of the ear. On the following days, kernel set followed a double bell-shaped curve with the peak shifting to the tip of the ear followed by a steady decrease. The minor peak, at the bottom of the ears, almost disappeared by day 4 of silking. More than 90% of the final number of kernels was set within five (2007) or three (2008) days. The visual marker system successfully traced the dynamics of silk emergence, its impact on kernel set, as well as its dependence on environmental conditions during flowering.
Introduction

Maize grain yield is closely associated with the number of ears per plant, number of kernels per ear and kernel weight. The number of kernels per ear is determined during flowering, whereas kernel weight depends on the capacity of the plant to fill grains. The rate of silk emergence and the synchrony with pollen shed determines the number of ovaries that are pollinated in a certain period of time. Consequently, it also influences the number of kernels produced per ear and, therefore, the grain yield.

The silks grow exponentially from the spikelet to the tip of the husk; once they extrude from the husk growth is linear. The rate of silk elongation occurs in a circadian rhythm and is highest early in the photoperiod and decreases gradually throughout the day (Westgate and Boyer, 1985). The dynamics of extension of individual silks are similar at all positions of the ovaries (Cárcova et al., 2003). According to Bassetti and Westgate (1993a), the first silks that emerge from the husks are those of ovaries on the lower third of the ear; silks from the basal positions of the flowers emerge later (Bonnett, 1966; Kiesselbach, 1980). The silks of ovaries at the tip of the ear are the last to emerge (Cárcova et al., 2003).

More than 50 years ago, Peterson (1942) and Lonnquist and Jugenheimer (1943) reported that the growth of the silks and daily kernel set are highest on the first two days of silking. As the rate of silk growth declined thereafter, the daily kernel set diminished correspondingly. Almost 60% of the kernels were set on day 1 (i.e. the first day of silking), and by day 5 more than 95% of the kernels had been set (Peterson, 1942). Bassetti and Westgate (1993b) reported that kernel set was completed even faster, within four days. These studies assessed the dynamics of silk emergence indirectly by quantifying the number and the position of the kernels produced on ears, which were pollinated (only once) on a preselected day during silking. Under natural conditions, however, the silks can be pollinated right after emergence from the husks. Daily manual pollinations might better reflect the natural dynamics of silk emergence and pollination, but it is difficult to determine the formation of the kernels according to the day, on which the corresponding silks were pollinated and the ovaries fertilized.

Many methods in the former studies are destructive, which makes it impossible to relate the dynamics of silking of the plant to its final kernel number. Visual markers can overcome this limitation by using sweet and non-sweet maize. Sweet maize carries mutation at a particular gene, thus altering the carbohydrate content in the endosperm (Tracy, 2001). Alleles associated
with the sweet maize phenotype are recessive. Therefore, pollinating a sweet maize plant with pollen from non-sweet maize results in tough and starchy kernels, which are easy to distinguish from the shriveled kernels at maturity.

The objective of the present study was to investigate the dynamics of silk emergence and its impact on kernel set in maize with daily pollination of the extruded silks of each ear. Daily kernel set along the ear can be visualized by using two different pollen sources and by pollinating each ear on several consecutive days.

**Material and Methods**

*Plant material*

The tropical sweet maize hybrid Insee2 was the pollen receptor (mother plants) and the principal pollen donor. The tropical flint maize hybrid SW4452 (Aekatasanawan et al., 2005), released in 2003, was the pollen donor for pollination on specific days (see below). Insee2 (Aekatasanawan et al., 2001), released in 1999, represents a new generation of sweet maize hybrids, the vigor of which is comparable to that of normal grain types. Insee2 is a sweet maize class 1 mutant and carries the recessive allele at the \(sh_2\) locus. Both maize varieties were developed at the National Corn and Sorghum Research Center (Suwan Farm) in Thailand.

*Experimental site and experimental design*

The experiments were conducted at Suwan Farm, Thailand, in the dry season (November to April) 2006/07 and 2007/08. The climate at Suwan Farm (14.5°N, 101°E, 360 m above sea level) is a tropical lowland climate (Gerpacio and Pingali, 2007). The soil at Suwan Farm is a Rhodic Kandiustox Oxisol (USDA taxonomy) (Land Development Department, 2009).

The experiments were arranged in two blocks, each with six plots. Each plot consisted of six rows, 6 m long and 0.75 m apart, with 21 plants per row. The distance between adjacent plants in a row was 0.3 m, resulting in a population density of 4.44 m\(^{-2}\). Three seeds were sown manually in each mound and redundant plants were removed at the 4-leaf stage. Prior to sowing, 25 kg N ha\(^{-1}\) and 30 kg P ha\(^{-1}\) were applied; 115 kg N ha\(^{-1}\) was applied one month after sowing. Herbicides and insecticides were applied as required according to local practices. The experiments were sprinkler-irrigated four times during the first three weeks after sowing.
Thereafter, the experiments were furrow-irrigated once a week (~30 mm). Several rows of pollen donor plants (sweet and flint maize) were grown around the plots in both years. The air temperature, rainfall, relative air humidity, the speed and direction of the wind, and water evaporation were measured every three hours at the experimental station Suwan Farm, where also the daily sunshine duration was recorded. Thermal time (growing degree days) was calculated as the sum of the daily mean temperature above 8 °C.

*Pollination procedure*

Twenty-eight homogeneous plants were randomly chosen from the four middle rows in each plot and tagged two weeks before flowering. Their ear primordia were covered with glassine bags and checked daily for extruded silks. The growing ears were pollinated on seven consecutive days, starting separately for each plant on the day when the first silks appeared. Seven sets of four ears per plot received pollen from sweet maize on six days and pollen from flint maize on one day. The days during silking, on which the ears were pollinated with flint pollen varied from one to seven (seven pollination treatments). In practice, the ears were uncovered, pollinated and immediately covered again with paper bags until the next day or, after the seventh pollination day, until harvest.

*Phenotypic data*

The average time from sowing to male and female flowering of the plants in each experimental plot and of the pollen donor plants was recorded as the number of days from sowing to the day, on which 50% of the plants in the middle two rows had started to shed pollen and to extrude silks, respectively. The first day of pollination (i.e. the date of first silk emergence) of each hand-pollinated plant was also recorded.

All the hand-pollinated ears were harvested as soon as it was possible to clearly distinguish between the hard flint and the shriveled sweet maize kernels. The ears were air-dried for about one week with the objective of increasing the contrast between the two kernel types. Then they were divided into kernel-wide segments, and the shriveled and hard kernels in each segment were counted. Thus, the total kernel number and the number of shriveled and hard kernels as well as the number of kernel rows and the number of kernel-bearing positions per ear were
automatically recorded. The hard kernels represent the daily kernel set, according to the day, on which the ears had been pollinated by pollen of flint maize.

In 2008, the ears of 60 representative and uniform plants (five plants per plot) were covered with glassine bags before silking. During the first week of silking, the length of the extruding silks was recorded daily and the silks were cut back to the tip of the husk leaves. The non-pollinated ears were harvested two weeks after flowering, and the ovaries per position along the ear were counted.

Statistical analysis

The following traits were determined from the number and distribution of the shriveled and hard kernels per ear: (i) the total number of kernels per ear, (ii) the number of kernels per ear set on the day of pollination by flint maize (daily kernel set), (iii) the number of kernels per position (i.e. the distribution of kernels along the ear), and (iv) the number of kernels per position that were set on the day of pollination by flint maize (i.e. the distribution of daily kernel set along the ear). All the ears that produced kernels with a clearly unusual, sometimes incomplete daily grain set were removed before the analysis. There were about 18 and 4% such ears in 2007 and 2008, respectively.

Data on ovaries and kernels were analyzed by mean of the np package (nonparametric kernel smoothing methods for mixed data types; Hayfield and Racine, 2008) in R (R Development Core Team, 2009). The regression models included different explanatory variables: (i) “day” in the case of daily kernel set, (ii) “position along the ear” in the case of distribution of ovaries and kernels along the ear, and (iii) “day” and “position along the ear” in the case of distribution of daily kernel set along the ear. Before computing the kernel regression estimates, data-driven bandwidths were computed with npregbw(), using a local-linear regression estimator and the Kullback-Leibler cross-validation method. The tolerance on the value and on the position of located minima of the cross-validation function were left at their default values, except for the analyses that included “position along the ear” as an explanatory variable. In those cases, the values were set to $10^{-4}$. Each process to find extrema of the cross-validation function was repeated five times. The resulting bandwidths were used to evaluate the regression model with npreg() and to make predictions for all combinations of the supplied explanatory variables. The
significance of explanatory variables was tested with npsigtest(). The number of bootstrap replications was left at its default value.

The daily kernel set and distribution along the ear, which resulted from the above-mentioned analyses, are referred to as absolute data. While the absolute daily kernel set was determined for seven sets of ears (one for each of the seven pollination treatments), the (average) total number of kernels per ear of the entire set was recorded in each year. Since the sum of the seven values of absolute daily kernel set did not necessarily equal the total number of kernels per ear, the absolute daily number of kernels was divided by the sum of the respective seven values and multiplied by the average number of total kernels. The resulting data are referred to as predicted daily kernel set and enabled a meaningful comparison of daily kernel set per pollination treatment with respect to the entire data set. This procedure was carried out for the daily kernel set per ear and per position along the ear.

The data for daily silk elongation were grouped according to the day of silking, irrespective of the female flowering date. Data analysis was done by mean of the np package (Hayfield and Racine, 2008) in R (R Development Core Team, 2009), calculating data-driven bandwidths before calculating the kernel regression estimate. The explanatory variable for silk length was “day”.

Results

Meteorological data

Table 1 gives the meteorological data of both experiments. During the first three weeks after sowing, the temperatures and the relative air humidity were higher in 2007 than in 2008, whereas later during the vegetative development, from 22 to 63 days after sowing (DAS), the temperatures were lower. During the flowering period, there were more windy days and there was more sunshine in 2007 than in 2008. At the same time, the relative air humidity was lower and evaporation higher.
Table 1: Average daily minimum and maximum temperatures (Temp), cumulative growing degree days (GDD), average daily minimum and maximum relative air humidity (RH), cumulative sunshine duration (Sun), average daily water evaporation from the soil surface (Evap), total precipitation (Rain), average daily wind speed (Wind), and number of days with wind speed above 5 m/s (Windy days) during vegetative plant growth (1-21 and 22-63 days after sowing (DAS)) and during the flowering period (64-78 DAS) in two field experiments conducted at Suwan Farm.

<table>
<thead>
<tr>
<th>Year</th>
<th>DAS</th>
<th>Temp</th>
<th>RH</th>
<th>Windy days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Min</td>
<td>GDD</td>
<td>Max</td>
</tr>
<tr>
<td>°C</td>
<td>°C</td>
<td>°Cd</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>2007</td>
<td>1-21</td>
<td>32.0</td>
<td>21.1</td>
<td>347.8</td>
</tr>
<tr>
<td></td>
<td>22-63</td>
<td>29.2</td>
<td>17.3</td>
<td>556.7</td>
</tr>
<tr>
<td></td>
<td>64-78</td>
<td>28.8</td>
<td>18.0</td>
<td>200.6</td>
</tr>
<tr>
<td>2008</td>
<td>1-21</td>
<td>28.2</td>
<td>17.1</td>
<td>266.1</td>
</tr>
<tr>
<td></td>
<td>22-63</td>
<td>31.2</td>
<td>18.2</td>
<td>617.6</td>
</tr>
<tr>
<td></td>
<td>64-78</td>
<td>30.1</td>
<td>19.9</td>
<td>225.0</td>
</tr>
</tbody>
</table>

Flowering and silk elongation

The average time to male flowering was 66.0 days in 2007 and 66.6 days in 2008, compared to which female flowering was delayed by 1.5 and 2.0 days. The average cumulative length of the silks per ear was 29.9 cm (2008), with a standard error of 0.47 cm. Silk elongation was at a maximum on the first and second days of silking (5.4 cm d⁻¹) and decreased thereafter (from 5.2 cm d⁻¹ on day 3 to 2.4 cm d⁻¹ on day 7), corresponding to the percentage of daily kernel set (see below).

Table 2: Average values ± standard errors for the number of ovaries per non-pollinated ear and the total number of kernels per mature ear in 2007 and 2008 (KNO). Ears: number of evaluated ears; KR: percentage of ears with 12, 14 or 16 kernel rows.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±</td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Non-pollinated ears</td>
<td>557.7 ± 7.0</td>
<td>60</td>
<td>51.7</td>
<td>45.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Mature ears (2007)</td>
<td>452.1 ± 4.5†</td>
<td>271</td>
<td>46.9</td>
<td>48.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Mature ears (2008)</td>
<td>421.3 ± 4.3†</td>
<td>320</td>
<td>43.8</td>
<td>45.9</td>
<td>10.3</td>
</tr>
</tbody>
</table>

†Bootstrapping with npsigtest() revealed a significant effect of the year (P = 0.01) on KNO.
Figure 1: Average number of ovaries per position on the non-pollinated ears (ovaries 2008) (average of 60 ears) and number of kernels per position along mature ears in 2007 (kernels 2007) (average of 271 ears) as well as in 2008 (kernels 2008) (average of 320 ears). Vertical segments indicate ± standard errors.

Kernel set in general

The 271 and 320 hand-pollinated ears produced, on average, 452 (± 4.5, standard error) kernels in 2007 and 421 (± 4.3) kernels in 2008 (Table 2). About 82 and 76% of the ovaries developed into harvestable kernels in both years, compared to the average number of ovaries on non-pollinated ears (554 ± 7.0) recorded in 2008. Most of the hand-pollinated ears produced 12 or 14 rows of kernels along the cob. Less than 11% of the ears had 16 kernel rows (Table 2). Similar values were observed for the number of rows of ovaries along the non-pollinated ears. The average number of ovaries per position remained constant at about 13 (± 0.16) from the bottom of the ears up to ovary position 35. Between positions 36 and 49, the values decreased sigmoidally to zero. They fell below 12 at position 39 (Fig. 1).

The distribution of kernels along the ears was somewhat different, compared to the distribution of ovaries. The number of kernels per position started decreasing at much lower positions and decreased more slowly than the number of ovaries did. The values fell below 12 already at position 27 in 2007 and at position 24 in 2008 (Fig. 1) and decreased below 0.5 at positions 42 and 43, indicating that kernel set decreased at the tip of the ear, but not at the bottom or in the middle of the ear.
**Table 3:** Number of kernels representing absolute daily kernel set (ADK) and the corresponding percentages in relation to the sum of the seven values of daily kernel set (%ADK) or in relation to the total number of kernels per ear (%KNO) (see Table 2) in 2007 and 2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>Traits</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>ADK†</td>
<td>157.3</td>
<td>141.9</td>
<td>88.8</td>
<td>60.1</td>
<td>28.7</td>
<td>22.4</td>
<td>12.0</td>
<td>511.2</td>
</tr>
<tr>
<td></td>
<td>%ADK</td>
<td>30.8</td>
<td>27.8</td>
<td>17.4</td>
<td>11.8</td>
<td>5.6</td>
<td>4.4</td>
<td>2.3</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>%KNO</td>
<td>34.8</td>
<td>31.4</td>
<td>19.6</td>
<td>13.3</td>
<td>6.3</td>
<td>5.0</td>
<td>2.6</td>
<td>113.0</td>
</tr>
<tr>
<td>2008</td>
<td>ADK†</td>
<td>188.3</td>
<td>153.4</td>
<td>70.5</td>
<td>29.1</td>
<td>4.8</td>
<td>1.3</td>
<td>1.1</td>
<td>448.5</td>
</tr>
<tr>
<td></td>
<td>%ADK</td>
<td>42.0</td>
<td>34.2</td>
<td>15.7</td>
<td>6.5</td>
<td>1.1</td>
<td>0.3</td>
<td>0.2</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>%KNO</td>
<td>44.7</td>
<td>36.4</td>
<td>16.7</td>
<td>6.9</td>
<td>1.1</td>
<td>0.3</td>
<td>0.3</td>
<td>106.4</td>
</tr>
</tbody>
</table>

† Bootstrapping with npsigtest() revealed significant effects of the year (\(P = 0.001\)) and the pollination day (\(P = 0.01\)) on ADK.

**Daily kernel set**

The daily kernel set was highest on the first day of silking in both years. From pollinations on day 1, 157 (± 12) kernels resulted in 2007 and 188 (± 14) kernels in 2008 (Table 3), corresponding to 31 and 42% of the total absolute daily kernel set. On the following days, kernel set decreased gradually and accounted for less than 12% of the total of the absolute daily kernel set on day 4 and later in both years. Although the ranking of the pollination days was identical in both years, the relative importance of individual days differed. While the percentage of kernels resulting from pollination on days 1 and 2 were smaller in 2007 than in 2008, it was higher on days 3 to 7. As a consequence, the cumulative percentage of absolute daily kernel set reached 90% by day 5 of silking in 2007 and by day 3 in 2008. The sum of the predicted daily values overestimated kernel set by 13% in 2007 and 6.4% in 2008.

Absolute kernel set along the ear on day 1 followed a bell-shaped curve with a peak at position 10. On the following days, kernel set followed a double bell-shaped curve, the main peak of which moved towards the tip of the ear and became smaller the longer pollination was delayed (data not shown). This development over time was observed in both years. However, the individual curves differed. For example, the highest probability of being pollinated on day 2 was found for ovaries between positions 18 and 25 in 2007 and for ovaries between positions 19 and 28 in 2008. The peak of the curve on day 2 in 2007 was three positions below the peak
on day 2 in 2008 (positions 20 and 23, respectively). The probability of being pollinated on day 3 was highest for ovaries between positions 26 and 32 in 2007 and between positions 29 and 34 in 2008 (data not shown).

The sum of the seven curves of daily kernel set approximated the total kernel set in the middle and at the tip of the ear in 2008, which proved the high quality of the model. At lower positions, the absolute kernel set exceeded the total number of kernels per position in both years. This overestimation was largest for some positions at the bottom of the ear where it accounted for up to 22% in 2007 and 12% in 2008 (data not shown).

**Figure 2:** Average individual and cumulative curves of predicted daily kernel set per position along ears of Insee2 on day 1 to day 6 of silking in 2007 and 2008. The cumulative curves of days 1 to 7 are to the same as those in Fig. 1. Vertical segments represent ± standard errors.
Figure 2 shows both the individual and the cumulative curves of predicted daily kernel set along the ear. The number of kernels, the ovaries of which were pollinated on day 1 was highest in the lower middle part of the ear, between positions 4 and 15, in both years. Between positions 3 and 19, more than 80% of the final number of kernels had been pollinated by day 2 in 2007. The cumulative kernel number per position on the first three days was greater in 2008 than in 2007, and the segment along the ear, where more than 12 kernels were set, was considerably longer, which reflects the faster emergence of silk during the first three days of silking in 2008. The cumulative curves on day 4 were similar in both years. The higher total number of kernels per ear in 2007 resulted from improved kernel set toward the tip of the ear (between positions 22 and 44), which resulted from the pollination on days 5 to 7. In 2007, kernels were still being initiated on those days, although the number of pollinated ovaries on days 6 and 7 was not significantly higher than on day 5 (data not shown). In contrast, in 2008, kernel set was practically completed within four days of silking (Table 3). These results indicate that the silks emerged faster in 2008 and that silk emergence was completed earlier than in 2007. The lower temperatures at flowering, the relatively dry air and the rather windy conditions in 2007 were probably responsible for these differences in silking dynamics.

**Discussion**

The observed rate of silk elongation, which was highest during the first three days of silking and decreased thereafter continuously, is in agreement with previously reported rates (Anderson et al., 2004; Bassetti and Westgate, 1993a; Lonnquist and Jugenheimer, 1943; Sadras et al., 1985). The period, during which the silks grow, can last up to 10 days (Anderson et al., 2004), depending on the variety and the environmental conditions. In the present study the silks probably continued to grow for the same length of time without being pollinated. However, it is not possible to characterize this period further, because the measurements were stopped after seven days. Nevertheless, the data on silk elongation complement the data on daily kernel set. While the data on silk elongation give the average silk growth per day, the data on kernel set gives the approximate number of silks that had been extruded and were receptive on the respective days. Assuming a strong relationship between the number of emerged silks and the number of kernels, as shown by Bassetti and Westgate (1993b), the data
of predicted daily kernel set can be regarded as an approximation of the number of receptive silks.

Both the rate of silk elongation and daily kernel set were higher on the first and second days of silking. Silk elongation diminished notably after the third day, but not as quickly as daily kernel set. Thus, a fast rate of silk elongation early during the silking period ensures that a large number of silks emerge and are pollinated as soon as possible, but it does not guarantee high kernel set on the following days. The method of measuring silk elongation did not allow us to differentiate among ovary positions. Nevertheless, it is deduced from the distribution of daily kernel set along the ear that the silks of ovaries on the upper part of the ear elongated more slowly than the silks of ovaries on the lower part; the latter were fecundated earlier, and the rate of silk growth decreased from day 3 to day 7. This is in agreement with the results of Cárcova and Otegui (2007), who showed that there are considerable differences in the rate of silk elongation, depending on the position of the ovaries; the rate of silk growth at the ear tip were always lower than at the base and in the middle part of the ear. Furthermore, Tollenaar and Daynard (1978) showed that the development of kernels at the tip of the ear lagged behind the development of kernels at the base of the ear from the start of silk growth to the onset of linear dry matter accumulation of the kernels. Therefore, the ontogenetic delay in the development of ovaries at higher positions was aggravated by the lower rate of silk growth.

This has several consequences: The ear is a weak sink at flowering, and the early development of kernels depends on a continuous supply of assimilates from concurrent photosynthesis (Zinselmeier et al., 2000). Ovaries fertilized early (at the base of the ear) can absorb more assimilates than ovaries fertilized late (at the tip of the ear), and there is strong competition for assimilates between developing kernels. Kernels at higher positions on the ear may be aborted because of this competition, even though they have already been initiated (Anderson et al., 2004; Otegui and Melon, 1997; Schussler and Westgate, 1991; Zinselmeier et al., 2000).

The ears of the plants in the present study were pollinated on seven consecutive days, avoiding an impact of the varying pollen supply within a natural plant stand. Thus, the differences in the onset of kernel development along the ears probably resulted in strong competition for assimilates, which, in turn, probably caused many kernels at the tip of the ears to abort. It has been shown that the timing of pollination affects kernel set. Struik and Makonnen (1992) and Cárcova and Otegui (2001) showed that the number of kernels per ear and, in particular, the
number of kernels on the ear tip, was higher when ears were pollinated only once when silks of all or almost all of the ovaries were visible, than when ears were pollinated continuously from the first day of silking onwards. In other words, delayed pollination of early-silking ovaries allows a greater number of late-silking ovaries to set kernels (Cárcova et al., 2000; Cárcova and Otegui, 2001). However, kernels on the ear tips may also abort when the silks of all the ovaries are pollinated at the same time (Ogiwara et al. 1995). Moreover, the differences between the two years showed how environmental conditions can influence kernel number and the temporal dynamics of kernel set, even when the field management is standardized.

As well as physiological and environmental determinants, the fact that only about 75% of the average number of ovaries per non-pollinated ear developed into harvestable kernels (in 2008) may also be due to the fact that pollinations were carried out without cutting back the silks. As Hallauer and Sears (1966) reported, when the silks of ears were cut back to 0.5" below the tip of the husk on the first day of silking and were pollinated 0, 2 or 4 days later, the ears produced 26% more kernels than ears, the silks of which were not cut back. Although a high grain yield is usually desirable, the objective of this study was not to maximize kernel set by controlled pollination. The objective was to model the temporal dynamics of kernel set along the ear, which resulted from daily pollinations in an attempt to simulate continuous pollination under natural conditions.

The ranking of daily kernel set was identical in both years with the highest number of kernels set on day 1. However, the predicted values of daily kernel set differed between the years (Table 3, Fig. 2). The lower values of daily kernel set during the first three days of silking evidenced slower silk growth in 2007 than in 2008. This was probably due to the higher temperatures at flowering in 2008, which accelerated plant development. Lizaso et al. (2007) reported that fast silk extrusion is associated with higher kernel number. Moreover, the low relative air humidity and the high wind speed that was observed in 2007 could have additionally reduced plant development and silk growth during flowering in 2007. Despite the slower silk extrusion, the final kernel number per ear was still larger in 2007 than in 2008. It is possible that the environmental conditions before flowering in 2007 favored the development of larger ears with more ovaries. The higher temperatures during the first three weeks after sowing in 2007, compared to 2008 (Table 1), probably allowed for a faster establishment of the canopy. Later during vegetative growth, the lower temperatures might have slowed down the
development of plants in 2007, compared to 2008. It has been shown that a slower vegetative development of maize can lead to a larger number of spikelets per ear and to higher grain yield (Chase, 1964). It is therefore likely that the plant produced more ovaries in 2007 than in 2008 because their growth was slower at the time when the tassels and the ears were initiated. The larger number of ovaries per ear might have resulted in higher kernel numbers, despite of lower temperatures, lower relative air humidity, elevated evapotranspiration and higher wind speed during flowering in 2007, compared to 2008. However, since ovaries were only examined in 2008, this hypothesis cannot be verified.

Two different kernel types (flint and sweet) enabled us to estimate the proportion of kernels fertilized at each position on the ear on each of the seven days of silking. Thus, the temporal dynamics of silk emergence could be visualized for each position along the ear. The visual marker system offers a reliable tool for studying the impact of a range of environmental conditions and genotypic impacts on grain set.
Chapter 3

Genotypic differences in the temporal dynamics of kernel set in tropical sweet maize (Zea mays L.) determined by visual markers

Abstract
Because of their vigor, modern sweet maize hybrids can be grown on a large scale and in a broad range of areas. Nonetheless, the genotypes/hybrids differ in potential grain yield as well as in the size and shape of ears. A fast and synchronous emergence of silks is the key to high kernel set and high grain yield. A non-destructive examination of the dynamics of silk emergence of two tropical sweet maize hybrids was conducted. Flint kernels were the visual markers on ears of sweet maize, which had been pollinated with flint maize pollen once during the seven days following silk emergence and six times with sweet maize pollen. The highest number of kernels always resulted from pollination that occurred on the second day of silking (day 2). The distribution of kernel set on day 1 generally followed a bell-shaped curve with a peak for the lower part of the ear. On the following days, the curve was a double bell shape with the main peak shifting to the tip of the ear while becoming smaller; the minor peak was found for the bottom of the ear and soon disappeared. The pattern of daily kernel set and the distribution of kernels along the ear were characteristic of both hybrids. Kernel set progressed faster for Sugar73 than for Hibrix10 on days 1 to 4. Nevertheless, with time, Hibrix10 produced more kernels, and there were no significant differences in the final kernel number per ear of both genotypes. More than 90% of the final number of kernels per ear was set during the first five days, with the exception of Sugar73 in 2005, which reached 90% within four days.
Introduction

Large increases in the grain yield of maize were mainly the result of the improved stress tolerance of modern hybrids and their adaption to high plant density, whereas the grain yield per plant did not or only slightly increase (Duvick, 2005). The lower the plant density of a maize stand, the more grain yield per area depends on the potential yield of single plants. The latter is considered to be the potential yield of the genotype, when only one genotype (e.g. one hybrid) is grown in the field or where different genotypes are harvested separately. Low to modulate population densities (up to approx. 5 plants m$^{-2}$) are, therefore, best suited to investigate the process of kernel set on individual ears, a major determinant of grain yield. This process depends on the temporal dynamics of silk extrusion when the supply of viable pollen is not limited and when the plants do not experience yield-limiting biotic or abiotic stresses (e.g. drought); a strong correlation between the number of extruded silks and the number of kernels is assumed (Bassetti and Westgate, 1993b).

Bassetti and Westgate (1993a) reported that the percentage of exposed silks during the first eight days of silking was similar for all flower positions on the ears of two maize genotypes, and that the rate of silk elongation was always highest when the silks appeared from the husks and gradually decreased thereafter. However, the rate of silk elongation of the genotypes on the first three days of silking varied by up to a factor of 2. Cárcova et al. (2003) observed that the shape of the ear also influenced the dynamics of silk emergence. Silk growth of a long-eared hybrid was later than that of a short-eared hybrid (Cárcova et al. 2003). Borrás et al. (2007); Pagano et al. (2007) also reported genotypic differences in silk elongation, which were positively associated with the rates of plant growth and ear growth. The above-mentioned relationship between the number of emerged silks and the number of kernels on the ear suggests that a high rate of silk elongation favors the rate of kernel set and, therefore, high grain yield.

We have optimized a methodology, whereby the process of kernel set of sweet maize can be visualized by means of controlled pollination with pollen of sweet and flint or dent maize on specific days. Sweet maize is the model for quantifying the grain set of maize over time without any direct disturbance by the pollen receptor plant, which would hinder the development of the kernels.
The objectives of the present study were to assess (i) whether the temporal dynamics of kernel set differ between two genotypes, which produce ears of unequal size and shape, and (ii) whether such differences between genotypes are stable across years.

**Material and Methods**

*Plant material*

The tropical sweet maize hybrids Hibrix10 (Pacific Seeds Ltd.), released in 1999, and Sugar73 (Novartis Seeds Ltd.), released in 1999, were the pollen receptors (mother plants) and the principal pollen donors. The tropical flint maize hybrid SW3851 (Aekatasanawan et al., 1998), released in 1997, was the pollen donor on specific days. All the hybrids were released in 1999. Both of the sweet maize hybrids belong to a new and very vigorous class of sweet maize genotypes. Hibrix10 produces long ears (~20 cm long at the milk stage (R3), Pacific Seeds Ltd.) compared to Sugar73, which produces shorter ears (~18 cm long at milk stage (R3), Novartis Seeds Ltd.). Both hybrids are mutants of class 1 and carry the recessive allele at the \textit{sh2} locus. This allele is responsible for the formation of large, inflated, translucent kernels, which are crisp and sweet at the milk stage but collapse on drying and become angular and brittle (Tracy, 2001). Therefore, kernels of sweet maize plants pollinated with pollen of non-sweet maize are easy to distinguish from the shriveled kernels resulting from pollination with pollen of sweet maize.

*Experimental site and experimental design*

Two experiments were conducted at the National Corn and Sorghum Research Center, Thailand (Suwan Farm) in the dry season (November to April) in 2003/04 and 2004/05. They were arranged in two blocks with six plots. Each plot consisted of six rows, 6 m long and 0.75 m apart, with 21 plants per row. The distance between adjacent plants in a row was 0.3 m, resulting in a population density of 4.44 plants m$^{-2}$. The four middle rows contained sweet maize, the two border rows flint maize. Both sweet maize hybrids were assigned randomly to three plots within each block. Three seeds were sown manually in each mound and redundant plants were removed at the 4-leaf stage. Prior to sowing, 25 kg N ha$^{-1}$ and 30 kg P ha$^{-1}$ were applied; 115 kg N ha$^{-1}$ were applied one month after sowing. Herbicides and insecticides were
applied as required according to local practices. The experiments were sprinkler-irrigated four times during the first three weeks after sowing. Thereafter, they were furrow-irrigated once a week (~30 mm). Several rows of pollen donor plants (sweet and flint maize) were grown around all the blocks in both years.

**Pollination procedure**

The ear primordia of plants in the four middle rows of each plot were covered with glassine bags and checked daily for extruded silks. Seven plants, the first silks of which extruded on the same day, were randomly chosen in each plot, leading to a total of 42 ears per experiment. During the week following the emergence of the first silks, these growing ears were pollinated daily with pollen from sweet maize on six days and pollen from flint maize on one day. Each of the seven plants per plot was pollinated by pollen of flint maize on a different day (i.e. days 1 to 7). The ears were immediately covered after the pollinations.

**Phenotypic data**

All the hand-pollinated ears were harvested as soon as it was possible to distinguish between the hard flint and the shriveled sweet maize kernels. The ears were air-dried for about one week with the objective of increasing the contrast between the two kernel types. Then they were divided into kernel-wide segments and the shriveled and hard kernels in each segment were counted. Thus, the total kernel number and the number of shriveled and hard kernels as well as the number of kernel rows and the number of kernel-bearing positions per ear were automatically recorded.

**Data analysis**

The hard kernels represent the daily kernel set, according to the day of silking on which pollen of flint maize was supplied. The following traits were determined from the number and distribution of the shriveled and hard kernels along the ear: (i) the total number of kernels per ear, (ii) the number of kernels per ear set on the day, on which flint-type pollen had been supplied (daily kernel set), (iii) the number of kernels per position (i.e. the distribution of kernels along the ear) and (iv) the number of kernels per position that were set on the day of pollination by flint-type pollen (i.e. the distribution of daily kernel set along the ear). All the
ears with incomplete or unusual grain set were removed before the analysis (about 9 and 11% in 2004 and 2005, respectively).

Data for kernels were analyzed by means of the package \textit{np} (nonparametric kernel smoothing methods for mixed data types; Hayfield and Racine, 2008) in R (R Development Core Team, 2009). The regression models included different variables: (i) “genotype” in the case of total number of kernels per ear, (ii) “day” and “genotype” in the case of daily kernel set, (iii) “genotype” and “position along the ear” in the case of distribution of kernels along the ear and (iv) “day”, “genotype” and “position along the ear” in the case of distribution of daily kernel set along the ear.

Before computing the kernel regression estimates, data-driven bandwidths were computed with \textit{npregbw()} using a local-linear regression estimator and the Kullback-Leibler cross-validation method. The tolerance for the value and the position of located minima of the cross-validation function were kept at default values, except for the analyses, which included “position along the ear” as a variable. In the latter cases, the tolerance value was set at $10^{-4}$. Each calculation to determine the extrema of the cross-validation function was repeated five times. The resulting bandwidths were used to evaluate the regression model with \textit{npreg()} and to make predictions for all combinations of the supplied variables. The significance of explanatory variables was tested with \textit{npsigtest()}. The number of bootstrap replications was left at its default value.

The daily kernel set and distribution along the ear, which resulted from the above-mentioned analyses, are referred to as absolute data. While the absolute daily kernel set was determined for seven sets of ears (one for each of the seven pollination days), the (average) total number of kernels per ear of the entire set was recorded in each year. The sum of the seven values of absolute daily kernel set did not, therefore, necessarily equal the total number of kernels per ear. The absolute daily number of kernels was divided by the sum of the respective seven values and multiplied by the average number of total kernels per ear. The resulting data are referred to as the predicted data of daily kernel set and enabled a meaningful comparison of daily kernel set with respect to the entire data set. This procedure was carried out for both the daily kernel set per ear and per position along the ear.
Results

Meteorological data
There were more windy days during the flowering period in 2004 than in 2005, and the temperature and relative air humidity were lower (Table 1). Although the experiments were conducted during the dry season, it rained on five days during flowering in 2005, corresponding to about two thirds of the water applied at each irrigation. There was no rainfall during flowering in 2004.

Table 1: Average daily minimum and maximum temperatures (Temp), relative air humidity (RH), water evaporation from the soil surface (Evap), wind speed (Wind) and the number of days with wind speed above 5 m/s (Windy days) and total amount of rainfall around flowering (Period) in two field experiments conducted at Suwan Farm in 2004 and 2005.

<table>
<thead>
<tr>
<th>Year</th>
<th>Period</th>
<th>Temp Max</th>
<th>Temp Min</th>
<th>RH Max</th>
<th>RH Min</th>
<th>Evap mm</th>
<th>Wind m/s</th>
<th>Windy days d</th>
<th>Rainfall mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>14</td>
<td>31.7</td>
<td>17.5</td>
<td>83.2</td>
<td>32.3</td>
<td>7.0</td>
<td>2.8</td>
<td>3</td>
<td>0.0</td>
</tr>
<tr>
<td>2005</td>
<td>12</td>
<td>32.9</td>
<td>21.8</td>
<td>90.3</td>
<td>61.6</td>
<td>6.7</td>
<td>2.0</td>
<td>0</td>
<td>21.8</td>
</tr>
</tbody>
</table>

Kernel set in general
At least 37 ears were evaluated per genotype and year. The average kernel number of the genotypes per ear (KNO) was similar in both years but differed between years, being higher in 2005 than in 2004 (Table 2). The higher KNO was accompanied by a higher percentage of ears with 16 kernel rows, particularly in the case of Sugar73. Most of the ears produced 14 or 16 rows of kernels along the cob. Less than 14% of the ears had 12 kernel rows. There were no significant differences between years in the ratio of ears with 12, 14 and 16 kernel rows between hybrids (Chi-square = 5.1, P-value = 0.40 in 2004 and Chi-square = 2.9, P-value = 0.87 in 2005).
Table 2: Average values ± standard errors of the number of kernels per ear in 2004 and 2005 (KNO), the number of evaluated ears (Ears) and the percentage of ears with 12, 14 or 16 kernel rows (KR).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Year</th>
<th>KNO</th>
<th>Ears</th>
<th>12 KR %</th>
<th>14 KR %</th>
<th>16 KR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibrix10</td>
<td>2004</td>
<td>548.8 ± 10.3</td>
<td>37</td>
<td>13.5</td>
<td>67.6</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>593.2 ± 11.9</td>
<td>37</td>
<td>7.9</td>
<td>47.4</td>
<td>42.1</td>
</tr>
<tr>
<td>Sugar73</td>
<td>2004</td>
<td>557.7 ± 10.0</td>
<td>37</td>
<td>5.4</td>
<td>70.3</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>593.0 ± 11.9</td>
<td>37</td>
<td>2.7</td>
<td>27.0</td>
<td>67.6</td>
</tr>
</tbody>
</table>

† Bootstrapping with npsigtest() revealed a significant effect of the year (P = 0.01) on KNO.

In 2004, the average number of kernels per position along the ear remained constant at approximately 14 (± 0.2) from the bottom of the ears to position 26 (Fig. 1 G). Between positions 27 and 48 the values decreased sigmoidally to zero and fell below 12 at position 36 without significant differences between genotypes. In 2005, both genotypes produced slightly more kernels per position from the bottom of the ear up to position 30, which was due to the larger proportion of ears with 16 kernel rows. Hibrix10 had significantly more kernels than Sugar73 toward the tip of the ears (i.e. between positions 37 and 48) in 2005. The results proved that kernel set at the bottom of the ears of both genotypes responded to almost the same extent to year-specific environmental conditions. At the tip of the ears, however, this was clearly not the case.

Daily kernel set

The daily kernel set was highest on the second day of pollination for both genotypes (Table 3). In 2004, the absolute daily kernel set was the same for Hibrix10 and Sugar73. The 141 (± 35) and 150 (± 29) kernels that resulted from pollinations on day 1 and day 2, respectively, corresponded to about 26% and 27% of the sum of the seven daily values. The percent of absolute daily kernel set (%ADK) decreased from day 2 to day 7. In 2005, the %ADK of Hibrix10 was lower than that of Sugar73 on days 1 and 2 but higher on days 3 to 7 because the percentage of kernels set after day 2 decreased rapidly for Sugar73. More than 90% of the kernels resulted from pollination during the first five days of silking, with the exception of Sugar73 in 2005, where this percentage was reached one day earlier. The sum of the ADK
values showed good correspondence to the total kernel number of both genotypes in 2004, indicating an excellent predictive value of ADK with respect to KNO. In 2005, the sum of the ADK values of Hibrix10 and Sugar73 underestimated KNO by 9% and 36%, respectively.

**Table 3:** Absolute daily kernel set (ADK), percent of absolute daily kernel set (%ADK) and percent of the total number of kernels per ear (%KNO) of two hybrids in 2004 and 2005.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Year</th>
<th>Traits</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibrix10</td>
<td>2004</td>
<td>ADK</td>
<td>141.3</td>
<td>150.3</td>
<td>104.8</td>
<td>79.4</td>
<td>47.8</td>
<td>21.3</td>
<td>7.3</td>
<td>552.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%ADK</td>
<td>25.6</td>
<td>27.2</td>
<td>19.0</td>
<td>14.4</td>
<td>8.6</td>
<td>3.9</td>
<td>1.3</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%KNO</td>
<td>25.7</td>
<td>27.4</td>
<td>19.1</td>
<td>14.5</td>
<td>8.7</td>
<td>3.9</td>
<td>1.3</td>
<td>100.6</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>ADK</td>
<td>140.7</td>
<td>167.1</td>
<td>101.7</td>
<td>67.6</td>
<td>36.6</td>
<td>16.8</td>
<td>10.4</td>
<td>540.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%ADK</td>
<td>26.0</td>
<td>30.9</td>
<td>18.8</td>
<td>12.5</td>
<td>6.8</td>
<td>3.1</td>
<td>1.9</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%KNO</td>
<td>23.7</td>
<td>28.2</td>
<td>17.1</td>
<td>11.4</td>
<td>6.2</td>
<td>2.8</td>
<td>1.8</td>
<td>91.2</td>
</tr>
<tr>
<td>Sugar73</td>
<td>2004</td>
<td>ADK</td>
<td>141.3</td>
<td>150.3</td>
<td>104.8</td>
<td>79.4</td>
<td>47.8</td>
<td>21.3</td>
<td>7.3</td>
<td>552.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%ADK</td>
<td>25.6</td>
<td>27.2</td>
<td>19.0</td>
<td>14.4</td>
<td>8.6</td>
<td>3.9</td>
<td>1.3</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%KNO</td>
<td>25.3</td>
<td>26.9</td>
<td>18.8</td>
<td>14.2</td>
<td>8.6</td>
<td>3.8</td>
<td>1.3</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>ADK</td>
<td>110.1</td>
<td>139.1</td>
<td>67.7</td>
<td>38.7</td>
<td>15.3</td>
<td>6.7</td>
<td>3.2</td>
<td>380.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%ADK</td>
<td>28.9</td>
<td>36.5</td>
<td>17.8</td>
<td>10.2</td>
<td>4.0</td>
<td>1.8</td>
<td>0.8</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%KNO</td>
<td>18.6</td>
<td>23.4</td>
<td>11.4</td>
<td>6.5</td>
<td>2.6</td>
<td>1.1</td>
<td>0.5</td>
<td>64.1</td>
</tr>
</tbody>
</table>

Figure 1 shows the cumulative curves of the predicted daily kernel set along the ear. The kernels resulting from pollination on day 1 of silking were located on the lower middle part of the ear. Their number was greater for both hybrids between position 10 and 20 in 2005 than in 2004. Kernel set progressed faster in 2005 than in 2004, without taking into account the differences between the two hybrids, especially on days 2 and 3 (Fig 1 B, C), as proven by the higher values for those segments along the ear where the kernel number varied from 0 to 12, indicating that the silks grew faster in 2005 than in 2004 on the first three days of silking.
Figure 1: The cumulative curves of predicted daily kernel set per position along the ear of Hibrix10 (H10) and Sugar73 (S73) in 2004 and 2005. A: day 1; B: days 1 and 2; C: days 1-3; etc.; F: days 1-6; G: days 1-7. Vertical segments represent ± standard errors of the corresponding absolute number of kernels of Hibrix10.

The kernel set of Hibrix10 lagged behind that of Sugar73 on days 2 and 3 in both years, as well as on day 4 in 2004 (Fig. 1 B, C, D). The segment along the ear where at least 12 kernels were initiated by day 2 was from position 8 to 16 for Hibrix10 in 2004, 9 positions shorter than that the segment of Sugar73. In 2005, the respective segment was considerably longer in both
genotypes but still shorter for Hibrix10 (position 4 to 24) than for Sugar73 (position 1 to 26). The length of the segment, along which 12 kernels or more were initiated by day 3 ranged from 23 (Hibrix10 in 2004) to 31 (Sugar73 in 2005) kernel positions. Kernel set of Sugar73 was very fast during the first days of silking. By day 3, more than 15 kernels were initiated at each position from 5 to 19, which corresponded to almost 100% of the final kernel number at these positions.

On later days, however, kernel set of Hibrix10 was faster. By day 5, the differences between the genotypes had vanished (in 2004), or Hibrix10 produced even more kernels than Sugar73 at the tip of the ear (in 2005) but at the cost of a slightly lower kernel number at lower positions (i.e. 0 to 30).

**Discussion**

The data for daily kernel set approximates the number of silks that had been extruded and were receptive on the respective day of silking (i.e. during the silking period) (Bassetti and Westgate, 1993b). The general pattern of kernel set along the ear was quite stable, starting on the lower part of the ear on the first day of silking. The largest number of kernels resulted from pollination on days 1 and 2 of silking; most of these kernels were located on the lower half of the ear. Kernel set along the ear on day 1 followed a bell-shaped curve with a peak near position 10 (counted from the bottom of the ear). On the following days, the daily kernel set was approximated by a double bell-shaped curve, the major peak of which shifted towards the tip of the ear over time with the minor peak located at the bottom of the ear, which decreased rapidly (data not shown). As well as confirming the general pattern of kernel set presented in chapter 2, the data provided evidence that the environmental conditions (of a given year) accelerated or slowed down average silk extrusion, while the genotype-specific differences remained relatively constant (Fig. 1). Both genotypes responded similarly to the conditions in the two years, although stress was at a minimum in both experiments due to artificial irrigation and high nutrient supply. The dry and windy weather during the flowering period in 2004 was probably responsible for the lower number of kernels per ear of both genotypes. A similar influence of the climatic conditions during the flowering periods on the number of kernels per ear was found in two other experiments, which also differed with respect to wind and air humidity (chapter 2). As discussed there, high wind speed increases transpiration and water
loss from the plants (Gliessman, 2007) and decreases the water-utilization efficiency of
photosynthesis.
Interestingly, our results showed that the progress of kernel set differed substantially between
genotypes and years. Hibrix10 responded positively to the environmental conditions in 2005
and produced more kernels at the tip of the ear. The genotype-specific temporal dynamics of
kernel set along the ear corresponded to the observation by Bassetti and Westgate (1993a) that
the duration of silk development was characteristic of a hybrid. Anderson et al. (2004) also
found differences between hybrids in the duration of pollination, required to maximize kernel
set as well as in the rate of silk elongation and the decline in silk receptivity over time.
Sugar73, the hybrid with shorter ears, completed kernel set faster than Hibrix10, the hybrid
with longer ears. Apparently, the silks of Sugar73 extruded faster than those of Hibrix10.
Cárcova et al. (2003) also observed that the growth of silks of a long-eared hybrid (with a high
number of spikelets per ear) was delayed compared to a short-eared hybrid and that its silks
grew more slowly. However, the final kernel number per ear and the distribution of these
kernels along the ear were quite similar for both hybrids, which reflect a similar potential
kernel number per plant. However, genotypic differences in the progress of silk emergence
over time may influence grain yield significantly under stress conditions (e.g. low nitrogen,
high plant densities, or drought). The observed genotypic differences in daily kernel set and the
distribution of kernels along the ear could become more pronounced under stress conditions.
Robust growth of the ear is an important aspect of enhanced tolerance to abiotic stress (Otegui
and Andrade, 2000).
Targeted pollination of sweet maize ears by flint maize pollen proved to provide a reliable
visual marker, which can aid in answering questions about maize grain set whenever sweet
maize genotypes are similar in vigor to field maize. The dynamics of kernel set were
influenced by year-specific environmental conditions, which affected the total kernel number
and the percentage of daily kernel set of both genotypes. Although the two modern sweet
maize varieties produced a similar final number of kernels per ear, daily kernel set and the
distribution of daily kernel set along the ear differed between both genotypes, reflecting
different rates of silk elongation and emergence that could affect total grain set under stress
conditions. Despite the presence of some genotype-by-environment interactions, the progress
of daily kernel set along the ear was characteristic for the two hybrids.
Chapter 4

The effects of mild pre-anthesis drought stress on the temporal dynamics of kernel set in tropical sweet maize (Zea mays L.) determined by visual markers

Abstract
Drought is one of the most costly limitations of crop production. The grain yield of maize is affected most by drought stress, particularly during flowering because of the delayed emergence of the silk and reduced silk receptivity, as a consequence of which the kernel number per ear is reduced. The objective was to model the effect of mild drought stress on the temporal dynamics of kernel set. The experiments were conducted on a large scale non-destructively in three tropical sweet maize hybrids that served as a model for maize in general by using flint maize pollen once in seven consecutive days to follow daily kernel set. This system enabled the non-destructive study of the dynamics of silk emergence. The kernel set reductions by mild drought stress two to three weeks before and throughout flowering were relatively low. In two years, drought stress modulated the dynamics of kernel set of all the genotypes and decreased the number of kernels per ear, reflecting a reduction in the number of exposed silks. The lower number of kernels per ear resulted from both a reduction in the number of kernel-bearing positions along the ear and in the number of kernels per position on each day of pollination. The changes in the dynamics of kernel set by drought stress differed among genotypes and suggested different levels of drought tolerance. The visual marker enabled us to pursue the dynamics of silk emergence and its impact on kernel set under varying environmental conditions.
Introduction

Drought stress is one of the most important abiotic constraints to rainfed agriculture worldwide and is expected to become even more severe (Burke et al., 2006). The deleterious effects of drought stress on maize yields are particularly strong at flowering when the kernel number is determined (Andrade et al., 1999; Saini and Westgate, 2000; Salter and Goode, 1967). There is a reduction in the uptake of nutrients from the soil and photosynthesis under drought. The limited availability of assimilates limits plant growth. Tassel growth, pollen production and the time of pollen release (male flowering) are hardly affected by adverse environmental conditions. Thus, the supply of pollen does not usually limit kernel set. In contrast, silk growth and the time of silk emergence are susceptible to these limitations. The rate of growth of the ear and the silk are lower, and the emergence of silks (female flowering) is delayed under stress. Delayed silk emergence can lead to poor kernel set because the silks may emerge too late to be pollinated. It is also possible that silks are not pollinated because they do not appear at all (Edmeades et al. 2000). Furthermore, the water potential of the silks may also be reduced. The silks lose their receptivity for pollen (Schoper et al., 1986) and the ovaries may not be pollinated even if the silks do emerge and come into contact with viable pollen. Both delayed emergence of silk and reduced receptivity of the silk lead to reductions in the number of kernels per ear and, thus, in grain yield. Furthermore, ovaries that had been successfully pollinated and were developing into kernels may abort due to the reduced availability of photosynthetic assimilates under drought stress. Drought-induced kernel abortion is, thus, another reason for the intolerance of maize to drought stress at flowering during early reproduction (Hall, 2001).

We present a visual marker system, for the study of the temporal dynamics of silk emergence of sweet maize by pollinating on certain days with flint maize pollen (chapter 2). It was shown that the largest proportion of kernels per ear resulted from pollination on the first two days of silking (i.e. the period of silk emergence). These kernels were located at the bottom and in the lower middle part of the ear. Most of the kernels that initiated on later days of silking were located towards the tip of the ear. Although the environment (the year) influenced the number of kernels produced per ear as well as the rate of daily kernel set, genotype-specific patterns of kernel set were identified (chapter 3). As outlined above, drought stress at flowering largely affects the growth of silks and their receptivity for pollen and, thus, reduces grain yield due to a
decrease in the number of kernels per ear. Therefore, the aim of this study was to assess the effect of drought stress on kernel set by means of a non-destructive quantification system, which relies on flint kernels to visualize the daily kernel set on ears of tropical sweet maize. The specific questions were: (i) Does drought stress cause proportional reductions in daily kernel set on individual days during the silking period? (ii) Do the reductions in kernel number per ear result mainly from a shorter kernel-bearing segment along the ear (i.e. fewer kernel positions) or from a lower number of kernels per position?

**Material and Methods**

*Plant material*

The sweet maize hybrids Hibrix10 (Pacific Seeds Ltd.), released in 1999, and Sugar73 (Novartis Seeds Ltd.), released in 1999, were the pollen receptors and the pollen donors in 2004 and 2005. The sweet maize hybrid Insee2 (Aekatasanawan et al., 2001), released in 1999, was grown in 2007 and 2008. The tropical flint-dent hybrid SW3851 (Aekatasanawan et al., 1998), released in 1997, was the pollen source for pollination on certain days (see below) in 2004 and 2005, whereas the flint hybrid SW4452 (Aekatasanawan et al., 2005), released in 2003, was the pollen donor on certain days in 2007 and 2008. Hibrix10 produces long ears (~20 cm long at the milk stage (R3), Pacific Seeds Ltd.) compared to Sugar73, which produces shorter ears (~18 cm long at milk stage (R3), Novartis Seeds Ltd.). The ears of Insee2 were about 17 cm long at the milk stage (R3) (Aekatasanawan et al., 2001). All three tropical hybrids are new vigorous types of sweet maize (class 1), which carry the recessive allele at the sh2 locus. This allele is responsible for the formation of large, inflated, translucent kernels, which are crisp and sweet at the milk stage but collapse when dry, becoming angular and brittle (Tracy, 2001). Because of the recessive action of the sh2 allele, sweet maize produces hard kernels upon fertilization with pollen of maize without this mutation.

*Experimental site and experimental design*

All experiments were conducted at Suwan Farm in Thailand in the dry season (November to April) in 2003/04, 2004/05, 2006/07 and 2007/08. There is a tropical lowland climate at Suwan Farm (14.5°N, 101°E, 360 m above sea level) (Gerpacio and Pingali, 2007). The distribution of
rainfall is bimodal with a minor peak in May and September. The total annual rainfall is about 1,000 to 1,200 mm. The soil at Suwan Farm is a Rhodic Kandiustox, Oxisol (USDA taxonomy) (Land Development Department, Thailand, 2009).

Three seeds were sown manually in each mound and redundant plants were removed at the 4-leaf stage. Prior to sowing, 25 kg N and 30 kg P were applied per hectare; 115 kg N per hectare were applied one month after sowing. Herbicides and insecticides were applied as required, according to local practices. Several rows of pollen donor plants (sweet and flint maize) were grown around all the plots in all the experiments.

In 2004 and 2005, the experiments were arranged in six blocks each, with six plots. Each plot consisted of six rows, 6 m long and 0.75 m apart, with 21 plants per row. The four middle rows contained sweet maize, the two border rows flint-type maize. Hibrix10 and Sugar73 were randomly assigned to three plots within each block. Two blocks were assigned to each of three water regimes: one well-watered regime (WW) and two drought stress regimes. Drought stress was induced by withholding water from one week (D1) or two weeks (D2) before flowering until the end of flowering in 2004 or from two (D2) or three (D3) weeks before flowering until the end of flowering in 2005.

In 2007 and 2008, the experiments were arranged in four blocks each, with six plots. Each plot consisted of six rows of sweet maize, 6 m long and 0.75 m apart, with 21 plants per row. There were two water treatments applied to two blocks: well-watered (WW) and drought-stressed (D3), in which water was withheld from three weeks before flowering until the end of flowering. The first four irrigations were done with sprinklers; thereafter furrow irrigation was applied once a week (~30 mm), with the exception of the above-mentioned periods in the drought-stress treatments.

*Determinaton of kernel set*

In 2004 and 2005, the ear primordia of plants in the four middle rows of each plot were covered with glassine bags. Seven plants, the first silks of which extruded on the same day, were randomly selected in each plot. The growing ears received pollen (collected from well-watered plants) of sweet maize on six days and pollen of flint maize on one of the seven days after emergence of the first silks (seven pollination treatments) in order to visualize daily
kernel set with hard kernels among the shriveled ones. In each experiment, this resulted in six ears per genotype, water regime and pollination treatment (day).

In 2007 and 2008 experiments, 28 homogeneous plants were randomly chosen from the four middle rows of each plot and were tagged two weeks before flowering. Their ear primordia were covered with glassine bags and checked daily for extruded silks. The growing ears of 14 plants per plot were pollinated on seven consecutive days with pollen of well-watered donor plants and the growing ears of the other 14 plants with pollen of drought-stressed donor plants, starting on the day when the first silks of the individual plants appeared. Seven sets of two growing ears per plot in both pollen-source treatments received pollen of sweet maize on six days and pollen of flint maize on one of the seven days after the emergence of the first silks (seven pollination treatments). In both experiment, 672 growing ears were pollinated as described above, 24 ears per water regime, pollen source and pollination treatment (day).

The ears were harvested as soon as hard flint kernels and the shrieveled sweet maize kernels were distinguishable. The ears were air-dried for about one week with the objective of increasing the contrast between the two kernel types. Then, they were divided into kernel-wide segments, and the shrieveled and hard kernels in each segment were counted, to give the total kernel number and the number of shrieveled and hard kernels as well as the number of kernel rows and the number of kernel-bearing positions per ear. The hard kernels represent the daily kernel set according to the day, on which the ears had been pollinated by pollen of flint maize.

*Additional phenotypic measurements*

In 2007 and 2008, the average time from sowing to male and female flowering of plants in each experimental plot and of the pollen donor plants was recorded as the number of days from sowing to the day, on which 50% of the plants in the middle two rows had started to shed pollen and to extrude silks. The first day of pollination (i.e. the date of first silk emergence) of each hand-pollinated plant was also recorded.

In 2008, the ear primordia of 120 representative and uniform plants (five plants per plot, 60 plants per water regime) were covered with glassine bags before silking. During the first week of silking, the length of the extruding silks was recorded daily and the silks were cut back to the tip of the husk leaves. The non-pollinated ears were harvested two weeks after flowering, and the ovaries per position along the ear were counted.
In 2008, pollen of 25 sweet and 25 flint-type pollen donor plants per water regime was collected at the beginning and at the end of the pollination period. The pollen grains were incubated in petri dishes in a medium containing 300 mg/l CaCl$_2$, 100 mg/l H$_3$BO$_3$, 120 g/l sucrose and 7 g/l phytagel (Cook and Walden, 1965) at room temperature for one hour. The total number of pollen grains and the number of germinated pollen grains were counted under a binocular microscope (frame size: 7x10 mm). In 2007, pollen viability was assessed by counting the proportion of stained pollen grains in a solution of iodine-potassium iodide (I$_2$-KI) (Song et al., 2001).

*Environmental data*

The air temperature, rainfall, relative air humidity, the speed and direction of the wind and water evaporation were measured every three hours at Suwan Farm. The soil water potential was measured by placing Watermark® soil moisture sensors at three soil depths: 30, 60 and 90 cm at one position in the well-watered treatment and three positions in the drought-stress treatment in 2008 and at one position in both treatments in 2007. The daily soil water potential was recorded from one week before the last irrigation until one week after flowering had ceased. During the same period, the leaf water potential of the youngest fully expanded leaf of 10 randomly selected plants in three plots per treatment was measured in 2008. The measurements were done twice a day, before daybreak and around 2 p.m. using a Scholander pressure bomb (Boyer, 1995; Tanguilig et al., 1987).

*Statistical analysis*

The following traits were determined from the number and distribution of the shriveled and hard kernels per ear: (i) the total number of kernels per ear, (ii) the number of kernels per ear set on the day on which pollen of flint maize had been supplied (daily kernel set), (iii) the number of kernels per position (i.e. the distribution of kernels along the ear) and (iv) the number of kernels per position that were set on the day of pollination by flint maize (i.e. the distribution of daily kernel set along the ear). All the ears that produced kernels with unusual and sometimes incomplete daily grain set were removed before the analysis: about 9 (2004), 11 (2005), 20 (2007) and 4% (2008) ears.
Data for ovaries and kernels were analyzed by means of the package *np* (nonparametric kernel smoothing methods for mixed data types; Hayfield and Racine, 2008) in *R* (R Development Core Team, 2009). The regression models included the following variables: (i) “irrigation” in the case of number of ovaries per ear (only in 2008), (ii) “irrigation” and “genotype” in the case of total number of kernels per ear, (iii) “day”, “irrigation” and “genotype” in the case of daily kernel set, (iv) “irrigation”, “genotype” and “position along the ear” in the case of distribution of kernels along the ear and (v) “day”, “irrigation”, “genotype” and “position along the ear” in the case of distribution of daily kernel set along the ear. The factor “pollen source” was included in a preliminary analysis of the data in 2007 and 2008 instead of the factor “genotype”, depending on the design of the experiments. However, because there were no differences in the viability (staining) or germination of pollen grains (see below) or in the results of kernel set in the two pollen-source treatments, the factor “pollen source” was not included in the regression models in these years.

Before computing the kernel regression estimates, data-driven bandwidths were computed with *npregbw()* using a local-linear regression estimator and the Kullback-Leibler cross-validation method. The tolerance on the value and on the position of located minima of the cross-validation function were left at their default values, except for the analyses that included “position along the ear” as a variable. In those cases, the value was set at $10^{-4}$. Each determination of extrema of the cross-validation function was repeated five times. The resulting bandwidths were used to evaluate the regression model with *npreg()* and to make predictions for all combinations of the supplied variables. The significance of explanatory variables was tested with *npsigtest()* using the default number of bootstrap replications.

The daily kernel set and distribution along the ear, which resulted from the above-mentioned analyses, are referred to as absolute data. While the daily kernel set was determined for seven sets of ears (one set for each of the seven pollination treatments), the total number of kernels per ear of the entire set was recorded in each combination of genotype, pollen source, and water regime. The sum of the seven values of absolute daily kernels set did not, therefore, necessarily equal the total number of kernels per ear. The absolute daily number of kernels was divided by the sum of the respective seven values and multiplied by the average number of total kernels. The resulting data are referred to as predicted data of daily kernel set and enabled a meaningful comparison of daily kernel set with respect to the entire data set. This procedure
was carried out for both the daily kernel set per ear and per position along the ear. Furthermore, to account for differences in kernel set between well-watered and drought-stressed treatments, the absolute number of daily kernels in the drought-stressed treatments was adjusted to the total number of kernels per ear in the well-watered treatment of the respective genotype and pollen source treatment in the same experiment.

The data for daily silk elongation were grouped according to the day of silking, irrespective of the date of the female flowering. Data analysis was done by means of the `np` package, calculating data-driven bandwidths before calculating the kernel regression estimate. The explanatory variables for silk length were “irrigation” and “day”.

**Results**

*Environmental data*

Table 1 gives the meteorological data for all experiments. In 2004 and 2007, the temperature and relative air humidity were lower than in 2005 and 2008. At the same time there were more windy days. There was no rain in 2004 and 2007. In 2005 and 2008 there was less wind, the relative air humidity was higher and there was about 22 mm of rainfall in 2005 and 18 mm in 2008. The soil water potential decreased over time in the drought-stressed treatments and was lowest at the end of stress period: -99.9 (at 30 cm), -90.8 (at 60 cm) and -33.2 kPa (at 90 cm) in 2007 and -193.3 (30 cm), -107.7 (60 cm) and -37.7 kPa (90 cm) in 2008. The values were lower in 2008, despite the fact that the duration of the stress was similar in both years (25 days in 2007 and 27 days in 2008). The predawn leaf water potential in 2008 during the stress period was the same in the well-watered and the drought-stressed treatments, with the exception of the end of the stress period when the average value in the drought-stressed treatment (-63 ± 4 kPa) was lower than in the well-watered treatment (-32 ± 2 kPa).
Table 1: Average daily minimum and maximum temperatures (Temp), relative air humidity (RH), water evaporation from the soil surface (Evap), wind speed (Wind) and the number of days with wind speed above 5 m/s (Windy days) and total amount of rainfall during the flowering period (Period) in four field experiments at Suwan Farm from 2004 to 2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>Period</th>
<th>Temp Max °C</th>
<th>Temp Min °C</th>
<th>RH Max %</th>
<th>RH Min %</th>
<th>Evap mm</th>
<th>Wind m/s</th>
<th>Windy days d</th>
<th>Rainfall mm</th>
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<td>17.5</td>
<td>83.2</td>
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<td>2.8</td>
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</tr>
<tr>
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<td>32.9</td>
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<td>90.3</td>
<td>61.6</td>
<td>6.7</td>
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<td>0</td>
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<td>27.7</td>
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<td>69.5</td>
<td>35.8</td>
<td>8.3</td>
<td>4.5</td>
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</tr>
<tr>
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<td>88.3</td>
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<td>4.6</td>
<td>2.3</td>
<td>2</td>
<td>17.9</td>
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</table>

Flowering, silk elongation and pollen viability

The average time from sowing to male flowering was about 66 days in 2007 and 66.6 days in 2008. In comparison, female flowering was delayed by 1.5 and 2 days on average. While male flowering under drought stress occurred only one day later than under well-watered conditions, the anthesis-to-silking interval was somewhat longer under stress, where it lasted about three days in both years. The average cumulative length of the silks per ear was 29.9 cm (± 0.5 cm; standard error) under well-watered conditions and 28.0 cm (± 0.5 cm) under drought stress (data of 2008). Silk elongation was maximal on the first and second days of silking (5.3 cm d⁻¹) and decreased thereafter (from 5.1 cm d⁻¹ on day 3 to 2.4 cm d⁻¹ on day 7). Silk elongation was significantly shorter in the drought-stressed treatment on days 2 to 4. Pollen viability (data of 2007) and pollen germination (data of 2008) were not affected by drought stress (data not shown).

Kernel set under well-watered conditions

The average kernel number (KNO) per ear was lower in 2004 than in 2005 and higher in 2007 than in 2008 (Table 2). The higher KNO was accompanied by a higher percentage of ears with more kernel rows (Table 2). Hibrix10 and Sugar73 produced, on average, about 14 kernels at each position on the lower part of the ears (i.e. kernel rows) in 2004 and about 15 kernels in 2005 (Fig. 1 A-D). Insee2 produced fewer kernel rows on average (Fig. 1 E-F).
Table 2: Average values ± standard errors of the number of kernels per ear (KNO) in the well-watered (WW) and drought-stressed (D1, D2, D3) treatments. Stress was induced by withholding water from one (D1), two (D2) or three (D3) weeks before flowering until the end of the flowering period. Two genotypes were evaluated together in 2004 and 2005; another genotype was studied in 2007 and 2008. Ears: number of evaluated ears; %WW: number of kernels per ear in relation to the number of kernels in the well-watered treatment; KR: percentage of ears with 12, 14 or 16 kernel rows.

<table>
<thead>
<tr>
<th>Year</th>
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<th>Ears</th>
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<th>%WW</th>
<th>12 KR</th>
<th>14 KR</th>
<th>16 KR</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
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<td>67.6</td>
<td>16.2</td>
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<tr>
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<td></td>
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<td>74.4</td>
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<tr>
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<td></td>
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<td>462.8 ± 13.6</td>
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<td>59.0</td>
<td>10.2</td>
</tr>
<tr>
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<td>WW</td>
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<td>100</td>
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<td>70.3</td>
<td>24.3</td>
</tr>
<tr>
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<td></td>
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<td>39</td>
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<td>12.8</td>
<td>61.5</td>
<td>25.6</td>
</tr>
<tr>
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<td></td>
<td>D2</td>
<td>38</td>
<td>522.2 ± 13.1</td>
<td>93.3</td>
<td>10.5</td>
<td>60.5</td>
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</tr>
<tr>
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<td>WW</td>
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<td>7.9</td>
<td>47.4</td>
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</tr>
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<td>581.0 ± 10.0</td>
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<td>43.6</td>
<td>41.0</td>
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<td>52.9</td>
<td>35.3</td>
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<tr>
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<td>Sugar73</td>
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<td>100</td>
<td>2.7</td>
<td>27.0</td>
<td>67.6</td>
</tr>
<tr>
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<td></td>
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<td>99.2</td>
<td>7.9</td>
<td>50.0</td>
<td>36.8</td>
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<td>99.2</td>
<td>7.9</td>
<td>57.9</td>
<td>28.9</td>
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<td>Insee2</td>
<td>WW</td>
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<td>451.8 ± 4.6</td>
<td>100</td>
<td>46.9</td>
<td>48.3</td>
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<td></td>
<td>D3</td>
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<td>397.3 ± 4.7</td>
<td>87.9</td>
<td>44.6</td>
<td>49.2</td>
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<td>Insee2</td>
<td>WW</td>
<td>320</td>
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<td>100</td>
<td>43.8</td>
<td>45.9</td>
<td>10.3</td>
</tr>
<tr>
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<td>D3</td>
<td>326</td>
<td>423.1 ± 4.1</td>
<td>100</td>
<td>46.3</td>
<td>48.5</td>
<td>5.2</td>
</tr>
</tbody>
</table>

†Irrigation, genotype and year had significant effects on KNO in 2004 and 2005 (P ≤ 0.01) and irrigation and year had significant effects on KNO in 2007 and 2008 (P ≤ 0.001).
Figure 1: Average number of kernels per position along the ear of Hibrix10 and Sugar73 in 2004 (A and B) and 2005 (C and D) and along the ear of Insee2 in 2007 (E) and 2008 (F) in the well-watered treatment (WW) and in the drought stressed treatments (D1, D2, D3). Vertical segments indicate standard errors for the WW treatment.

Furthermore, Insee2 produced considerably fewer kernels towards the tip of the ears than both Hibrix10 and Sugar73, indicating a lower yield potential of Insee2. For all genotypes, more than 90% of the produced kernels resulted from pollination during the first four (Sugar73) or five (Hibrix10 and Insee2) days of silking, except for Hibrix10 in 2005 and Insee2 in 2008, where this percentage was reached already by days 4 and 3, respectively (data not shown) due to the higher proportion of kernels set on the first and second days of silking. The day, on which the highest proportion of kernels was initiated depended on the genotype and the year and was always the first or second day of silking. Hibrix10 and Insee2 always produced the largest number of kernels on days 1 and 2, respectively, while Sugar73 produced the largest
number of kernels on day 1 in 2004 and on day 2 in 2005. In principle, the distribution of daily kernels set along the ear of Hibrix10 and Sugar73 was similar to that of Insee2 (compare with data in chapters 2 and 3). The number and distribution of kernels initiated on day 1 followed a bell-shaped curve with a peak for the lower part of the ear (Fig. 2 A). On the following days, kernel set followed a double bell-shaped curve, the major peak of which shifted to the tip of the ear and the minor peak, for the bottom of the ear, decreased rapidly (data not shown). However, there were genotype-specific deviations from this general pattern of daily kernel set along the ear (shown in part in Fig. 2). Taking the data of 2004 and 2007 as an example, kernel set of Sugar73 progressed faster during the first four days of silking than kernel set of the other genotypes (Fig. 2 A-D); the highest cumulative number of kernels per position was initiated by day 4 for this genotype. The curves of kernel set were similar for Hibrix10 and for Insee2 on days 1 and 2. From day 3 onwards (Fig. 2 C-F), fewer kernels were initiated on the ears of Insee2 than on the ears of the other two genotypes, primarily at the tip of the ears. Thus, the above-mentioned lower total number of kernels per ear of Insee2 (WW) resulted from a disproportional reduction in the number of kernels initiated later during the flowering period.

Changes in kernel set induced by drought

The effects of drought stress on kernel number per ear were relatively weak in all years; kernel number per ear was reduced by 15% or less compared to the well-watered treatment. There were no significant effects in 2005 and 2008 (Table 2). The stress-induced reductions in the total number of kernels per ear resulted mainly from reductions in the number of kernels produced at the tip of the ear (Fig. 1 A, B, E). In the case of Hibrix10 in 2004, for example, the number of kernels per position fell below 12 at position 35 in the WW treatment and at position 26 in the D2 treatment (Fig. 1 A). Hibrix10 reacted somewhat more strongly to drought stress than Sugar73, because its kernel number was slightly lower in the D2 treatment in 2004. Drought stress also induced a significant shift in the ratio between the number of ears with 12, 14 and 16 kernel rows of Hibrix10 in 2004 (Chi-squared = 15.4; P-value = 0.02) and Sugar73 in 2005 (Chi-squared = 18.3; P-value = 0.02). Correspondingly, the average number of kernels at the bottom of the ears was slightly lower under drought stress than under well-watered conditions (Figure 1 A, D). However, these changes did not necessarily influence the total number of kernels per ear (Sugar73 in 2005). For Hibrix10 in 2005, Sugar73 in 2004 and
Insee2 in 2007 and 2008, the average kernel number at positions at the bottom and the lower middle part of the ears was relatively constant across water regimes (Fig. 1 B, C, E, F).

**Figure 2:** The cumulative curves of the predicted daily kernel set per position along the ear of Hibrix10 (H10) and Sugar73 (S73) in 2004 and Insee2 (I2) in 2007 in the WW treatment (filled symbols) and the D1, D2 and D3 treatments (open symbols). A: day 1; B: days 1 and 2; C: days 1 to 3; etc.; F: days 1 to 6. The cumulative curves of days 1 to 7 (not shown) are identical to those in Fig. 1.
Table 3: Number of kernels representing absolute daily kernel set (ADK) and the corresponding percentages in relation to the sum of the seven daily values (%ADK) in relation to the total number of kernels per ear for the respective year, hybrid and water regime (%KNO) or in relation to the total number of kernels per ear for the respective year and hybrid in the WW treatment (%KNOww).

<table>
<thead>
<tr>
<th>Year</th>
<th>Genotype</th>
<th>Water regime</th>
<th>Traits</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Total</th>
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<tbody>
<tr>
<td>2004</td>
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<td>WW</td>
<td>ADK†</td>
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<td>125.8</td>
<td>102.1</td>
<td>79.6</td>
<td>47.0</td>
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<td>4.5</td>
<td>494.7</td>
</tr>
<tr>
<td></td>
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<td>25.4</td>
<td>20.6</td>
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<td>2.7</td>
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</tr>
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<td>0.9</td>
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</tr>
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<td></td>
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†Genotype had a significant effect on KNO in 2004 (P ≤ 0.001).
‡Day had a significant effect on ADK in 2007 (P ≤ 0.001).
Table 3 and Figure 2 show the data for daily kernel set in 2004 and 2007, the years in which a significant effect of drought stress on kernel number per ear was observed. Drought stress reduced the cumulative number of daily kernels per position for all genotypes but to different extents. As an exception, a higher number of kernels on ears of Sugar73 on day 1 were initiated under drought compared to the well-watered treatment, which indicates a more synchronous and somewhat faster emergence of silk. The observed differences in kernel number per position between water regimes were greater towards the tip of the ears and, because the curves show cumulative values, on later days of silking. The differences in the percentage of daily kernel set between WW (%ADK) and D1 and D2 in 2004 and D3 in 2007 (%KNOww), however, were usually largest at the beginning of silking (Table 3). The reductions in the cumulative number of kernels per position in the drought-stressed treatments led to shorter segments along the ears on, which an arbitrary threshold of kernels (e.g. 6 or 12) were initiated by a given day. For example, the length of the segment with more than 12 kernels initiated by day 4 was reduced from 23 to 19 positions for Insee2 in 2007 (Fig. 2 D) and from 30 to 24 positions for Hibrix10 in 2004 (Fig. 2 D). By day 7, the shortest with more than 12 kernels was observed for Sugar73 (Fig. 1 B), which again indicates that Sugar73 tolerates drought stress better than the other genotypes. As a consequence of the more severe drought stress, the segment along the ear with 12 or more kernels initiated by day 5 was shorter in the D2 than in the D1 treatment in 2004 compared to the corresponding segment in the WW treatment (data not shown). The differences between the stress treatments and the well-watered treatment increased on the following days of pollination for Hibrix10 but remained constant for Sugar73 and Insee2.

Discussion
Drought stress caused relatively small but significant reductions in the average number of kernels per ear in 2004 and 2007 but not in 2005 and 2008, even though irrigation was stopped one week earlier in 2005 than in 2004. The two main reasons for this are that the relative air humidity during the flowering period was higher and there was less wind in 2005 and 2008 than in the other years. The higher average minimum and maximum temperatures were apparently less relevant with regard to the plant response to stress. When the air is humid, maize requires less water than when the air is dry (Maximov, 1929), because the stomata may
respond to air humidity, irregardless of the leaf water status (Ackerson and Krieg, 1977; Hall and Hoffman, 1976). Moreover, wind can greatly enhance water loss through transpiration by constantly removing the layer of saturated air around the leaf (Wooley, 1961). All in all, the intensity of drought stress was too low to induce a substantial reduction in grain yield (e.g. of 30-40%). However, it was not the objective of this study to induce severe drought stress, leading, for example to a 70 to 80% reduction in yield, as the focus lay on the modeling of flowering dynamics at mild stress that occurs regularly in many maize growing regions, reducing the yield consistency.

Although the duration of silking is usually shorter under drought stress (Bassetti and Westgate, 1993c; Hall et al., 1982), this was not the case in this study. However, the number of silks extruded per day was reduced, as indicated by the lower number of kernels per ear of Insee2 in 2007. The data for 2008 shows that silk elongation was more sensitive to drought stress than kernel set. While drought stress reduced daily silk elongation, both the total kernel number per ear and daily kernel set did not change compared to WW conditions. These observations are in agreement with those of Bassetti and Westgate (1993c), who observed that silks could be pollinated even after elongation had ceased.

Despite the rather weak stress, it was possible to model the effects of drought stress on daily kernel set and the distribution of kernels along the ear in 2004 and 2007. Drought stress affected the number of daily kernels in two ways: i) by reducing the number of kernels per position and ii) by reducing the number of kernel-bearing positions along the ear. There are numerous reports of variation in the number of kernel rows per ear among genotypes and in response to abiotic stress such as drought or nitrogen deficiency (Elmore and Abendroth, 2006; Daniel, 1963; Alexander, 1952). The drought-induced reductions in the number of kernel rows along the ears of Hibrix10 resulted from reductions in kernel set along the ear on all seven days of pollination (data not shown). The number of kernel rows per ear of the other hybrids was quite stable under drought stress compared to the well-watered treatment. The number of kernel rows is usually a highly heritable trait, the expression of which is stable as long as environmental stress is not particularly severe (Daniel, 1963).

The reduction in kernel number on the upper part of the ear under drought stress was relatively large for Hibrix10 compared to Sugar73, which reveals a stronger response of the latter genotype to drought (Fig. 1 A, B). The reduction in the kernel number of Insee2 (Fig. 1 E) was
comparable to that of Hibrix10. However, since the stress was probably more severe in 2007 than in 2004 (three weeks without irrigation for Insee2 in 2007 compared to one or two weeks for Hibrix10 and Sugar73 in 2004), Insee2 might be more drought-tolerant than Hibrix10. All in all, the reduction in the number of kernel-bearing positions along the ear depended on both the genotype and the severity of the drought stress and was the result of cumulated reductions on all seven days of pollination.

This study shows that the model system of sweet and flint type kernels could reveal effects of drought stress on the dynamics of kernel set. Kernel set was influenced not only by the length of the irrigation-free period, but also significantly by the year-specific environmental conditions during the flowering period. In the experiments, in which drought stress was strong enough to reduce the number of kernels per ear, the reductions resulted from a reduction in kernel number per position and/or from a reduction in kernel-bearing positions, which were initiated daily. However, the stress response of the genotypes differed, which showed that the extent of their tolerance to stress differed.
Chapter 5

General discussion and conclusion

The dynamics of silk emergence and its impact on kernel were successfully investigated by the visual marker system. This method traces the distribution of kernels along the ear. Assuming a strong relationship between the number of emerged silks and the number of kernels (Bassetti and Westgate 1993b), the data on kernel set approximates the number and the position of silks that had been extruded and were receptive on respective days. The distribution of kernels along the ear that were set on day 1 followed a bell-shaped curve at lower part of the ear. The distribution of kernels along the ear that were initiated on the following days corresponded to double bell-shaped curves whose major peak moved toward the tip of the ear and whose minor peak remained at bottom of the ear. Genotypic differences in and environmental effects on the distribution of kernel set were determined in this study, particularly under water stressed conditions.

The total number of kernels per ear and the percentage of daily kernel set varied across years. In years with high relative air humidity, the percentage of kernel set on the first two days of silking was high (chapters 2 and 3). In years with rather low relative air humidity, high wind and high water evaporation from the soil surface, the cumulative number of daily kernels that were initiated between day 5 and day 7 accounted for more than 12% of the total number of kernels, which reflected a slower silk emergence during the first four days of silking under well-watered conditions. High wind speed provokes an increased loss of water through transpiration by constantly removing the layer of saturated air around the leaf (Wooley, 1961). Because the water potential of the silks is associated with the leaf water potential (Westgate and Boyer, 1986; Westgate and Grant, 1989), silk growth is reduced when water loss through transpiration is large because of windy weather.

The curves of the distribution of daily kernel set looked similar among the three hybrids and across the years but they were shifted by up to 12 positions along the ear. This was in agreement with Bassetti and Westgate (1993a), who reported that the cumulative curves of the daily percentage of exposed silks of two genotypes were similar in shape. However, there were differences in the present study between the three hybrids with respect to the duration of silk
emergence. Considering only the years with high relative air humidity (i.e. 2005 and 2008), more than 90% of the kernels were produced within 5, 4 and 3 days in case of Hibrix10, Sugar73 and Insee2, respectively (chapter 2 and 3). The ears of Insee2 were characterized by a lower number of kernel positions (43 kernels) along the ear than those of Hibrix10 (49 kernels) and Sugar73 (48 kernels). These results were in agreement with Cárcova et al. (2003) who reported that the silk growth of a long-eared hybrid (with high number of spikelets per ear) was delayed, compared to a short-eared hybrid. Likewise, the percentage of daily kernels differed between the three hybrids and the genotype-specific differences remained relatively constant across years. In this study, the genotype also influenced the day, on which the largest number of kernels was initiated, although the largest number of kernels was always set on one of the first two days of silking (chapter 2 and 3), which was in agreement with many reports in literature (Anderson et al., 2004; Bassetti and Westgate, 1993a; Lonnquist and Jugenheimer, 1943; Sadras et al., 1985).

In specific years, drought stress reduced silk growth and the number of kernels per ear, and therefore grain yield; these years were characterized by low relative air humidity. However, drought stress was too weak to induce yield reductions in the years in which the air was more humid and there was less wind. Silk elongation was delayed under drought-stress conditions in 2008, even though the length of the silking period and the number of kernels per ear were not reduced. Nevertheless, there were significant differences in the number of kernels per ear and the distribution of kernels along the ear between droughted and well-watered conditions in 2004 and 2007. Drought stress affected the number of daily kernels in two ways: by reducing the number of kernels per position and by reducing the number of kernel-bearing positions along the ear. The decreasing of number of kernels depended on both the length of the drought stress period and the drought resistance of the hybrids. In 2004, two hybrids responded differently to drought stress. Similarly, Hall et al. (1982) reported that the reduction in the number of kernels per ear and the duration of silk emergence depended on the genotype under drought stress. The reduction in the number of kernel-bearing positions was the result of cumulated reductions that were observed on all seven pollination days.

It is the great advantage of our pollination method, i.e. visual marker of daily grain set that it allows assessing daily kernel set non-destructively. In contrast to other methods (e.g. Anderson et al., 2004; Lonnquist and Jugenheimer, 1943; Peterson, 1942) it was possible to relate the
number and the position of ovaries whose silks were extruded on a particular day during silking with grain production in the field. A disadvantage in the actual application of the method might have been that the silks were not cut back before the manual pollinations. The pollination of late-appearing silks might therefore have been hampered. There is a great potential for our visual marker system in further research to unravel the role of the dynamics of kernel set under various biotic or abiotic stresses such as high plant density or nitrogen deficiency.
References


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