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THESIS

EFFECTS OF MAGNESIUM CHLORIDE ON SOME PHOTOSYNTHESIS – RELATED COMPOUNDS IN MAIZE (*Zea may* L.)

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Two maize cultivars, a feeder maize (Nakhon Sawan 2; NS 2) and a sweet maize (Hawaiian Sugar Supersweet; HSS), were treated with different concentrations of $MgCl_2$. The most appropriate time to apply $MgCl_2$ at vegetative stage and reproductive stage was determined. After the beginning of both stages, the date for $MgCl_2$ treatment was designated as days 25 to days 31 which had a stable quantity of phosphoenolpyruvate carboxylase enzyme (PEPC). After $MgCl_2$ treatment, changes in photosynthesis-related compounds, i.e., chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid, PEPC as well as sugar and protein, were evaluated. It was found that foliar application of $MgCl_2$ to either vegetative or reproductive stage of both feeder and sweet maizes grown in pots under partial shade condition led to random increases in all six photosynthesis-related compounds analyzed. The inconclusive effects of magnesium were likely due to the unfavorable growing conditions.

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EFFECTS OF MAGNESIUM CHLORIDE ON SOME PHOTOSYNTHESIS – RELATED COMPOUNDS IN MAIZE (*Zea may* L.)

INTRODUCTION

Maize (*Zea may* L.) is one of the most important agronomic crops in the world. Its grain provides food, feed, and a resource for many unique industrial and commercial products. In Thailand, maize production occupies almost 6% of the Thai farmland which is predominantly owned by smallholders. In 2002, Thailand was the fourth largest exporter of various products from maize in the world. In the production year of 2000-2001, Thailand has 36,960.48 hectares (231,003 Rai) of maize cultivation land and of 33,429.76 hectares (208,936 Rai) for sweet maize harvest land. The total produce was 384,961 tons and the average produce was 12,450 kilogram/hectare (1,992 kilogram/Rai). In 2004, there were over 320,000 households producing maize. One third of these households farmed less than 1.6 hectares (10 Rai) and earned less than US$900 (30,000 baht) of annual income. Another 35% of these households farmed 3-6.5 hectares (20-50 Rai) and had annual income that ranged between US$1,500-2,350 (50,000-80,000 baht). Actually, maize contributed, on average, 70% of total income across all those households (Anonymous, 2006). Increasing yield quantity and quality would enhance the income generated from maize plantation.

Magnesium ion (Mg^{2+}), a macronutrient, is a component of several enzymes mainly participating in carbohydrate metabolism. Furthermore, Mg^{2+} is required for photosynthesis process (Leegood, 1985). In many soils, the release of Mg^{2+} by weathering is able to balance the removal by leaching because the uptake rates of magnesium ions by crops are relatively low. Foliar-applied magnesium is readily absorbed and rapidly transferred to use (Terry and Ulrich, 1974). A primary effect of Mg^{2+} deficiency is to disturb polypeptide synthesis in the ribosomes thereby restricting protein production and growth (Mengel *et al*., 2001). Mg^{2+} is essential for

the activity of phosphoenolpyruvate carboxylase (PEPC). Westhoff and Gowik (2004) reported that PEPC needs Mg^{2+} as an essential cofactor and requires that the inorganic carbon be supplied as bicarbonate. Moreover, it is a constituent of the chlorophyll molecule. Mg^{2+} is also required for the normal structural development of the chloroplast (Terry and Ulrich, 1974). Addition of Mg^{2+} to maize metabolic system, thus, has a potential to increase bath quantity and quality of maize kernel.

OBJECTIVES

1. To determine the appropriate days of spraying $MgCl_2$ at vegetative and reproductive stages of feeder maize (Nakhon Sawan 2; NS 2) and sweet maize (Hawaiian Sugar Supersweet; HSS).

2. To the most effective concentration of $MgCl_2$ and harvest time to increase the photosynthetic factors, i.e., chlorophyll *a*, chlorophyll *b*, carotenoid, phosphoenolpyruvate carboxylase and total soluble sugar and total protein contents in maize leaves.

LITERATURE REVIEW

1. Botany of maize

Zea mays L. belongs to the grass family Poaceae (Gramineae), tribe Maydeae. "Maize" is the most widely used name for a monoecious plant which develops inflorescences with unisexual flowers having 10 chromosomes (Anonymous, 2007). Mengel *et al.* (2001) reported that Mexico and Central America are the center of origin of maize; hence it can be grown in a semi arid climate with high light intensity. At present, the various cultivars of maize are adapting under different climatic conditions where the temperature is warm enough. Maize may be grouped into seven types on the basis of endosperm and glume characteristics, as maize is sensitive to low temperatures (Acquaah, 2007)

1. Dent maize (*Z. mays indentata*), is characterized by a depression (dent) in the crown caused by the rapid drying and shrinkage of the soft starch at the crown. Of the multiple colors available, the yellow or white kernels dominate commercial production.

2. Flint maize (*Zea mays indurate*) is predominantly comprised of corneous of hard starch that encloses the soft starch at the center. The kernels are smooth, hard, and usually rounded at the top.

3. Flour maize (*Zea mays amylacea*) consists almost entirely of soft starch, making the kernels soft. The kernel colors are white, blue, and variegated.

4. Popcorn (*Zea mays everta*) is an extreme form of flint maize. It has a very hard corneous endosperm with only a small portion of soft starch.

5. Sweet maize (*Zea mays saccharata*) is characterized by a translucent and wrinkled appearance upon drying, and a sweet taste when immature. Standard sweet maize is a mutant of the dent maize with a mutation at the *sugary (sy)* locus.

6. Waxy maize (*Zea mays kuleshov*) has the starch that consists of amylopectin, the result of *waxy (wx)* mutation.

7. Pod maize (*Zea mays tunicate*) has primitive features, each kernel being enclosed in a pod or husk, before the entire ear is enclosed in husks like other maize.

2. Physiology of growth and development of maize

Maize is a C_4 plant of the NADP-malic enzyme class which is characterized by a distinctive anatomy and possession of a CO_2 pumping system that aids the C_3 cycle in photosynthesis. In the mesophyll cells, CO_2 is initially incorporated into C_4 acids which are transported to the bundle sheath tissues where CO_2 is liberated in the vicinity of the C_3 cycle (Pastori *et al.*, 2000). The CO_2 pumping of C_4 metabolism does not depend on the specific function of a membrane transporter but relies on a prefixation of CO_2, after conversion to bicarbonate (HCO_3^-), by reaction with PEP to form oxaloacetate (OAA) in the mesophyll cells. The malate diffuses through the plasmodesmata into the bundle sheath cells after the conversion of this OAA to malate. As a result, CO_2 is released as a substrate for RubisCo. The reaction of HCO_3^- with PEP is catalyzed by the enzyme PEPC (Heldt, 2005).

Maize is a fast growing crop producing a large biomass. Macronutrient fertilization is essential for optimum yield and quality. The most important macronutrients essential for maize is probably phosphorus. Phosphorus is important for root development and early growth. For micronutrient, zinc plays an crucial role in the early development and can have a serious effect on yield potential and grain quality. Zinc is important for the correct functioning of many enzyme systems, the synthesis of nucleic acids and auxin metabolism and normal maize development and growth. Other micronutrients are also required (Hay and Porter, 2006).

The interpretation of positional signaling information is likely to be of particular importance in the elaboration of Kranz anatomy in the leaves of C4 plants such as maize. In maize, two morphologically and biochemically distinct photosynthetic cell types, bundle sheath and mesophyll cells, are arranged in concentric rings around leaf veins. Each cell type carries out a subset of the reactions that lead to photosynthetic carbon fixation. The maize method of photosynthesis can

be interpreted as a set of adaptations to a combination of high temperature and irradiance (Hay and Porter, 2006).

3. Role of magnesium (Mg^{2+}) in plant growth

3.1 Role of Mg in plant growth on photosynthetic pigment (chlorophyll *a* and *b* and carotenoid)

The conversion of primary energy occurs in the chloroplast in porphyrinic nuclei of chlorophylls which have the capacity to absorb photon energy. As a result, the energy is trapped in the nucleus. The radiant energy in the form of photons is absorbed by two groups of pigments and associated compounds representing photosystems I and II (Maiti and Wesche-Edeling, 1998). Chlorophyll of different forms plays an important role as a part of photosynthetic apparatus of all phototrophic organisms (Ferus and Arkosiova, 2001).

Higher plants contain chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*), accessory pigments and several other forms of chlorophyll (Ferus and Arkosiova, 2001). The ratio Chl *a* to Chl *b* is about three to one. Only Chl *a* is a constituent of the photosynthetic reaction centers and, therefore, it can be regarded as the central photosynthesis pigment. The light energy absorbed by Chl *b* can be transferred very efficiently to Chl *a*. Chl *b* enhances the plant's efficiency for utilizing sunlight energy (Heldt, 2005).

The basic structure of Chl *a* and *b* is a ring of four pyrroles. This tetrapyrrole is named porphyrin. At the center of the ring, magnesium ion (Mg^{2+}) is present at the central nucleus. Mg^{2+} is covalently bound with two N-atoms and coordinately bound to the other two atoms of the tetrapyrole ring (Heldt, 2005). At low concentrations of magnesium, the rate of photosynthesis is relatively independent of the chlorophyll content. As the magnesium concentration increases, the rate of photosynthesis rises rapidly. The rise is relatively independent of the chlorophyll content. Evidence indicates that magnesium plays a part in the process of

photosynthesis, in addition to its necessity for chlorophyll formation (Fleischer, 2008).

Maiti and Wesche-Edeling (1998) showed that the Chl *a* and Chl *b* content could vary due to the cultivars, growth stage as well as other climatic factors, i.e., temperature, light, and growing conditions, i.e. field grown or container grown. Ferus and Arkosiova (2001) reported that spring barley (*Hordeum vulgare* L.) grown in the shade gave lower chlorophyll content than those grown under the sunlight. The chlorophyll content in maize at reproductive stage reached the maximum at 19^{th} day of the stage, followed by a steady decrease (Martinez-Barajas, 1997). In general, maize showed the average of 0.65 – 1.46 mg Chl *a* /g fresh weight and of 0.17 – 0.56 mg Chl *b* /g fresh weight (Zaidi *et al*., 2003; Amujoyegbe *et al*., 2007). The recent report observed that levels of chlorophyll in the Mg^{2+} deficient *Mentha pulegium* leaves decreased significantly between the 3rd and 17th days and showed a positive correlation with Mg^{2+} concentration over the treatment period (Candan and Tarhan, 2003).

The carotenoids are one of the most abundant groups of naturally occurring pigments. They are present in all green tissue, where they are constituents of the chloroplast, as well as being responsible for most of the yellow to red colours of flowers and fruits (Lea, 1990). The function of carotenoids in reproductive tissues is to provide colour to flowers and fruit for attraction of pollinators and seed dispersal agents (Davies, 2004). Furthermore, carotenoids are a class of secondary plant compounds that act as accessory photosynthetic pigments. Plant carotenoids are divided into two groups; the xanthophylls and the carotenes (Zaripheh and Erdman**,** 2002; Eonseon *et al*., 2003). These compounds serve many functions including light harvesting, structure stabilization, and excess energy dissipation (Frank *et al.*, 1996; Young *et al*., 1997). However under normal circumstances, carotenoids serve a protective function. It protects the photosynthetic membrane from photooxidation by effectively scavenging singlet oxygen and the quenching triplet state of chlorophyll (Candan and Tarhan, 2003). Chlorophyll will not accumulate if carotenoids are not present (Bozarth and Kennedy, 1985).

3.2 Role of Mg^{2+} on photosynthesis related enzyme (PEPC)

Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) is a key regulatory enzyme in CO_2 fixation in C_4 plants (such as maize). It makes possible the high photosynthetic efficiency of such plants (Wu and Wedding, 1992; Foyer *et al.*, 1998; Buchanan and Wolosiuk, 2002; Izui *et al.*, 2004; Xu *et al.*, 2006). PEPC from higher plants are regulated by both allosteric effects and reversible phosphorylation. It plays primarily an anaplerotic role by replenishing C4-dicarboxylic acids utilized for the energy and biosynthetic metabolisms (Ettema *et al*., 2004; Izui *et al.*, 2004; Takahashi-Terada *et al*., 2005). CO_2 is initially fixed, by PEPC in the mesophyll cells, into the C4 acids. The acids, malatic acid and/or aspartatic acid, which are then transported to the bundle-sheath (Westhoff and Gowik, 2004). PEPC is also involved in the maintenance of the pH balance in guard cells and the accumulation of organic acids in fruits (Takahashi-Terada *et al*., 2005).

PEPC catalyses the irreversible β-carboxylation of phosphoenolpyruvate (PEP) in the presence of HCO^{3-} to form oxaloacetate (OAA) and inorganic phosphate (Pi) in the presence of Mg^{2+} (Mancera, n.d.; Sangwan *et al*., 1992; Ettema *et al*., 2004; Izui *et al.*, 2004; Westhoff *et al*, 2004; Sebei *et al*., 2006) or Mn^{2+} as a cofactor during C_4 photosynthesis (Mancera, n.d.; Ettema *et al*., 2004; Westhoff and Gowik *et al*, 2004; Sebei *et al*., 2006). It is also suggested that the binding mode of PEP and its analogs involves the electrostatic interaction of both the phosphate and carboxylate groups with the Mg^{2+} ion (Mancera, n.d.). PEPC of monocotyledonous plants is activated by glycine (Wagner *et al*., 1987; Takahashi-Terada *et al*., 2005). But in maize, metabolic activators such as glucose 6-P, glycine, alanine and serine are much more effective in protecting the enzyme against malate inhibition (Woo and Xu, 1996; Mancera, n.d.).

This enzyme is inactivated when binds to the chloroplast membrane or else interacts with a protein isolated from the membrane. As the concentration of Glc-6-P (Glucose-6-Phosphate) increased, the inactivation of PEPC decreased. While,

phosphorylation of PEPC results in an increase in maximal enzyme activity and a decrease in the sensitivity of the enzyme to malate. Malate is a competitive inhibitor when PEPC is assayed at pH 7.3 in the presence of 1 mM PEP (Buchanan and Wolosiuk, 2002).

In addition, a protein isolated from a maize leaf chloroplast membrane preparation inactivated PEPC. It was also found that C_4 PEPC is unstable at low temperatures (Buchanan and Wolosiuk, 2002). PEPC is more active in the light than in the dark, as a result of reversible phosphorylation (Gardeström and Lgamberdiev, 2002). Activation ranging from 20 to 110% over the activity values of PEPC in the dark was found in *Atriplex tatarica* L., *Atriplex halimus* L., *Cyperus rotundus* L., *Salsola kali* L., *Digitaria sanguinalis* (L.) Scop, *Setaria verticillata* (L.) Beauv., *Eleusine indica* (L.) Gaetner, and *Portulaca oleracea* L. In contrast, no activation could be ensured under light in *Cynodon dactylon* (L.) Pers., *Saccharum ravenae* (L.) Murray, *Amaranthus* sp., *Zea mays* L., and *Sorghum bicolor* (L.) Moench (Karabourniotis *et al.*, 1983). Thus, cytosolic pH and PEP concentration are factors that may determine the physiological significance of the apparent light effect on PEPC in maize (Kalt-Torres *et al.*, 1987). The maximal catalytic activity of PEPC significantly increased in water-stressed maize leaves compared with well-watered controls. The decrease in the PEPC transcript pool occurred with carbohydrate accumulation in water-stressed maize leaves (Foyer *et al*., 1998).

3.3 Role of Mg^{2+} on sugar metabolism in plant

Sugars, which are the first products of photosynthesis, are converted into starch, protein, oil, cellulose, lignin, and thousands of other chemical compounds. The accumulation of sugar in maize leaves during the day was found to rapidly deplete close to the end of the photoperiod (Kalt-Torres *et al*., 1987). It is also known that photosynthetically active source of tissues, such as mature leaves produce more carbohydrates than they require and thus export assimilates as sucrose to photosynthetically less active or inactive tissues, such as young leaves, roots, fruits or canes. However, source/sink relations are not static. During vegetative stage, most

carbohydrates are imported into the roots and young leaves, whereas reproductive stage, carbohydrates are mainly directed into the developing fruits tuber and storage roots (Roitch *et al*., 2003). When photosynthesis was inhibited, the starch broke down and allowed ovary development to continue with the released sugars (Zinselmeier *et al*., 1999). Decreasing photosynthesis thus had an immediate inhibitory effect on the sugar stream to the ovaries of maize (Westgate and Boyer, 1986; Boyle *et al*., 1991; Schussler and Westgate, 1995). While the decreased sugar stream was coupled with decreased carbon processing inside the ovaries, kernel number was markedly decreased. Later in kernel development, sugar reserves were abundant in the stems and leaves of the parent plant, and the sugars were mobilized to support kernel development (McPherson and Boyer, 1977; Jurgens et al., 1978; Westgate and Boyer, 1985).

Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al.*, 2000). The increase in sugar concentration may be a result from the degradation of starch (Fischer and Höll, 1991). Starch may play an important role in accumulation of soluble sugars in cells. Starch depletion in grapevine leaves was responded to drought stress (Patakas and Noitsakis, 2001). Under the stress, the concentrations of soluble sugars increased at the same time as a decrease in the starch concentration. This means that the raised soluble sugar fraction was accompanied by a sharp decrease in the starch fraction as the water potential dropped (Mohammadkhani and Heidari, 2008).

During fruit maturation, when sugar transports from leaves to berries, the leaf starch accumulation decreased. When the fruit growth rate is maximum, the fruits are preferential sinks which receives a great amount of sucrose transported from photosynthesizing leaves. Therefore, leaves would present low invertase activity, consuming little sucrose during fruit ripening (Pimentel, 1998). Earlier reports mentioned that sugars protect the cells during drought by the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes

through hydrogen-bonding, thereby preventing protein denaturation (Leopold *et al*., 1994).

3.4 Role of Mg^{2+} on plant total protein

The protein synthesis in leaves can be separated temporarily and spatially from that of the intrinsic pigment content of the thylakoids during chloroplast development along the leaf lamina. Therefore, the changes in leaf protein contents were not parallel with the leaf pigment content (Dilnawaz *et al*, 2001). The primary effect of a lack of Mg^{2+} is to disturb polypeptide synthesis in the ribosomes there by restricting protein production and growth as proposed by Mengel and Kirkby (2001). Magnesium has functions in protein synthesis that can affect the size, structure, and function of chloroplasts. The requirement of magnesium in protein synthesis is apparent in chloroplasts, where magnesium is essential for the synthesis and maintenance of proteins in the thylakoids of the chlorophyll molecule. Hence, the degradation of proteins in chloroplasts in magnesium-deficient plants may lead to loss of chlorophyll as much as the loss of magnesium for chlorophyll synthesis (Barker and Pilbeam, 2007).

MATERIALS AND METHODS

Plant materials

Two maize varieties, Nakhon Sawan 2 (NSX 022031) and Hawaiian Sugar Supersweet (HSSS) were used. Plants were grown from seeds in 12 inches pots at the Plant Nursery, Botany, Kasetsart University in July 2006 to January 2007. The stems of HSSS are yellowish green with 150 – 180 cm when matured. There are 12 leaves and the silk become elongated stigma when the maize is 45 – 50 days old. It has a production of (ears without husk leaves) 5,625-7,500 kilogram/hectares (900 – 1,200 kilogram/Rai), sweetness 14.0 °Bx, with sweet and crisp yellow seeds. The green ears of sweet maize are consumed in various ways. However, HSS is not resistant to downy mildews (Nakapraves, 1989; Songchow, 1992; Insamapun, 2002; Anonymous, 2006).

One hybrid feeder maize which is also resistant to downy mildew (*Peromoscleros-pora sorghi*) is *Zea mays* L. cv NSX 022031 (Nakhon Sawan 2) has a higher product and stability in production within *in vitro* conditions than *Zea mays* L. cv NSX 9210 (Nakhon Sawan 72). It also NSX 022031 is a new single-cross that is harvested at 110-115 days. It is generated from cross-pollination homozygous lines feeder maize Nei 452008 (mother lines) and homozygous lines feeder maize Nei 9202 (T) (father lines). NS2 gives 1,110 kilogram of seed per 0.16 hectares, silking 55 days, tassel 53 days, and a level of weakness on maize downy mildews of 22.2%. It has fatty 6.78%, protein 11.66% and carbohydrate 67.46%.

The third leaves of 17-day old plants were collected every other day until the plants reached approximate 60 days. After removing the midribs, the samples were kept at -70ºC until use. Enzyme phosphoenolpyruvate carboxylase (PEPC) of each collecting days were analyzed to find the maximum PEPC in the vegetative and reproductive stages.

$MgCl_2$ treatment

Different concentrations of $MgCl_2$ solution (0.00, 0.05, 0.10, and 0.15 mM) were sprayed to the whole plants (500 ml/plant) one day before the designated maximum PEPC of either vegetative or reproductive stages as determined from the pretest. The third leaves of the $MgCl_2$-treated plants were collected on the 1, 3, 5, 7, 9, 11, and 21 days after spraying. The samples were analyzed for PEPC activity, protein content, chlorophyll *a* and *b* contents, carotenoid content, and total soluble sugar content.

PEPC activity and protein analysis

Approximately 0.5 g (fresh weight) of leaf samples were pulverized in liquid N_2 and dissolved in 5 ml buffer solution containing 10 mM PVPP, 100 mM Trizma®-HCl (pH 7.6), 1.5 mM EDTA, 1.5 mM $MgCl_2$, 1.5 mM $KHCO_3$, 2.5% (v/v) Tween 20, 10% (v/v) glycerol and 5 mM DTT. The homogenates were centrifuged twice at 18,900 g, 4 ºC, for 15 min. Supernatants were collected for analysis of PEPC activity and protein content. PEPC activity was determined according to the method of Anonymous (n.d.). The 2.9 ml mixing solution containing 0.1M Tris-HCl buffer (pH 8.0), 0.1 M Na_2CO_3, 32 mM K-phosphoenolpyruvate, 1 M $MgSO_4$, 1.4 mM NADH and ca.100U/ml MDH was left at room temperature for 5 min. The 0.1 ml sample was transferred to the mixing solution and gently mixed. The absorbance was measured at 340 nm. The protein content in the samples was determined by the method of Hartree-Lowry (1972) using bovine serum albumin as a standard (Appendix). The results were expressed as mg protein/g fresh tissue.

Chlorophyll *a*, *b* and carotenoid content

Chlorophyll *a*, *b* and carotenoid content were determined according to the method described by Bajracharya (2003). Approximately 500 mg of leaf samples were transferred into 20 ml of 80% acetone and ground for 5 min, then, filtered

(Whatman no.1). The remaining pulp on the filter paper was extracted again using the same procedure described above. Both extracts were pooled and added with 80% acetone to make the final volume of 50 ml. Absorbances were measured at 440, 645, and 663 nm. The results were presented as mg/g fresh weight. Chlorophyll *a*, *b* and carotenoid were calculated as follows:

$$\text{Chlorophyll } a = 9.78 \cdot A_{663} - 0.99 \cdot A_{645}$$

$$\text{Chlorophyll } b = 21.4 \cdot A_{645} - 4.65 \cdot A_{663}$$

$$\text{Carotenoid} = 4.69 \cdot A_{440} - 0.268 \cdot (20.2 \cdot A_{645} + 8.02 \cdot A_{663})$$

Total soluble sugar

Total soluble sugar was determined according to the method of Bajaracharya (2003). Approximately 500 mg of leaf samples were transferred to 5 ml distilled water and ground. They were filtered twice (Whatman no.1) as previously described. Both filtrates were combined and centrifuged at 10,000 g, 4 ºC, for 15 min. Supernatant was collected and added with distilled water to make 50 ml. An aliquot of 1 ml supernatant was gently mixed with 2 ml anthrone reagent (95 % sulfuric acid and 50 mg anthrone), immersed in 100 ºC water bath for 3 min and transferred to an ice-cold water bath. After complete cooling, the absorbance was measured at 620 nm. The total soluble sugar was determined as mg sugar/g fresh weight.

Statistical analysis

The experimental plots have been arranged in Completely Randomized Design with 3 replications. All the results (except the pretest) were recorded and analyzed statistically using SPSS 13.0 program. Significant differences between the days after spraying $MgCl_2$ and the concentration of $MgCl_2$ solution were also determined.

RESULTS

1. Phosphoenolpyruvate carboxylase (PEPC) activity in the maize leaves without $MgCl_2$ treatment

1.1 Vegetative stage

PEPC (EC 4.1.1.31) is a key regulatory enzyme playing an important role in CO_2 fixation in C_4 plants (Izui *et al*., 2004; Xu *et al*., 2006). PEPC activity profiles of both 'Nakhon Sawan 2' (NS 2) and 'Hawaiian Sugar Supersweet' (HSS) maizes were established to determine the appropriate spraying date of $MgCl_2$ for vegetative and reproductive stages. For vegetative stage (Figures 1), PEPC activity was highest on days 43 for the feeder maize (NS 2) and on day 45 for the sweet maize (HSS) which are almost the end of vegetative period. Therefore, the earliest date (day 25) with stable PEPC activity was selected for $MgCl_2$ treatment.

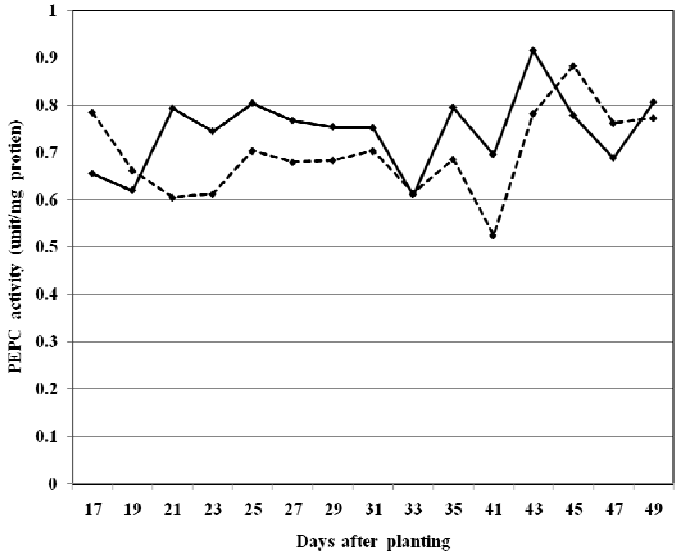

Figure 1 Changes in phosphoenolpyruvate carboxylase activity in the leaves of 'Nakhon Sawan 2' (—) and 'Hawaiian Sugar Supersweet' (·····) maizes at vegetative stage without treating with $MgCl_2$.

1.2 Reproductive stage

For reproductive stage (Figures 2), the highest PEPC activities for both varieties were recorded at the beginning of their reproductive period. The appropriate spraying times of $MgCl_2$ were, thus, designated to be day 62 and day 58 for 'Nakhon Sawan 2' and 'Hawaiian Sugar Supersweet' maizes, respectively.

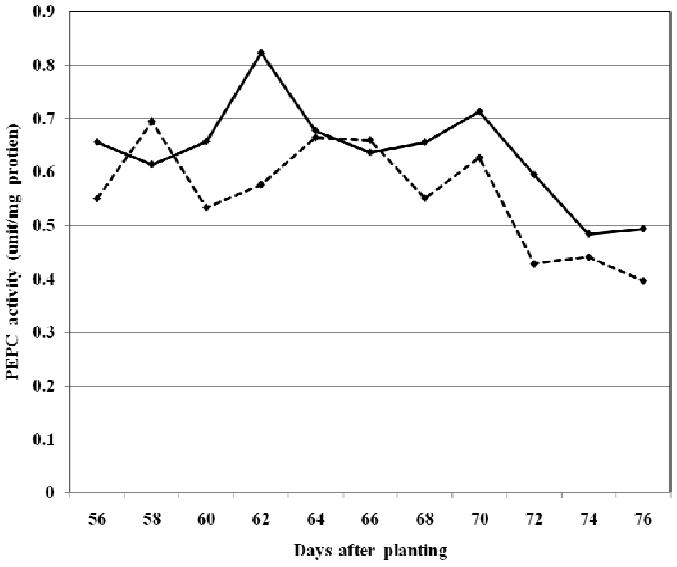

Figure 2 Changes in phosphoenolpyruvate carboxylase activity in the leaves of 'Nakhon Sawan 2' (—) and 'Hawaiian Sugar Supersweet' (·····) maizes at reproductive stage without treating with $MgCl_2$.

2. Chlorophyll *a* (Chl *a*) contents in the maize leaves after $MgCl_2$ application

2.1 Vegetative stage

The chlorophyll *a* and *b* take part in different pathways of photosynthesis, i.e., Chl *a* acts as a reaction center while Chl *b* transfer absorbed light energy to Chl *a* (Heldt, 2005). Together they are the main factors controlling photosynthetic mechanism. Chl *a* contents in the leaves of the feeder maize with and without $MgCl_2$ treatment were compared (Table 1). Among 7 recorded dates, the leaf Chl *a* contents in the treated plants were significantly different from those in non-treated plants only on day 21 after spraying. On the first day after spraying, leaf Chl *a* contents in the maizes treated with 0.05 mM $MgCl_2$ were less than those in the control plants. On the other hand, leaf Chl *a* contents in the maizes treated with 0.05 mM $MgCl_2$ were higher than those in the control plants on the 21^{st} day after spraying.

For the sweet maize (Table 2), leaf Chl *a* contents in the treated plants were significantly higher than those in non-treated plant on day 1, day 7 and day 9 after spraying. On the day 1 and the day 7, the Chl *a* contents in the plants sprayed with 0.10 and 0.15 mM $MgCl_2$ were significantly higher than those in the control plants. For the day 9, the leaf Chl *a* contents in all the plants sprayed with $MgCl_2$ were significantly higher than those in the control plants. Among the treated plant, the one sprayed with the highest concentration of $MgCl_2$ had the significantly highest leaf Chl *a* content.

2.2 Reproductive stage

As seen from Table 3, leaf Chl *a* contents in the treated NS 2 plants were significantly higher than those in the non-treated plants only on day 9 after spraying. On day 3 after spraying, leaf Chl *a* contents in the feeder maizes treated with 0.05 mM $MgCl_2$ were less than those in the non-treated plants. On the contrary, Chl *a* contents in the leaves on day 9 of the maizes treated with 0.15 mM $MgCl_2$ were higher than those in the non-treated plants.

For sweet maize (Table 4), Chl *a* contents in the leave of the maizes on day 11 after spraying, which treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the control plants. On the other hand, the leaf Chl *a* contents in the treated plants on day 21 sprayed with 0.05 and 0.10 mM $MgCl_2$ were significantly higher than those in the control plants.

Table 1 Chlorophyll *a* content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.459±0.029[1/]a[2/]	0.379±0.008	0.447±0.028	0.410±0.025	0.310±0.017	0.348±0.056	0.353±0.030b
0.05 mM	0.339±0.037b	0.408±0.011	0.260±0.033	0.312±0.029	0.409±0.009	0.432±0.006	0.475±0.008a
0.10 mM	0.460±0.015a	0.433±0.054	0.395±0.068	0.360±0.036	0.309±0.048	0.362±0.030	0.384±0.018b
0.15 mM	0.422±0.016ab	0.421±0.039	0.346±0.015	0.349±0.023	0.369±0.055	0.325±0.038	0.376±0.023b
F test	*	ns	ns	ns	ns	ns	*
C.V. %	13.56%	5.65%	22.02%	11.39%	13.97%	12.52%	13.50%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

***** = significant at the 95% level of confidence

Table 2 Chlorophyll *a* content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.382±0.006[1/]b[2/]	0.425±0.015	0.367±0.024	0.324±0.014b	0.197±0.017c	0.338±0.020	0.253±0.002
0.05 mM	0.387±0.059b	0.384±0.038	0.288±0.071	0.299±0.026b	0.371±0.037b	0.316±0.031	0.311±0.038
0.10 mM	0.603±0.034a	0.435±0.019	0.365±0.004	0.471±0.040a	0.370±0.012b	0.402±0.077	0.282±0.032
0.15 mM	0.613±0.070a	0.422±0.030	0.473±0.020	0.479±0.029a	0.509±0.069a	0.389±0.034	0.348±0.013
F test	*	ns	ns	**	**	ns	ns
C.V. %	25.99%	5.42%	20.34%	24.18%	35.25%	11.31%	13.63%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 3 Chlorophyll *a* content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.364±0.001	0.398±0.042[1/]a[2/]	0.407±0.007	0.347±0.025	0.316±0.049b	0.318±0.028	0.208±0.018
0.05 mM	0.396±0.024	0.283±0.023b	0.338±0.008	0.416±0.022	0.259±0.008b	0.432±0.004	0.269±0.043
0.10 mM	0.381±0.022	0.443±0.012a	0.447±0.019	0.336±0.018	0.349±0.043ab	0.346±0.028	0.303±0.003
0.15 mM	0.395±0.002	0.449±0.017a	0.445±0.084	0.416±0.062	0.444±0.031a	0.333±0.042	0.230±0.027
F test	ns	**	ns	ns	*	ns	ns
C.V. %	3.91%	19.57%	12.35%	11.48%	22.72%	14.35%	16.54%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

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Table 4 Chlorophyll *a* content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.274±0.033	0.353±0.034	0.411±0.006	0.291±0.034	0.339±0.037	0.258±0.011[1/]c[2/]	0.367±0.021a
0.05 mM	0.368±0.014	0.359±0.064	0.362±0.025	0.357±0.034	0.371±0.020	0.361±0.014ab	0.182±0.037b
0.10 mM	0.351±0.028	0.322±0.070	0.441±0.011	0.350±0.061	0.390±0.027	0.391±0.027a	0.173±0.017b
0.15 mM	0.380±0.025	0.427±0.031	0.396±0.026	0.435±0.030	0.323±0.018	0.318±0.025bc	0.280±0.049ab
F test	ns	ns	ns	ns	ns	**	*
C.V. %	13.95%	12.16%	8.11%	16.47%	8.50%	17.35%	36.47%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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3. Chlorophyll *b* (Chl *b*) contents in the maize leaves after $MgCl_2$ application

3.1 Vegetative stage

Chl *b* contents in the leaves of the treated and non-treated feeder maizes were compared (Table 5). On day 21 after spraying, leaf Chl *b* content of the maize treated with 0.05 mM $MgCl_2$ were higher than those in the control plants.

Table 6 shows Chl *b* contents in the leaves of sweet maize. Among 7 recorded dates, the leaf Chl *b* contents in the treated plants were significantly different from those in non-treated plants on day 1, day 5, day 7, and day 9 after spraying. For the day 1, the Chl *b* contents in the plants sprayed with 0.10 and 0.15 mM $MgCl_2$ were significantly higher than those in the control plants. On the day 5 and day 7, the leaf Chl *b* contents in the plants sprayed with 0.15 mM $MgCl_2$ were significantly higher than those in the control plants. On day 9, leaf Chl *b* contents in the treated plants were significantly higher than those in the control plants. It was found that Chl *b* contents of the plants sprayed with the highest concentration of $MgCl_2$ were the highest.

3.2 Reproductive stage

The leaf Chl *b* contents in the feeder maize were significantly higher than those in the control plants only on day 21 day after spraying (Table 7). The Chl *b* in maize treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the control plants.

For sweet maize, Chl *b* contents in the treated plants were significantly different from those in non-treated plants only on day 11 after spraying. As shown in Table 8, those in the plants treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the non-treated plants.

Table 5 Chlorophyll *b* content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.198±0.015	0.166±0.004	0.202±0.013	0.183±0.004	0.140±0.007	0.154±0.018	0.156±0.013b[1/][2/]
0.05 mM	0.151±0.014	0.176±0.010	0.120±0.011	0.157±0.014	0.180±0.001	0.192±0.002	0.238±0.017a
0.10 mM	0.191±0.010	0.187±0.022	0.175±0.031	0.171±0.013	0.132±0.017	0.157±0.012	0.176±0.003b
0.15 mM	0.184±0.008	0.183±0.018	0.146±0.009	0.159±0.009	0.163±0.020	0.139±0.012	0.178±0.005b
F test	ns	ns	ns	ns	ns	ns	**
C.V. %	11.37%	5.27%	22.13%	7.37%	14.40%	13.96%	19.00%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

****** = significant at the 99% level of confidence

Table 6 Chlorophyll *b* content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	0.143±0.013[1/]b[2/]	0.177±0.008	0.159±0.008b	0.150±0.009bc	0.090±0.012c	0.148±0.015	0.114±0.004
0.05 mM	0.182±0.026b	0.212±0.045	0.137±0.024b	0.123±0.195c	0.169±0.016b	0.146±0.010	0.143±0.018
0.10 mM	0.264±0.013a	0.190±0.005	0.160±0.001b	0.192±0.016ab	0.165±0.007b	0.183±0.033	0.124±0.014
0.15 mM	0.277±0.034a	0.188±0.018	0.209±0.006a	0.220±0.014a	0.245±0.036a	0.171±0.015	0.159±0.007
F test	*	ns	*	*	**	ns	ns
C.V. %	29.94%	7.66%	18.20%	25.39%	37.63%	11.14%	14.77%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 7 Chlorophyll *b* content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.116±0.027	0.167±0.020[1/]ab[2/]	0.184±0.003	0.155±0.012	0.143±0.019	0.134±0.013	0.103±0.002c
0.05 mM	0.182±0.016	0.124±0.013b	0.151±0.001	0.189±0.012	0.127±0.006	0.182±0.004	0.147±0.014b
0.10 mM	0.154±0.016	0.180±0.006a	0.196±0.011	0.157±0.006	0.170±0.018	0.156±0.013	0.181±0.002a
0.15 mM	0.179±0.011	0.210±0.012a	0.202±0.039	0.201±0.022	0.201±0.017	0.157±0.016	0.120±0.010bc
F test	ns	*	ns	ns	ns	ns	**
C.V. %	19.43%	20.86%	12.34%	13.20%	20.15%	12.46%	24.67%

Note: [1/] = the average of 3 replications ± S.E.

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Table 8 Chlorophyll *b* content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.124±0.014	0.154±0.014	0.176±0.002	0.123±0.018	0.163±0.009	0.108±0.005[1/]c[2/]	0.165±0.005
0.05 mM	0.204±0.035	0.147±0.029	0.155±0.009	0.161±0.016	0.176±0.008	0.175±0.016ab	0.100±0.018
0.10 mM	0.160±0.009	0.164±0.028	0.189±0.004	0.162±0.026	0.176±0.009	0.179±0.012a	0.091±0.004
0.15 mM	0.169±0.010	0.186±0.003	0.174±0.010	0.196±0.013	0.158±0.007	0.137±0.011bc	0.133±0.042
F test	ns	ns	ns	ns	ns	**	ns
C.V. %	20.06%	10.37%	8.15%	18.43%	5.52%	22.40%	31.96%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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4. Carotenoid content in the maize leaves after $MgCl_2$ application

4.1 Vegetative stage

Carotenoids play a variety of crucial roles in all photosynthetic organisms by absorbing light energy and transferring it to chlorophyll (Cuttriss and Pogson, 2004). Carotenoid contents in the vegetative stage of the feeder and the sweet maizes were in the range of 4.654 – 7.268 mg/g fresh weight (average 5.735 mg/g fresh weight) and 2.118 – 7.553 mg (average 4.972 mg/g fresh weight), respectively. The feeder maize with $MgCl_2$ treatment had statistically the same carotenoid content as the control plants in all dates recorded (Table 9).

The carotenoid contents in the sweet maize leaves on day 1, day 7, and day 9 after spraying were significantly higher than those without $MgCl_2$ treatment (Table 10). On day 1 and day 7, leaves carotenoid contents in the maizes treated with 0.10 and 0.15 mM $MgCl_2$ were higher than those in the control plants. On day 9, the carotenoid content in the control plants were the lowest.

4.2 Reproductive stage

Leaf carotenoid contents in the treated feeder maizes were significant higher than those in non-treated plants on day 1, 9, 11, and 21 after spraying (Table 11). For the day 1 and day 11, leaf carotenoid contents in the plants treated with 0.05 mM $MgCl_2$ were higher than those in non-treated plants. On day 9, only the leaf carotenoid contents in the plants sprayed with 0.15 mM $MgCl_2$ were significant higher than those in non-treated plants. In addition, the carotenoid content in the plants treated with 0.10 mM $MgCl_2$ were higher than those in non-treated plant on day 21.

The sweet maize achieved its raise carotenoid content in the plants treated with 0.05 and 0.10 mM $MgCl_2$ on day 11 after spraying, as compared to the non-

treated plant (Table 12). However, the leaf carotenoid contents in all the plants sprayed with $MgCl_2$ were less than those in the control plant on day 21.

Table 9 Changes in carotenoid content (mg/g fresh weight) in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	6.720±0.532[1/]	4.780±0.203	7.268±0.830	6.051±0.511	4.654±0.313	5.780±0.997	4.893±0.492
0.05 mM	5.778±0.739	7.106±0.509	4.021±0.845	4.341±0.452	7.180±0.169	6.361±0.082	7.266±0.821
0.10 mM	6.177±0.414	5.763±1.144	5.927±1.880	5.208±0.745	4.450±0.999	6.029±0.452	5.527±0.264
0.15 mM	5.733±0.157	7.966±0.907	5.675±0.077	4.748±0.597	5.077±1.136	4.787±0.864	5.371±0.641
F test	ns	ns	ns	ns	ns	ns	ns
C.V. %	7.50%	22.05%	23.29%	14.43%	23.48%	11.81%	17.98%

Note: [1/] = the average of 3 replications ± S.E.

ns = non-significance by LSD

Table 10 Carotenoid content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	6.085±0.338[1/]b[2/]	7.553±0.439	5.660±0.095ab	4.979±0.195b	2.118±0.306b	4.940±0.110	3.472±0.103
0.05 mM	6.893±1.105b	7.289±0.661	4.314±1.163b	4.474±0.213b	5.582±0.438a	5.089±0.498	4.786±0.461
0.10 mM	10.728±0.880a	6.814±0.532	6.757±0.269a	7.728±0.854a	6.779±0.288a	5.962±1.881	3.872±0.671
0.15 mM	11.996±1.584a	7.904±0.401	7.529±0.333a	8.685±0.966a	8.412±1.775a	5.861±0.388	4.998±0.183
F test	*	ns	*	**	**	ns	ns
C.V. %	32.26%	6.21%	23.03%	31.81%	46.63%	9.58%	17.01%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 11 Carotenoid content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	5.029±0.296[1/]bc[2/]	6.868±1.014a	7.041±0.246	4.749±0.615	4.850±0.832b	4.729±0.229b	2.857±0.294b
0.05 mM	6.482±0.530a	4.367±0.409b	5.141±0.491	6.510±0.882	4.114±0.541b	7.005±0.298a	4.245±0.437ab
0.10 mM	4.713±0.429c	7.834±0.543a	7.523±0.560	4.929±0.232	5.218±0.211b	6.169±0.534ab	5.312±0.165a
0.15 mM	6.147±0.169ab	7.906±0.496a	7.845±1.918	7.186±1.691	7.867±0.967a	5.516±0.623b	3.839±0.666b
F test	*	*	ns	ns	*	*	*
C.V. %	15.28%	24.52%	17.57%	20.44%	29.67%	16.51%	25.02%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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***** = significant at the 95% level of confidence

Table 12 Carotenoid content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	3.824±0.597	5.770±0.322	7.804±0.140	4.379±0.326	6.230±0.467	3.932±0.412[1/]c[2/]	6.388±0.285a
0.05 mM	6.568±0.339	6.407±1.462	5.690±0.517	5.014±0.429	5.793±0.391	5.840±0.287ab	2.561±0.978b
0.10 mM	4.995±0.670	5.868±1.401	7.830±0.343	5.967±1.079	6.613±0.424	6.511±0.774a	2.341±0.336b
0.15 mM	5.497±0.611	7.847±0.436	6.481±1.073	7.181±1.173	5.100±0.326	4.686±0.397bc	3.353±0.138b
F test	ns	ns	ns	ns	ns	*	**
C.V. %	21.82%	14.80%	15.11%	21.64%	10.94%	22.01%	51.07%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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5. Total soluble sugar (TSS) content in the maize leaves after $MgCl_2$ application

5.1 Vegetative stage

The leaf TSS contents in treated feeder maize plants were significantly different from those in the non-treated plants on day 1, day 5, and day 9 after spraying (Table 13). For the day 1, leaf TSS contents in the maizes treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the non-treated plants. However, leaf TSS contents of the plant treated with 0.15 mM $MgCl_2$ were less than those in the non-treated plants. On day 5, only leaf TSS contents in the plants treated with 0.10 mM $MgCl_2$ were higher than those in the control plants.

TSS contents in the leaves of the sweet maize with and without $MgCl_2$ treatment showed are in Table 14. The leaf TSS contents of the maizes treated with 0.05 and 0.10 mM $MgCl_2$ were less than the non-treated plant on day 3 after spraying. Moreover on day 11, the TSS contents in the plants sprayed with 0.05 and 0.15 mM $MgCl_2$ were significantly less than those in the control plants.

5.2 Reproductive stage

Leaf TSS contents in the treated feeder maizes were significantly higher than those in non-treated plants on day 1 and day 21 after spraying (Table 15). Although TSS contents in the maizes sprayed with 0.10 mM $MgCl_2$ were higher than those in the control plants on day 1, but those in the plants sprayed with 0.15 mM $MgCl_2$ less than those in the control plants. For day 21, leaf TSS contents in the all treated plants were significantly higher than those in the non-treated plants.

TSS contents in the leaves of the sweet maize with and without $MgCl_2$ treatment were compared (Table 16). Among 7 recorded dates, there were no significant difference between the treated and the non-treated plants.

Table 13 Total soluble sugar content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	2.224±0.018[1/]b[2/]	2.244±0.099	1.988±0.007b	2.210±0.029	3.751±0.859ab	2.076±0.023	2.621±0.394
0.05 mM	2.412±0.064a	1.995±0.042	1.901±0.064b	2.035±0.047	4.142±0.765a	2.028±0.128	2.062±0.018
0.10 mM	2.425±0.029a	2.096±0.062	2.123±0.037a	2.176±0.012	1.975±0.035bc	1.880±0.093	2.399±0.012
0.15 mM	1.874±0.012c	2.055±0.042	1.874±0.031b	2.264±0.150	1.874±0.000c	2.069±0.102	2.493±0.076
F test	**	ns	*	ns	*	ns	ns
C.V. %	11.51%	5.06%	5.69%	4.50%	40.17%	4.52%	9.98%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 14 Total soluble sugar content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	2.298±0.012	2.264±0.018[1/]a[2/]	2.096±0.152	2.197±0.012	2.001±0.076	2.237±0.023a	2.257±0.047ab
0.05 mM	2.331±0.117	1.995±0.062b	1.941±0.029	2.271±0.079	1.988±0.054	1.907±0.018b	2.385±0.059a
0.10 mM	2.203±0.064	1.988±0.087b	1.981±0.037	2.069±0.041	1.907±0.036	2.217±0.095a	2.197±0.020b
0.15 mM	2.264±0.184	2.170±0.037ab	1.894±0.012	2.015±0.102	2.015±0.111	2.042±0.013b	2.176±0.047b
F test	ns	*	ns	ns	ns	**	*
C.V. %	2.40%	6.45%	4.36%	5.47%	2.45%	7.42%	4.17%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Table 15 Total soluble sugar content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	2.156±0.012[1/]b[2/]	2.473±0.071	2.533±0.106a	2.102±0.041ab	2.486±0.034	2.486±0.105	2.076±0.012c
0.05 mM	2.190±0.007b	2.298±0.107	2.318±0.192ab	2.170±0.034a	2.217±0.062	2.298±0.035	2.197±0.000b
0.10 mM	2.331±0.018a	2.311±0.013	2.069±0.075b	1.901±0.108b	2.156±0.107	2.372±0.064	2.621±0.023a
0.15 mM	1.894±0.051c	2.271±0.036	1.995±0.031b	2.244±0.047a	2.224±0.197	2.526±0.058	2.553±0.044a
F test	**	ns	*	*	ns	ns	**
C.V. %	8.52%	3.90%	11.02%	7.01%	6.46%	4.33%	11.28%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 16 Total soluble sugar content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	2.082±0.047[1/]	2.419±0.062	2.540±0.012	2.325±0.084	2.264±0.029	2.385±0.133	2.439±0.051
0.05 mM	1.968±0.013	2.203±0.070	2.277±0.111	2.055±0.076	1.975±0.076	2.298±0.012	2.412±0.086
0.10 mM	1.954±0.062	2.365±0.205	2.156±0.172	2.089±0.087	2.291±0.178	2.351±0.117	2.486±0.024
0.15 mM	2.143±0.049	2.257±0.123	2.008±0.186	2.217±0.031	2.143±0.091	2.318±0.076	2.580±0.142
F test	ns	ns	ns	ns	ns	ns	ns
C.V. %	4.47%	4.25%	10.02%	5.69%	6.65%	1.65%	2.98%

Note: [1/] = the average of 3 replications ± S.E.

ns = non-significance by LSD

6. Protein content in the maize leaves after $MgCl_2$ application

6.1 Vegetative stage

As shown in Table 17, leaf protein contents in the feeder maizes treated with 0.05 and 0.10 mM $MgCl_2$ were less than those in non-treated plants on day 1 and day 3 after spraying. On day 1, leaf protein contents in the plants treated with 0.15 mM $MgCl_2$ were equal to those in the control plants. For day 3, TSS contents in all the treated plants were less than those in the non-treated plant. It was also found that, on day 21, leaf protein contents in the plant treated with 0.05 mM $MgCl_2$ were less than those the non-treated plants. On the other hand, leaf protein contents in the plant treated with 0.15 mM $MgCl_2$ were significantly higher than those in the non-treated plants.

For sweet maize (Table 18), protein contents in the leaves on day 9 of the maizes treated with 0.05 mM $MgCl_2$ were less than those in the control plants. On the contrary, leaf protein contents in the treated plants with 0.10 and 0.15 mM $MgCl_2$ were significantly higher than those in the non-treated plants on day 11 after spraying.

6.2 Reproductive stage

Among 7 recorded dates, the leaf protein contents in some treated feeder maizes were significantly higher than those in non-treated plants on day 5 and day 21 after spraying (Table 19). On day 5, the protein contents in the plant sprayed with 0.10 mM $MgCl_2$ were higher than those in the control plants. While, leaf protein contents in the plants treated with 0.05 mM $MgCl_2$ were higher than the control plants on day 21.

On day 5 after spraying, leaf protein contents in the sweet maizes treated with 0.10 mM $MgCl_2$ were higher than those in the non-treated plants (Table 20). However, the plants treated with 0.15 mM $MgCl_2$ had less leaf protein than the non-

treated plants. For day 11, leaf protein contents in all the treated plants were less than those in the control plants.

Table 17 Protein content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.930±0.019[1/]a[2/]	1.028±0.048a	0.589±0.045	0.667±0.025	0.579±0.044	0.554±0.021	0.580±0.031b
0.05 mM	0.781±0.052bc	0.874±0.041b	0.855±0.013	0.646±0.072	0.644±0.048	0.633±0.012	0.499±0.004c
0.10 mM	0.689±0.045c	0.827±0.037b	0.793±0.053	0.597±0.036	0.661±0.055	0.625±0.043	0.552±0.009bc
0.15 mM	0.837±0.031ab	0.885±0.017b	0.715±0.105	0.610±0.032	0.555±0.024	0.614±0.004	0.716±0.012a
F test	*	*	ns	ns	ns	ns	**
C.V. %	12.48%	9.59%	15.55%	5.16%	8.32%	5.88%	15.70%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 18 Protein content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.919±0.005	0.863±0.054	0.799±0.095	0.870±0.019	0.813±0.005[1/]a[2/]	0.494±0.003b	0.512±0.001
0.05 mM	1.150±0.077	0.849±0.067	0.766±0.011	0.826±0.093	0.591±0.025b	0.595±0.010ab	0.573±0.027
0.10 mM	0.859±0.102	0.815±0.058	0.776±0.033	0.835±0.014	0.735±0.037a	0.723±0.047a	0.535±0.026
0.15 mM	0.948±0.124	0.716±0.033	0.748±0.079	0.747±0.084	0.713±0.050a	0.732±0.097a	0.579±0.025
F test	ns	ns	ns	ns	*	*	ns
C.V. %	13.03%	8.16%	2.77%	6.37%	12.90%	17.81%	5.81%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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Table 19 Protein content (mg/g fresh weight) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

$MgCl_2$	Days after spraying						
	1	3	5	7	9	11	21
0.00 mM	0.711±0.009	0.683±0.027	0.686±0.029 b[1/,2/]	0.659±0.027	0.728±0.026	0.653±0.015	0.726±0.003b
0.05 mM	0.698±0.038	0.704±0.011	0.707±0.013b	0.666±0.037	0.685±0.025	0.609±0.017	0.834±0.012a
0.10 mM	0.700±0.005	0.715±0.007	0.809±0.003a	0.680±0.025	0.619±0.034	0.680±0.055	0.689±0.002b
0.15 mM	0.744±0.002	0.713±0.036	0.726±0.018b	0.763±0.050	0.874±0.107	0.707±0.019	0.694±0.023b
F test	ns	ns	**	ns	ns	ns	**
C.V. %	3.01%	2.07%	7.34%	6.90%	14.89%	6.31%	9.19%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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****** = significant at the 99% level of confidence

Table 20 Protein content (mg/g fresh weight) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.659±0.043	0.740±0.053	0.722±0.015[1/]b[2/]	0.656±0.044	0.722±0.024	0.852±0.057a	0.731±0.026
0.05 mM	0.719±0.064	0.729±0.017	0.784±0.028ab	0.764±0.004	0.847±0.054	0.740±0.004b	0.788±0.081
0.10 mM	0.693±0.021	0.723±0.004	0.837±0.008a	0.687±0.054	0.884±0.068	0.705±0.019b	0.884±0.053
0.15 mM	0.689±0.026	0.697±0.025	0.644±0.021c	0.645±0.026	0.781±0.026	0.686±0.014b	0.784±0.016
F test	ns	ns	**	ns	ns	*	ns
C.V. %	3.53%	2.51%	11.11%	7.85%	8.86%	9.99%	8.02%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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7. PEPC activity in the maize leaves after $MgCl_2$ application

7.1 Vegetative stage

PEPC activities in the leaves of the feeder maize with and without $MgCl_2$ treatment were compared (Table 21). On day 3 after spraying, leaf PEPC activities of the maizes treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the non-treated plants. On the other hand on day 11, the PEPC activities in the plants treated with 0.10 and 0.15 mM $MgCl_2$ were less than those in the non-treated plants. For day 21, the PEPC activity in the plants sprayed with 0.10 mM $MgCl_2$ were significantly higher than those in the control plants.

Table 22 shows PEPC activities in the leaves of the sweet maize. Among 7 recorded dates, the leaf PEPC activities in some treated sweet maize plants were higher than those in non-treated plants on day 5, day 7, and day 9. However, they were less than those in the control plants on day 11 and day 21. For the day 5, the PEPC activities in the plants sprayed with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the control plants. On day 7, PEPC activities in all the treated plants were higher than those in the control plants. For day 9 only, the leaf PEPC activities in plants treated with 0.05 mM $MgCl_2$ were higher those that in the control plants. On the day 11, PEPC activities of the maizes treated with 0.10 and 0.15 mM $MgCl_2$ were less those that in the control plants. In addition, the leaf PEPC activities in the treated plants with 0.05 and 0.15 mM $MgCl_2$ on day 21 were less than those in the control plants.

7.2 Reproductive stage

The PEPC activity, on day 1, in the leaves of the feeder maize treated with only 0.15 mM $MgCl_2$ were less than those in the non-treated plants (Table 23). For the day 3 and the day 5, leaf PEPC activities in the maizes treatd with 0.10 and 0.15 mM $MgCl_2$ were less than those in the control plants. On the contrary, it was found that the PEPC activities in the plants treated with 0.05 and 0.10 mM $MgCl_2$ on day 11

were higher than those in the control plants. On day 21, only plants treated with 0.05 mM $MgCl_2$ had less PEPC activities in the leaves than the non-treated plant.

The leaf PEPC activities in some treated plants were significantly different from those in non-treated plants on day 1, day 3, day 5, day 9, and day 11 (Table 24). On the day 1, leaf PEPC activities of the maizes treated with 0.05 and 0.10 mM $MgCl_2$ were higher than those in the non-treated plant. PEPC activities in the leaves of the maizes treated with 0.10 and 0.15 mM $MgCl_2$ on day 3 were higher than those in the control plants. On the day 5 and day 9, the PEPC activities in the plants sprayed with 0.15 mM $MgCl_2$ were significantly higher than those in the control plants. For day 11, leaf PEPC activity in the plants treated with 0.05 mM $MgCl_2$ was higher than those in the non-treated plants.

Table 21 Phosphoenolpyruvate carboxylase activity (unit/mg protein) in the leaves of 'Nakhon Sawan 2' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.698±0.035	0.553±0.017[1/]c[2/]	1.036±0.112	1.080±0.032	0.970±0.094	1.211±0.016a	0.939±0.026bc
0.05 mM	0.795±0.031	0.731±0.033a	0.730±0.017	0.928±0.064	1.083±0.060	1.110±0.071ab	1.147±0.064ab
0.10 mM	0.763±0.073	0.684±0.020ab	0.730±0.102	0.903±0.032	1.094±0.079	0.979±0.060b	1.215±0.022a
0.15 mM	0.834±0.046	0.629±0.030bc	0.744±0.059	0.847±0.084	0.984±0.101	1.007±0.007b	0.894±0.108c
F test	ns	**	ns	ns	ns	*	*
C.V. %	7.48%	11.79%	18.62%	10.59%	6.28%	9.81%	14.87%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

* = significant at the 95% level of confidence

** = significant at the 99% level of confidence

Table 22 Phosphoenolpyruvate carboxylase activity (unit/mg protein) in the leaves of 'Hawaiian Sugar Supersweet' maize, at vegetative stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.619±0.013	0.738±0.036	0.670±0.033[1/]b[2/]	0.625±0.016b	0.744±0.021b	0.821±0.015a	0.858±0.007a
0.05 mM	0.715±0.031	0.845±0.015	0.935±0.012a	0.893±0.038a	0.895±0.029a	0.745±0.015ab	0.763±0.006b
0.10 mM	0.668±0.009	0.856±0.014	0.896±0.009a	0.883±0.013a	0.817±0.026ab	0.639±0.047c	0.901±0.002a
0.15 mM	0.687±0.003	0.857±0.025	0.611±0.019b	0.979±0.027a	0.715±0.056b	0.652±0.022bc	0.746±0.020b
F test	ns	ns	**	**	*	**	**
C.V. %	5.99%	6.96%	20.77%	18.10%	10.19%	11.98%	9.14%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

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****** = significant at the 99% level of confidence

Table 23 Phosphoenolpyruvate carboxylase activity (unit/mg protein) in the leaves of 'Nakhon Sawan 2' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.788±0.019[1/]a[2/]	0.855±0.047a	0.788±0.061a	0.758±0.008	0.724±0.003ab	0.675±0.006b	0.714±0.011a
0.05 mM	0.786±0.043a	0.785±0.029ab	0.780±0.029a	0.718±0.053	0.714±0.017ab	0.834±0.022a	0.625±0.032b
0.10 mM	0.732±0.057a	0.747±0.049b	0.694±0.042b	0.696±0.010	0.799±0.043a	0.797±0.023a	0.685±0.038a
0.15 mM	0.674±0.059b	0.717±0.036b	0.681±0.028b	0.654±0.085	0.612±0.060b	0.681±0.048b	0.719±0.013a
F test	**	*	*	ns	*	**	**
C.V. %	7.23%	7.68%	7.59%	6.18%	10.80%	10.87%	6.30%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

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****** = significant at the 99% level of confidence

Table 24 Phosphoenolpyruvate carboxylase activity (unit/mg protein) in the leaves of 'Hawaiian Sugar Supersweet' maize, at reproductive stage after spraying with different $MgCl_2$ concentrations.

	Days after spraying						
$MgCl_2$	1	3	5	7	9	11	21
0.00 mM	0.743±0.009[1/]b[2/]	0.808±0.036b	0.832±0.009b	0.768±0.047	0.706±0.020b	0.676±0.029b	0.655±0.008
0.05 mM	0.928±0.069a	0.813±0.007b	0.759±0.020b	0.744±0.003	0.755±0.036b	0.745±0.007a	0.646±0.046
0.10 mM	0.916±0.015a	0.923±0.002a	0.841±0.012b	0.783±0.046	0.831±0.054ab	0.668±0.023b	0.595±0.014
0.15 mM	0.839±0.035ab	0.982±0.022a	1.066±0.048a	0.810±0.031	0.922±0.037a	0.574±0.011c	0.584±0.018
F test	*	**	**	ns	*	**	ns
C.V. %	9.96%	9.71%	15.20%	3.55%	11.70%	10.56%	5.75%

Note: [1/] = the average of 3 replications ± S.E.

[2/] = variation of alphabets within a column show significant differences at 95% level of confidence by LSD

ns = non-significance

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DISCUSSION

Phosphoenolpyruvate carboxylase (PEPC) catalyzes the carboxylation of PEP with HCO^{3-} to produce oxaloacetate in the presence of Mg^{2+} under physiological conditions (Ting and Osmond, 1973). This reaction is the first step in the assimilation pathway of atmospheric CO_2 in C_4 plants such as maize. The importance of this step in the photosynthetic metabolism of C_4 plants is underscored by the abundance of the PEPC protein in mesophyll cells of leaves of these plants, accounting for approximate 10±15% of the total soluble protein (Uedan and Sugiyama, 1976; Hague and Sims, 1980). The maize leaves from continuously irrigated plants (1 week) showed variable trends in catalytic activity of PEPC at vegetative stage (Foyer *et al*., 1998). The fining of this study is in accordance with Foyer *et al*. (1998) since PEPC at both vegetative and reproductive stages gave variable trends in catalytic activity. Moreover, the PEPC activity in the maize leaves of both cultivars declined gradually with leaf senescence. At the reproductive stage, He *et al*. (2002) also reported that PEPC activity in the leaves of maize declined gradually with leaf senescence.

Determination of PEPC activity in maize to find a suitable date for $MgCl_2$ spraying has never been reported before. This study found that the PEPC activity in maize was not correlated with the age. Considering from the stable range of PEPC activity at vegetative stage, the suitable time for treating $MgCl_2$ was 24 days after planting. While 54 days was more appropriate for the use of $MgCl_2$ at reproductive stage to both maize varieties. The time for treating $MgCl_2$ at vegetative stage in these results disagree with what reported by Krantev *et al*. (2006). Their experiment showed that the time for treating cadmium (Cd) in maize were 15 days, which aerial part of plants was proper to perceive the stimulus. However, Steucek and Koontz (1970) treated 21 day old barley plants, when the primary and secondary leaves were mature for Mg movement in the phloem with 20 mM $MgCl_2$ solution. According to Peacock and Christensen (1998), 20 mM $MgCl_2$ can cause leaf burn, especially with monocot. Therefore, applying the low range of $MgCl_2$ (0.05 mM $MgCl_2$ – 0.15 mM $MgCl_2$) to both vegetative and reproductive stages was selected for this study. The changes of

PEPC activity as well as other factors, i.e. chlorophyll *a*, chlorophyll *b*, carotenoid, total soluble sugar, and protein were recorded to determine the effect of $MgCl_2$. Since different types of chlorophyll (Chl *a* and *b*) take part in different pathways of photosynthesis, i.e. Chl *a* acts as a reaction center while Chl *b* transfer absorbed light energy to Chl *a* (Heldt, 2005), both were separately measured and compared.

After $MgCl_2$ application at vegetative stage, Chl *a* content in the treated feeder maize was 34.6 % higher (0.475 mg/g fresh weight) than the non-treated plant on day 21 after spraying with 0.05 mM $MgCl_2$. While Chl *a* content in the treated sweet maize was 60% higher (0.603 mg/g fresh weight) on day 1 and 5 after spraying with 0.15 mM $MgCl_2$. As for Chl *b* content in the treated feeder maize, it was 53.73% higher (0.238 mg/g fresh weight) than the non-treated plant on day 21 after spraying with 0.05 mM $MgCl_2$. Whilst Chl *b* content in the treated sweet maize was 94.26% higher (0.277 mg/g fresh weight) than the non-treated plant on day 1 after spraying with 0.15 mM $MgCl_2$. It is interesting that the feeder maize sprayed with 0.05 mM $MgCl_2$ obtain high Chl *a* and *b* content on the same day. Based on the report of Steucek and Koontz (1970), foliar-applied magnesium ($MgCl_2$) was readily absorbed by and exported from young and old barley leaves. Thus, it is likely that the high contents of both chlorophyll for feeder maize on day 21 may not affected by foliar application of $MgCl_2$. On the other hand, sweet maize readily responded to 0.10 - 0.15 mM $MgCl_2$ on day 1 after spraying. The results showed that Chl *a* at the vegetative stage of the non-treated feeder and sweet maizes (~ 0.387 and 0.327 mg/g fresh weight respectively) were well below the average Chl *a* range of 0.65 – 1.46 mg/g fresh weight (Zaidi *et al*., 2003; Amujoyegbe *et al*., 2007). The same trend was also found in Chl *b* content, showing low average (~ 0.171 and ~ 0.140 mg/g fresh weight) in the feeder and the sweet maizes compared to the average of 0.17 – 0.56 mg/g fresh weight in other maize cultivars (Zaidi *et al*., 2003; Amujoyegbe *et al*., 2007). These may be due to the unfavorable growing condition in this study; such as light condition and restricted rhizosphere in the pot. Ferus and Arkosiova (2001) reported that spring barley (*Hordeum vulgare* L.) grown in the shade had lower chlorophyll content than those grown under the full sunlight.

At reproductive stage (day 56 – 76 after planting), after $MgCl_2$ application, Chl *a* content in the treated feeder maize was 40.51% higher than that in the non-treated plant on day 9 after spraying with 0.15 mM $MgCl_2$. In addition, Chl *a* content in the treated sweet maizes was 51.55% higher than the non-treated plant on day 11 after spraying with 0.10 mM $MgCl_2$. The Chl *b* content in the treated feeder maizes was 75.73% higher than those in the non-treated plants on day 21 after spraying with 0.10 mM $MgCl_2$. While, Chl *b* content in the treated sweet maizes was 62.04% and 65.74% higher than those in the non-treated plants on day 11 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, correspondingly. It could be observed that Chl *a* and *b* contents in the treated feeder maizes were clearly increased on day 9 and 21, respectively. Moreover, the contents of both Chl *a* and *b* in the treated sweet maizes were increased on day 11. As a result, it seems certain that the high contents of both chlorophyll for both maizes on day 9, 11 and 21 may not affected by foliar application of $MgCl_2$. These might be owing to the unfavorable growing condition in this study; such as restricted rhizosphere in the pot and light condition. The study of Yuh-Jyuan and Chwen-Ming (1999) showed that rice grown in the pot had higher chlorophyll content than that grown in the field. This result is consistent with Amujoyegbe *et al.* (2007) which showed that the shaded maize leaves exhibit higher chlorophyll content than the normal leaves. Moreover, each genotype of the maize may interact with the growing environment differently.

Carotenoids are a class of secondary metabolites that act as accessory photosynthetic pigments (Zaripheh *et al.*, 2002). These compounds serve many functions including light harvesting, structure stabilization, and excess energy dissipation (Frank *et al.*, 1996). Xanthophylls are the oxygenated derivatives of carotenoids. All xanthophylls produced by higher plants, such as zeaxanthin which it is the principal pigment of yellow maize kernels (Nelis and DeLeenheer, 1991; Handelman *et al.*, 1999; Eonseon *et al.*, 2003; Humphries and Khachik 2003). Xanthophylls do not require light for synthesis, so that xanthophylls are present in all young leaves as well as in etiolated leaves (Zuber and Darrah, 1987).

Carotenoids contents at the vegetative stage of the non-treated of feeder and sweet maize were much higher than that found in the wild type maize (5.735 and 4.972 mg/g fresh weight compare to 0.780 mg/g fresh weight) reported by Braun *et al.* (2006). This could reflect the fact that the two experimental cultivars are the elite cultivars for better growth and yield than the wild type (Insamapun, 2002; Grudloyma *et al.*, 2007). After treating with $MgCl_2$ solution at vegetative stage carotenoids contents in the treated feeder maize were in significant different from the control plants. It may be owing to the limit availability of other growth factors, such as photosynthetic irradiance and other essential nutrients. Under this limit condition, increasing of carotenoids content was inhibited even though $MgCl_2$ was treated with the feeder maizes. Of pertinence are the recent studies of Goncalves *et al*. (2005), it found that carotenoids content in sugarcane leaves was decreased when growing under non-suitable condition; such as lower light.

On the contrary, carotenoids contents in the treated sweet maizes were 76.39% and 97.14% higher than that in the non-treated plants on day 1 after spraying with 0.10 mM and 0.15 mM $MgCl_2$, respectively. Increasing of carotenoids content observed only in the $MgCl_2$ treated sweet maize was probably due to Mg^{2+} required for the biosynthesis of carotenoids. In addition, carotenoids contents were 55.21% and 74.43% higher than those in the non-treated plant on day 7 after spraying with 0.10 mM and 0.15 mM $MgCl_2$, correspondingly. Furthermore, it found that the contents of carotenoids were 163.55%, 220.07%, and 297.17% higher than that in the non-treated plant on day 9 after spraying with 0.05, 0.10, 0.15 mM $MgCl_2$, respectively. The effect of magnesium observed on day 1 to 9 might suggested that the treated sweet maize maintained carotenoids changes for 8 to 9 days. The continuously decreasing content of carotenoids in the non-treated sweet maize from day 3 to 9 after treatment at vegetative stage might be the effect of unfavorable growing conditions. This result is consistent with the report of Braun *et al*. (2006) which found that carotenoids content in the wild type maize at vegetative stage grown in less light condition was decreased. On the other hand, the slight increase of carotenoids content in the sweet maize recorded on day 1, 7 and 9 after sprayed with 0.15 M $MgCl_2$, indicated that the 0.15 mM $MgCl_2$ may lead to the accumulation of carotenoids. Since the increase can

occur only when irradiance is greater than that required for photosynthesis, light saturation point of the feeder maize might be higher than that of the sweet maize.

On the contrary to vegetative stage, carotenoids content in the treated feeder maize at reproductive stage was 28.89% and 62.21% higher than those in the non-treated plants on day 1 after spraying with 0.05 mM $MgCl_2$ and on day 9 after spraying with 0.15 mM $MgCl_2$, respectively. In addition, they were also 48.13% and 85.93% higher than those in the non-treated plant on day 11 and 21 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, correspondingly. In this study, it has shown that male inflorescence (tassel) of the feeder maize developed from the top of the plant on 56 days after planting (on day 1 after spraying). This is in line with the assertion of Davies (2004) that the function of carotenoids in reproductive tissue is to provide colour to flowers and fruit for attraction of pollinators. It seems that low levels of carotenoids in the leaves lead to its accumulation in tassel (Egesel *et al.*, 2003). This may be the movement of Mg^{2+} from leaves to flowers for carotenoids synthesis (Chenard *et al.*, n.d.). In addition to this, change in physiology of the feeder maize at reproductive stage may affect carotenoids content after $MgCl_2$ application. Interestingly, this phenomenon was not observed in the sweet maize.

While, carotenoids contents in the treated sweet maize at reproductive stage were 48.52% and 65.59% higher than those in the non-treated plant only on day 11 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, respectively. These findings indicated that the environment on day 11 was suitable condition for increasing carotenoids contents; such as light saturation point.

Mg^{2+} is a component of several enzymes mainly participating in carbohydrate metabolism. Furthermore, Mg^{2+} is required for photosynthetic function (Leegood, 1985). Decreasing photosynthesis thus had an immediate inhibitory effect on the sugar stream to the ovaries of maize (Westgate and Boyer, 1986; Boyle *et al.*, 1991; Schussler and Westgate, 1995). When the decreased sugar stream was coupled with decreased carbon processing inside the ovaries, kernel number of maize was markedly decreased (McPherson and Boyer, 1977; Jurgens *et al.*, 1978; Westgate and Boyer,

1985). The accumulation of sugar in maize leaves during the day was found to rapidly deplete close to the end of the photoperiod (Kalt-Torres *et al*., 1987). In grass (*Aeluropus lagopoides*), it was found that the leaves could accumulate high total soluble sugar (TSS) content (Mohsenzadeh *et al.,* 2006) at vegetative stage.

In this study, it showed low TSS content in the leaves of non-treated maize at 2.193 and 2.445 mg/g fresh weight for the feeder and the sweet maize at vegetative stage. The low TSS content in this experiment may be due to unsuitable environmental factor. For example, Mg availability in soil may be insufficient for growth of the maizes. After $MgCl_2$ application, TSS content in the treated feeder maize was 8.45% and 9.04% higher than the non-treated plant on day 1 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, accordingly. Moreover, the content of TSS was 6.79% higher than the non-treated plant on day 5 after spraying with 0.10 mM $MgCl_2$. These findings indicated that 0.05 mM $MgCl_2$ was too low to affect TSS content on day 5. This also meant that either 0.15 mM $MgCl_2$ was unsuitable to increase TSS or the growing condition of the 0.15 mM $MgCl_2$ treated plants limited the effect of Mg^{2+}. On the other hand, the sweet maize did not response to $MgCl_2$.

Whilst, TSS content at reproductive stage in the treated feeder maize was 8.12% and 26.25% higher than the non-treated plant on day 1 and 21 after spraying with 0.10 mM $MgCl_2$, respectively. As opposed to this, there was no change in TSS content in the treated sweet maize after spraying with treating $MgCl_2$. Junqueira *et al.* (2004) found that sugars in maize leaves at reproductive stage (55 to 90 days old the seedlings) were high. In this study, kernel development of both maizes was present in approximate 63-day old plants. During in kernel development, sugar reserves were mobilized from the stems and leaves to support kernel development (Westgate and Boyer, 1985; Jurgens *et al.*, 1987; Mcpherson and Boyer, 1997). The result of the present study strongly disagree with the previous studies since TSS content in sweet maize at reproductive stage was not increase during kernel development. It might be owing to inefficient metabolizing enzyme of sugar was influenced by phenology.

The activity of the oxygen free radicals and the anti-oxidants depend on both the age of the whole shoot (coleoptyl and epicotyl) and the slight difference in developmental age between the different parts of the same shoot of wheat and maize and in the roots of table beet. The basis of standardisation (protein content or total mass) can cause great differences in the free radical and antioxidant values (Kess *et al.*, 2003). From the result of Kess *et al.* (2003), the addition of magnesium increased the amount of protein in germination of wheat and maize. Since leaf protein concentrations have been shown to improve the quality of protein in maize (QPM) (Maciejewicz-Rys and Hanczakowski, 1989), $MgCl_2$ was used to increase maize protein of the experimental cultivars. Our results showed that the protein content (17 to 50 days after planting) of the non-treated maize at vegetative stage was ~ 0.704 mg/g fresh weight in feeder maize and ~ 0.753 mg/g fresh weight in sweet maize. These values were considerably high when compared to other maize cultivars from China, i.e., XLX 520 and FT 9006. They had the protein contents (10 to 55 days after planting) in the range of 0.25 – 0.55 mg protein/g fresh weight (Junqueira *et al*., 2004). These may be due to the difference genotype and stress condition. In *Aeluropus lagopoides* (L.) Trin., the total protein contents increased proportionally in the shoot as drought period was extended (Mohsenzadeh *et al.*, 2006).

After $MgCl_2$ application at vegetative stage, protein content in the treated feeder maize was 23.45% higher than the non-treated plant on day 21 after spraying with 0.15 mM $MgCl_2$. While protein content in the treated sweet maize was 48.18% higher than the non-treated plant on day 11 after spraying with 0.15 mM $MgCl_2$. At reproductive stage, protein content in the treated feeder maize was 17.93% and 14.88% higher than the non-treated plant on day 5 and 21 after spraying with 0.10 mM and 0.05 mM $MgCl_2$, respectively. Whilst protein content in the treated sweet maize was 15.93% higher than the non-treated plant on day 5 after spraying with 0.10 mM $MgCl_2$. However, it could be observed that protein content in the both treated maizes has difference responded to $MgCl_2$ application, because of the condition during plant growth. Moreover, protein content in the treated maize was higher than the non-treated plant, owing to growing condition. Increase of protein content in the treated maize on 5, 11, and 21 could be because of acclimation to condition.

Mohsenzadeh *et al.* (2006) reported, increase protein content in *Aeluropus lagopoides* could be related to reprogramming to new conditions, particularly for maintenance of photosynthesis.

After $MgCl_2$ application at vegetative stage, PEPC activity in the treated feeder maize was 32.19% and 23.69% higher than the non-treated plant on day 3 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, respectively. Moreover, PEPC activity was 29.39% higher than the non-treated feeder maize on day 21 after spraying with 0.10 mM $MgCl_2$. The results shown than PEPC activity in the sweet maize was 39.55% and 33.73% higher than the non-treated plants on day 5 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, correspondingly. It also found that PEPC activity was 42.88%, 41.28%, and 56.64% higher than the non-treated sweet maizes on day 7 after spraying with 0.05 to 0.15 mM $MgCl_2$, respectively. Furthermore, PEPC activity was also 20.30% higher than the non-treated sweet maize on day 9 with 0.05 mM $MgCl_2$. PEPC activity in both non-treated maizes at vegetative stage is, however, contrary to the report of He *et al.* (2002) which showed that trends of PEPC activity in maize declined gradually with growth (day 1 to 50 after planting). Based on the study of Bandurski (1995), Mg^{2+} is essential for the activity of PEPC. It is concluded that the increase in PEPC activity in the treated maize was a result of $MgCl_2$ effect on PEPC. In addition to this, PEPC activity in the treated maize was increased on day 3 and 21 for the feeder maizes and on day 5, 7, and 9 for the sweet maizes. The results supported that PEPC activity was influenced by the external environment such as temperature and water (Li *et al.*, 2006).

PEPC activity after $MgCl_2$ application of the treated feeder maize at reproductive stage was 23.56% and 18.07% higher than the non-treated plant only on day 11 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, respectively. The response to $MgCl_2$ application of the sweet maize was clearly increased in PEPC activity by comparison with the feeder maize. PEPC activity was 24.90% and 23.28% higher than the non-treated sweet maize on day 1 after spraying with 0.05 mM and 0.10 mM $MgCl_2$, correspondingly. Moreover, on day 3 after spraying with 0.10 mM and 0.15 mM $MgCl_2$ was 14.23% and 21.53% higher of PEPC activity than the non-treated

sweet maize. Furthermore, PEPC activity in the treated sweet maize was 28.13% and 30.59% higher than the non-treated plant on day 5 and 9 after spraying with same $MgCl_2$ concentration, 0.15 mM. In addition to this, PEPC activity in the treated sweet maize was 10.21% higher than the non-treated plant on day 11 after spraying with 0.05 mM $MgCl_2$. It is concluded again that the increase in PEPC activity in the treated maize was the effect of $MgCl_2$ on PEPC. The results showed that PEPC activity in the treated maize was increased on day 11 for feeder maize and on day 1, 3, 5, 9, and 11 for sweet maize. Base on Li *et al.* (2006) who suggested that PEPC activity may provide an ecological advantage under condition of temperature and water storage. At reproductive stage, both cultivars had similar level of PEPC activity, the average of 0.758 unit/mg protein in the non-treated feeder maize and 0.741 unit/mg protein in the non-treated sweet maize, which were lower than 1.2 – 2.5 unit/mg protein found in six *Zea may* L. hybrids as reported by Ding *et al.* (2005). The PEPC activity in both maizes was lower than six *Zea may* L. hybrids even though treating with $MgCl_2$. The lower PEPC activity in the two cultivars could be due to the genotypes and growth condition of the plants (nutrient in soil, photoperiod, and temperature).

From the current experiment, the results did not clearly show effects of $MgCl_2$ on maizes as hypothesized. This might be the unsuitable growing condition that limits the growth, thus shadowed the effect of $MgCl_2$. An experiment in the future should provide sufficient nutrient in growing medium, proper spacing and the optimum temperature and light condition to the plants. Another source of variation that affected plant performance was the method of leaf sample collection. The whole plant was removed each day to get the material for the analysis. The result should be more reliable if the leaf of the same plant is collected for the entire period of study.

CONCLUSION

An appropriate date for $MgCl_2$ foliar application was determined based on phosphoenolpyruvate carboxylase activity. For vegetative stage, day 25 after planting was selected for both Nakhon Sawan 2 and Hawaiian Sugar Supersweet maizes. For reproductive stage, day 62 and day 58 after planting were chosen for Nakhon Sawan 2 and Hawaiian Sugar Supersweet maizes, respectively. Foliar application of $MgCl_2$ to either vegetative or reproductive stage of both feeder and sweet maize grown in pots under partial shade condition led to a random increase in all six photosynthesis-related compounds analyzed. The inconclusive effects of magnesium were likely due to the unfavorable growing conditions.

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APPENDICES

Appendix A

Reagents and solutions for protein analysis

Appendix A

Reagents and solutions for protein analysis

1. Hartree-Lowry reagent A:

   2 g sodium potassium tartrate·4 H_2O (Rochelle salt; 7 mM final)

   100 g Na_2CO_3 (0.81 M final)

   500 ml 1 N NaOH (0.5 N final)

   H_2O to 1 liter

   Store 2 to 3 months at room temperature in a plastic container

2. Hartree-Lowry reagent B:

   2 g sodium potassium tartrate·4 H_2O (0.07 M final)

   1 g CuSO4·5H_2O (0.04 M final)

   90 ml H_2O

   10 ml 1 N NaOH

   Store 2 to 3 months at room temperature in a plastic container.

3. Hartree-Lowry reagent C:

   Dilute 1 vol Folin-Ciocalteau reagent (Sigma) with 15 vol water.

(Hartree-Lowry assay for quantitation of total protein by Coligan *et al.*, 2003)

Standard curve of protein

1. Prepare bovine serum albumin as standard in the same buffer or solvent used to prepare the sample to give low to high concentrations.

2. Add 1.0 ml of the protein-containing sample, of each dilution of calibration standard, or of the buffer or solvent used to prepare the sample (reference standard) to 0.90 ml of Hartree-Lowry reagent A in separate test tubes. Incubate 10 min in a 50°C water bath.

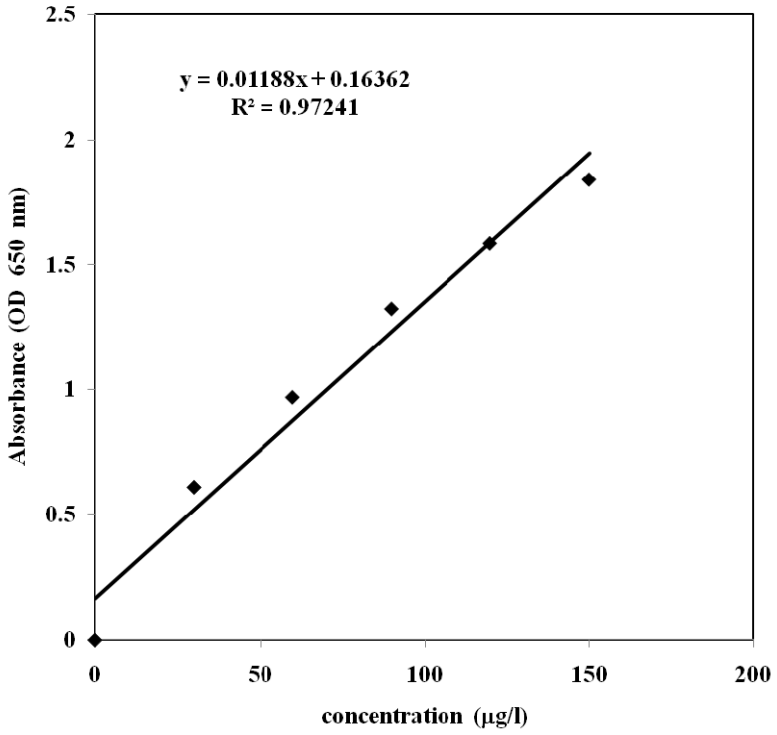
3. Cool the tubes to room temperature.

4. Add 0.1 ml of Hartree-Lowry reagent B to each tube and mix. Incubate 10 min at room temperature.

5. Rapidly add 3 ml Hartree-Lowry reagent C to each tube and mix thoroughly. Incubate10 min in a 50°C water bath, then cool to room temperature. The final assay volume is 5.0 ml.

6. Measure the net absorbance the sample, calibration standards, and reference standard at 650 nm (*A*650) in 1-cm cuvettes. If the spectrophotometer does not automatically give net absorbance readings, subtract the value for the reference solution from those obtained for the sample and calibration standards.

7. Prepare a calibration plot by graphing the net *A*650 values for the standards versus protein concentration (µg protein/5 ml f.a.v.). Determine the protein concentration of the sample by interpolation from the plot.



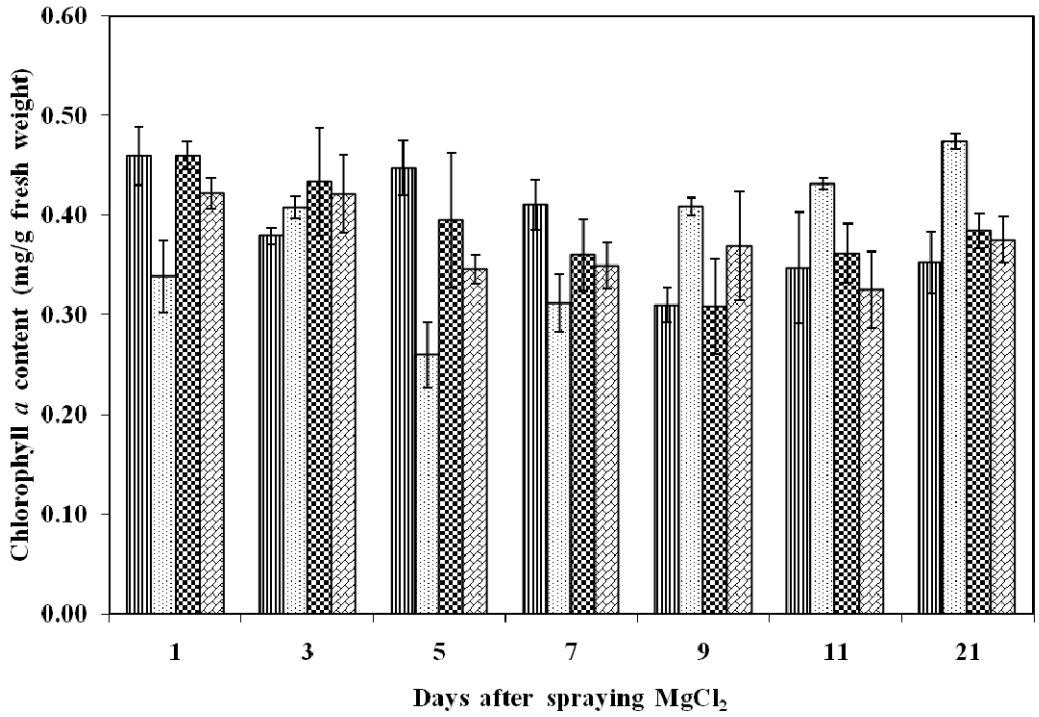
Appendix Figure A1 Standard curve of protein

Appendix B

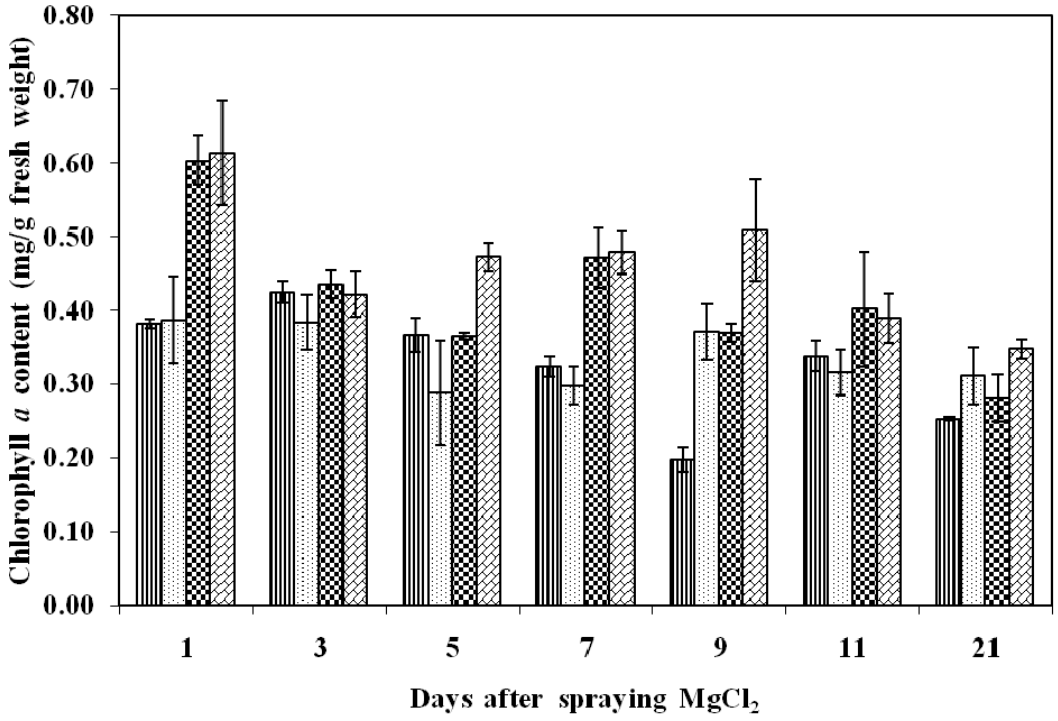
Experimental graph

Appendix B

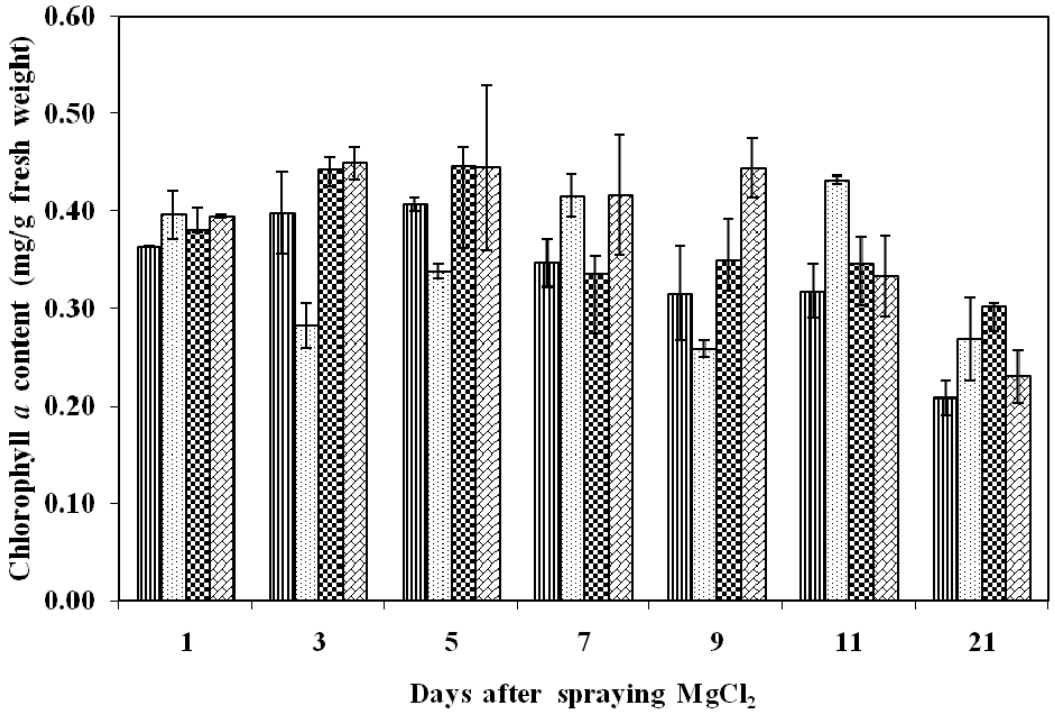
Experimental graph



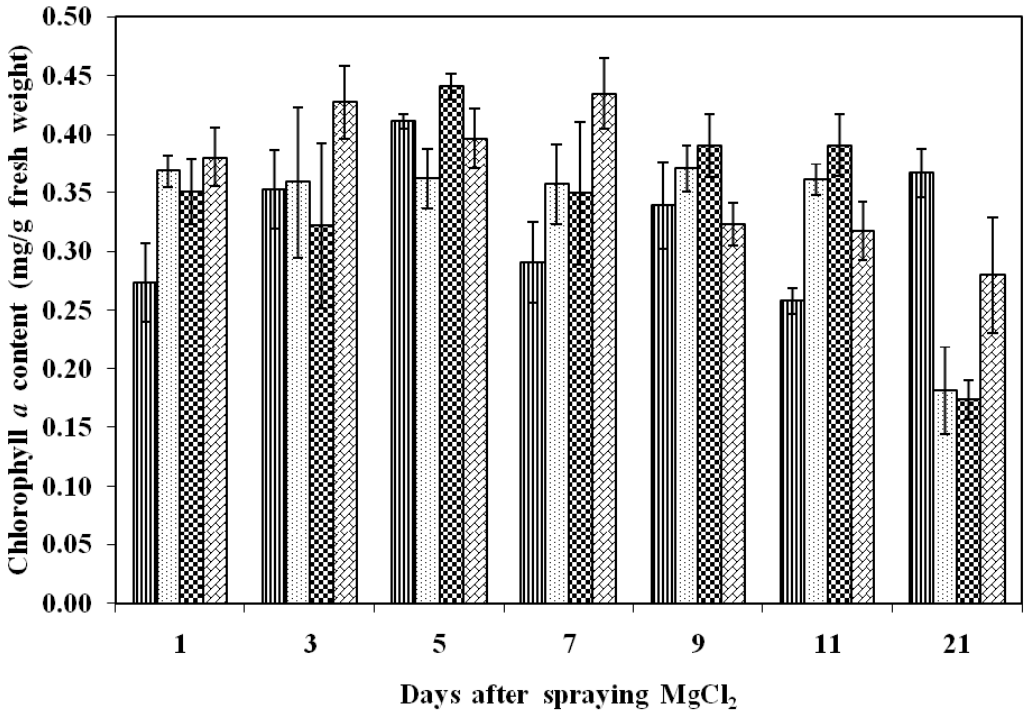
Appendix Figure B1 Changes in Chl *a* content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (⬚), and 0.15 mM (⬚). Data represent the mean ± S.E. (n = 3).



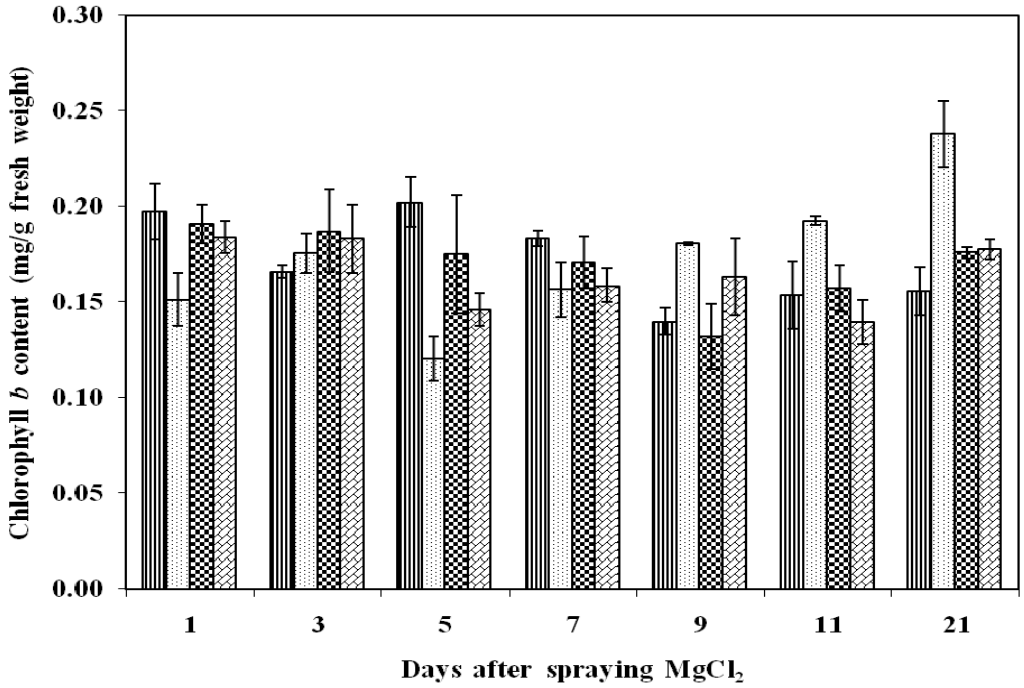
Appendix Figure B2 Changes in Chl *a* content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



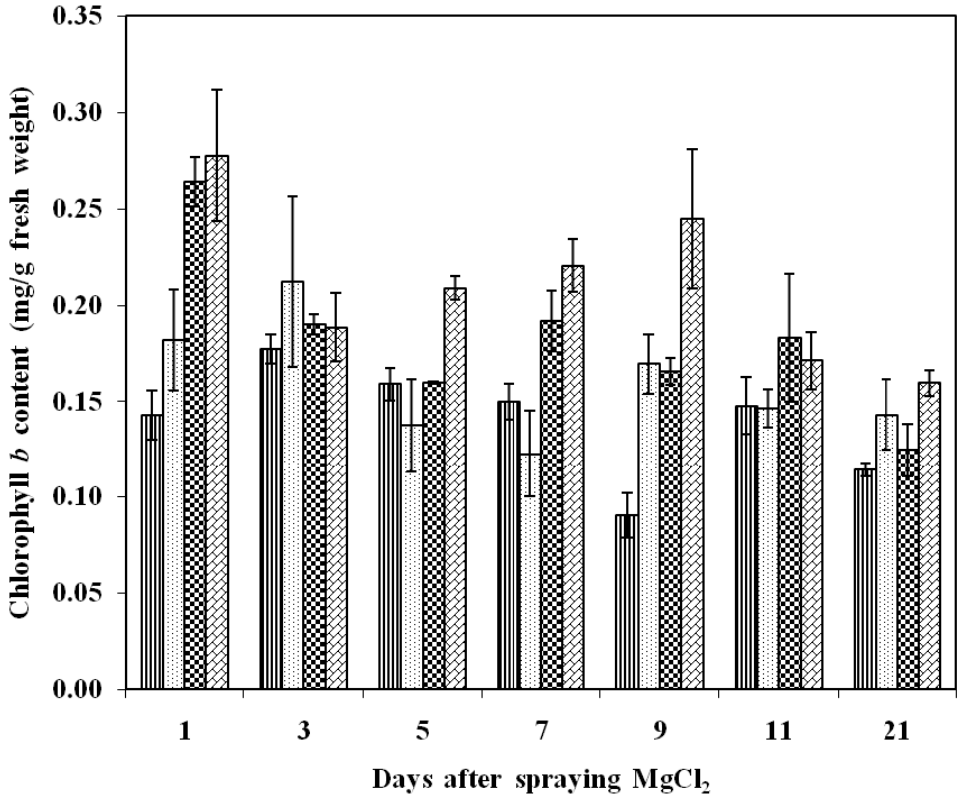
Appendix Figure B3 Changes in Chl *a* content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



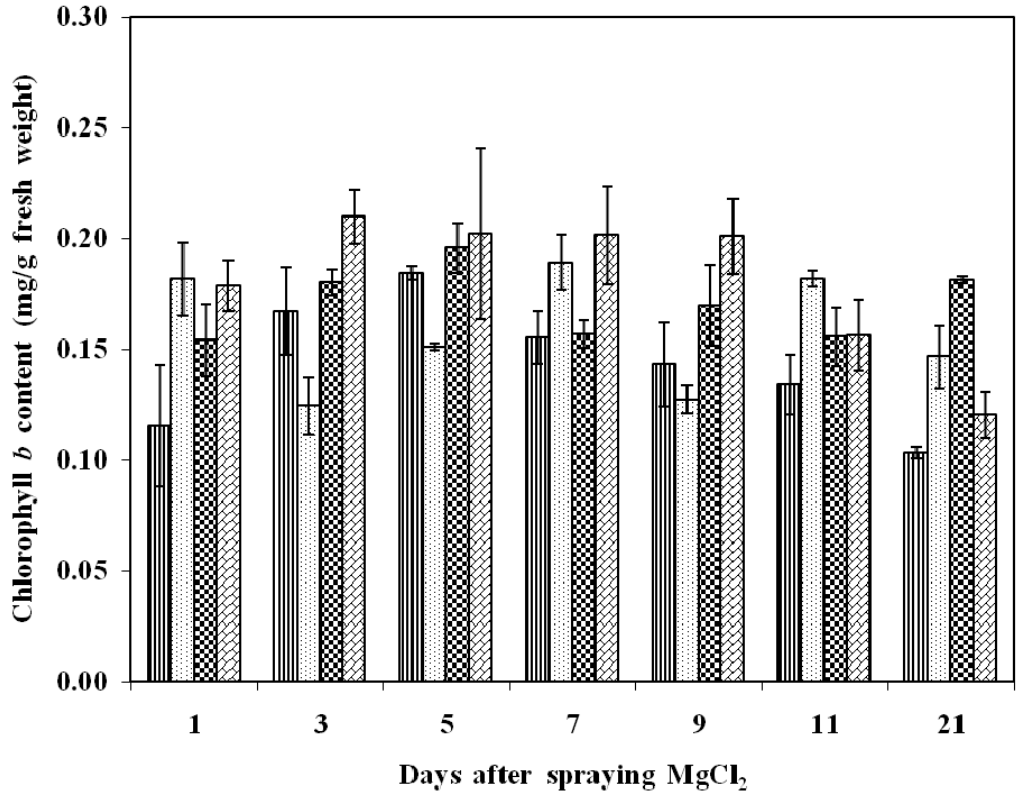
Appendix Figure B4 Changes in Chl *a* content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



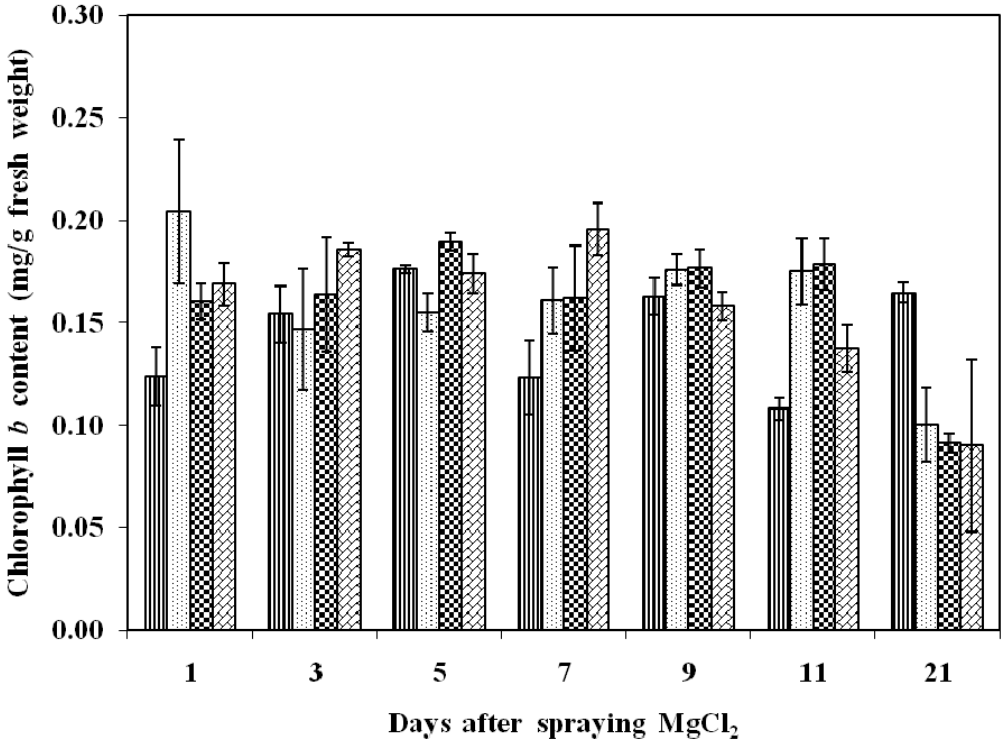
Appendix Figure B5 Changes in Chl *b* content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



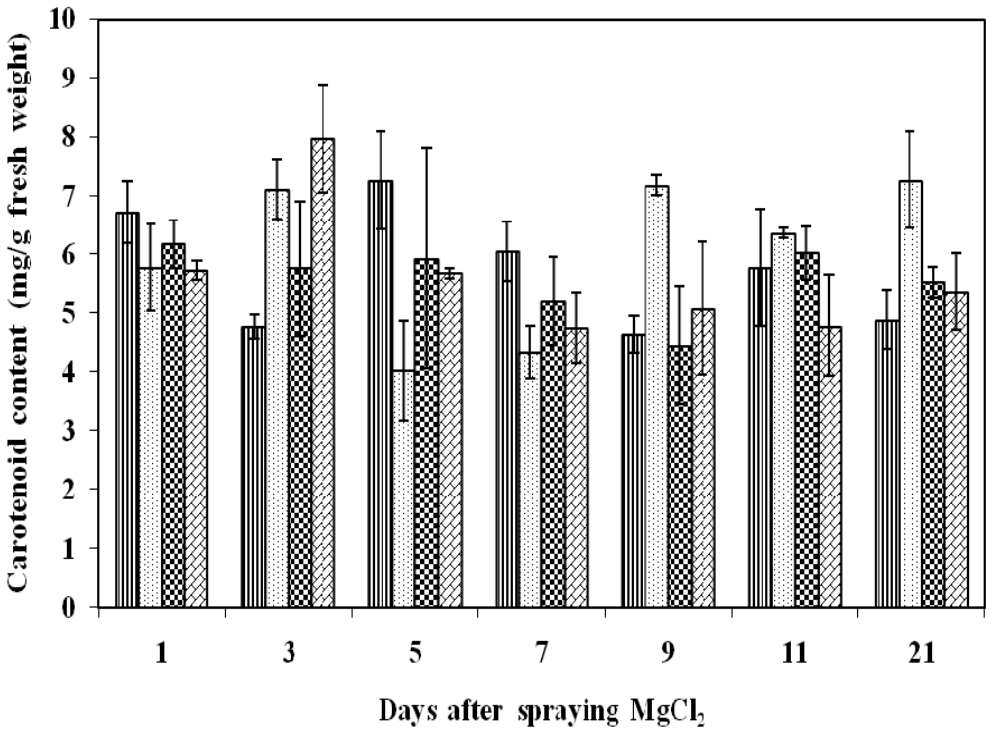
Appendix Figure B6 Changes in Chl *b* content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



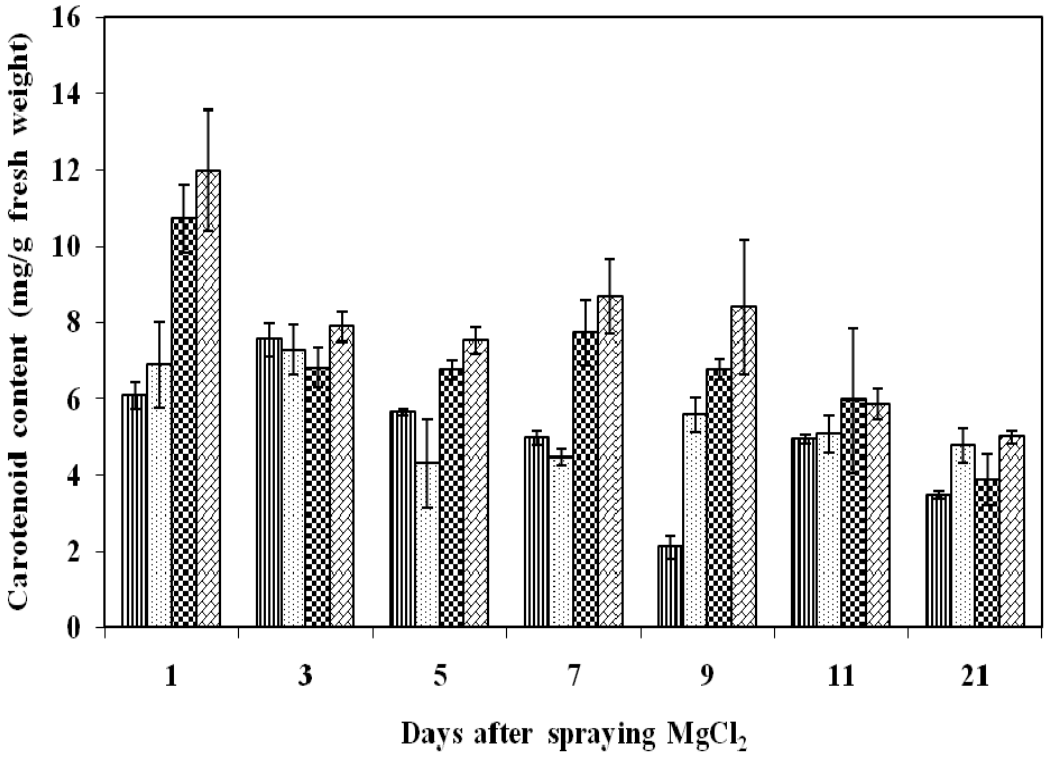
Appendix Figure B7 Changes in Chl *b* content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



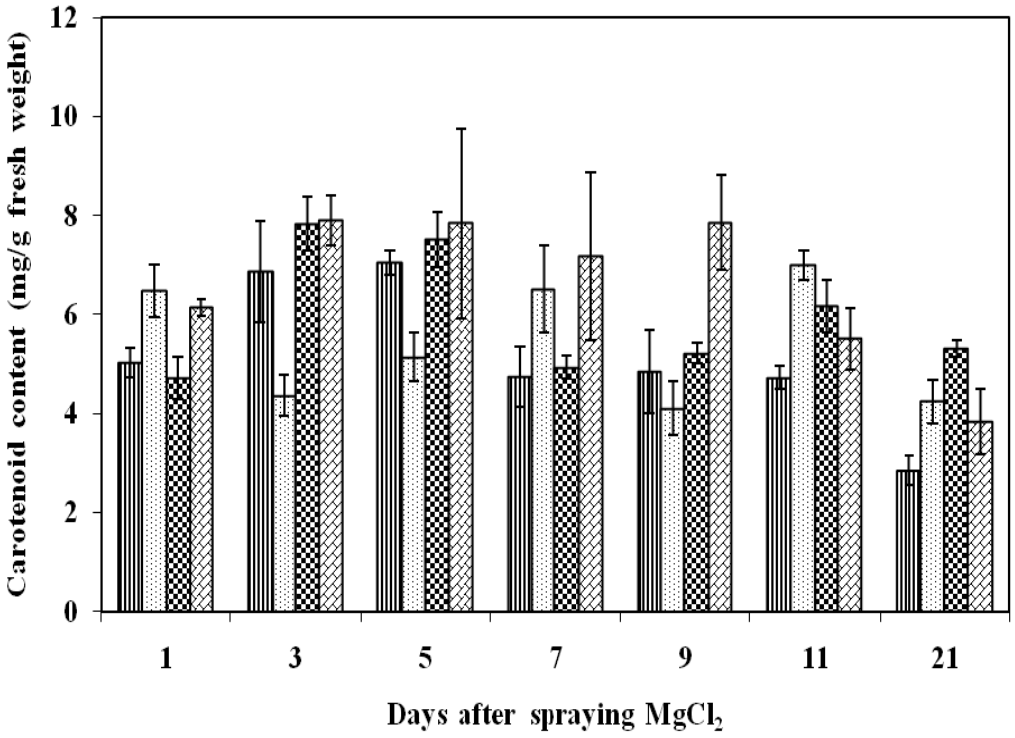
Appendix Figure B8 Changes in Chl *b* content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



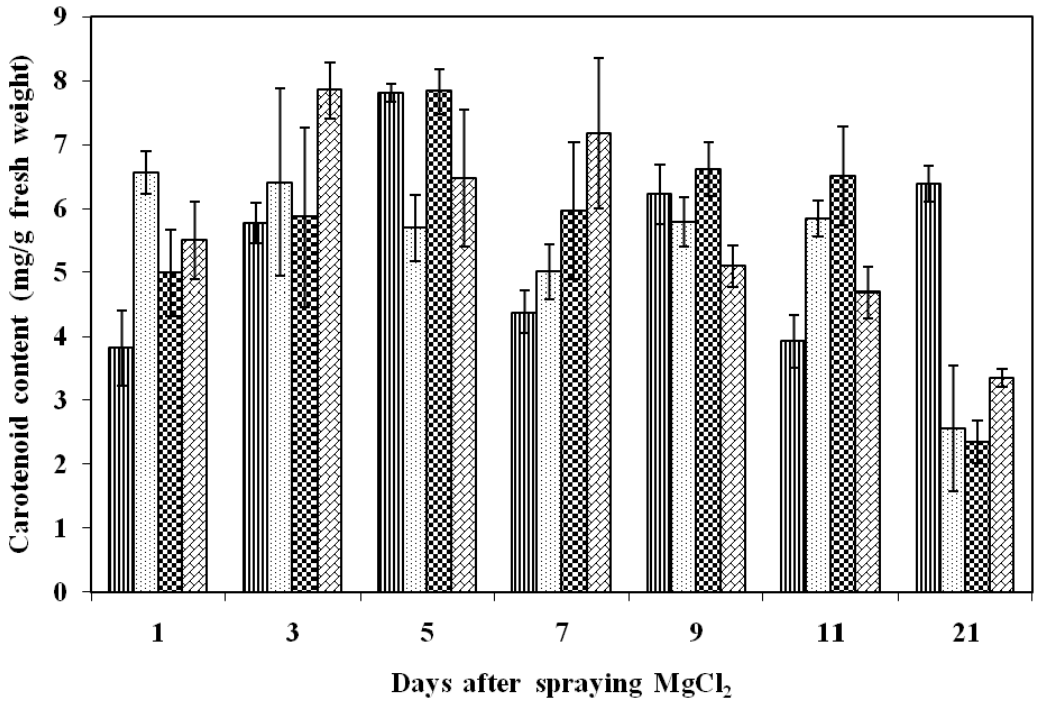
Appendix Figure B9 Changes in carotenoid content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



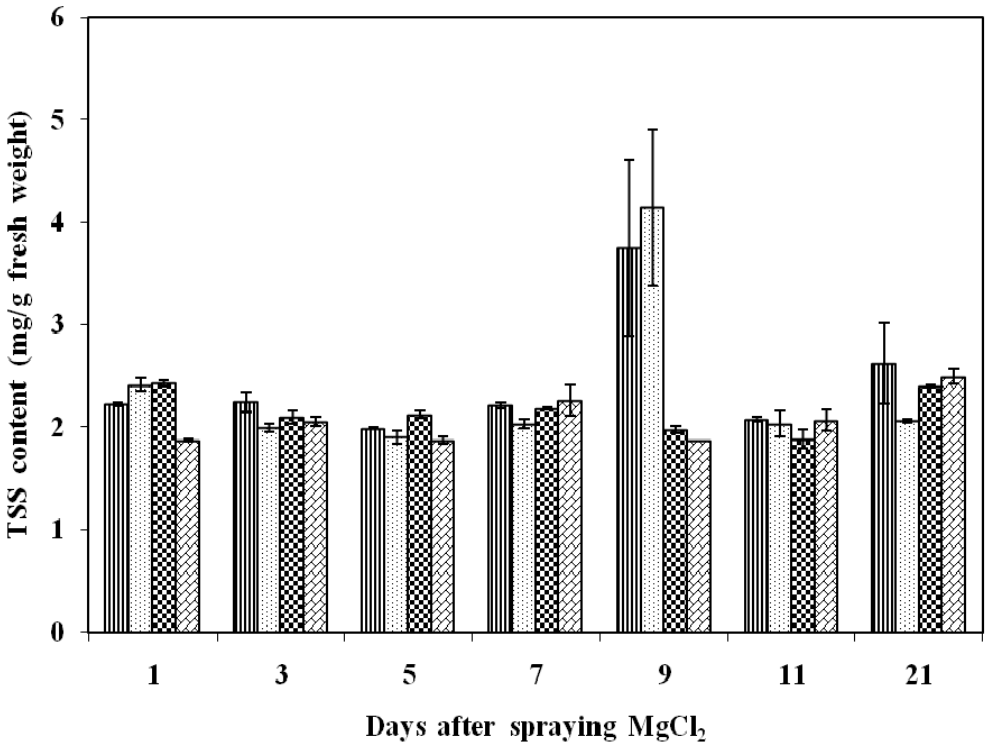
Appendix Figure B10 Changes in carotenoid content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



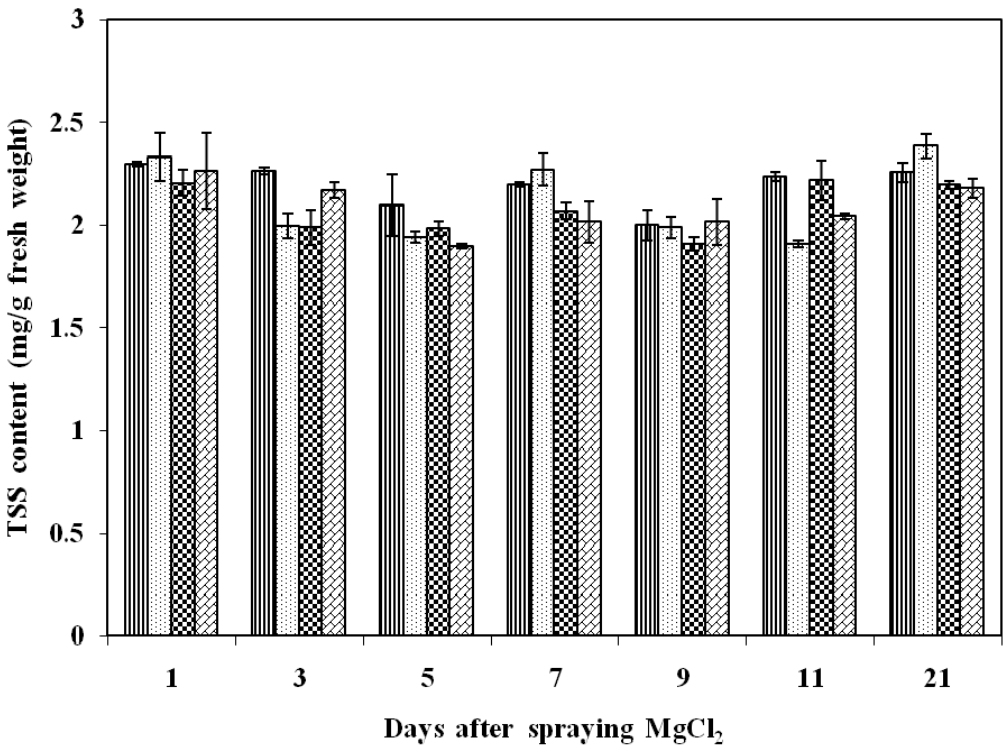
Appendix Figure B11 Changes in carotenoid content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



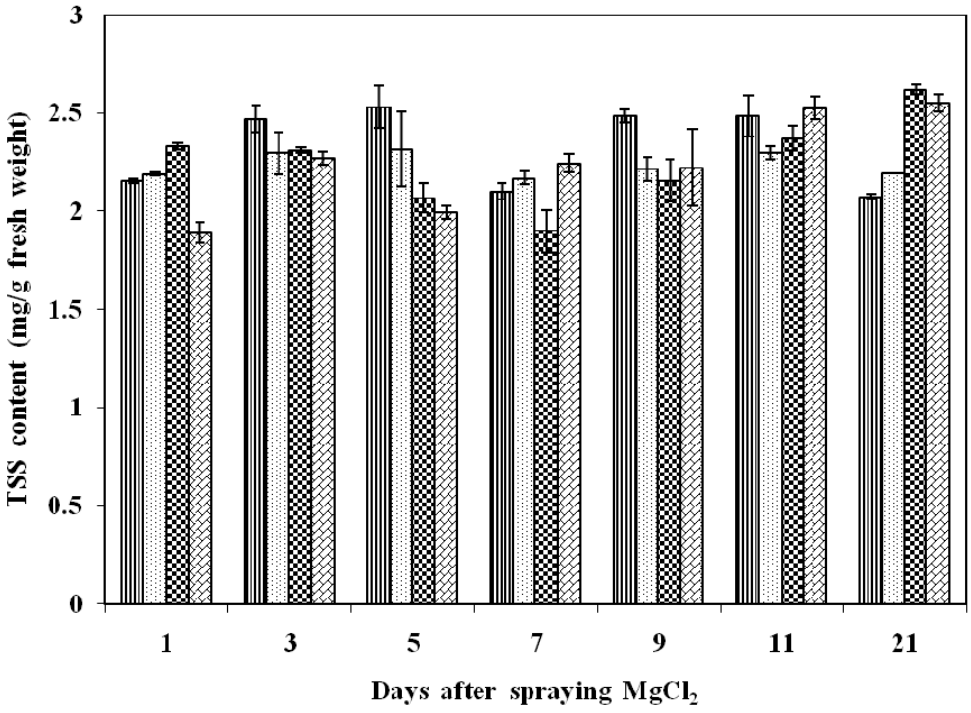
Appendix Figure B12 Changes in carotenoid content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



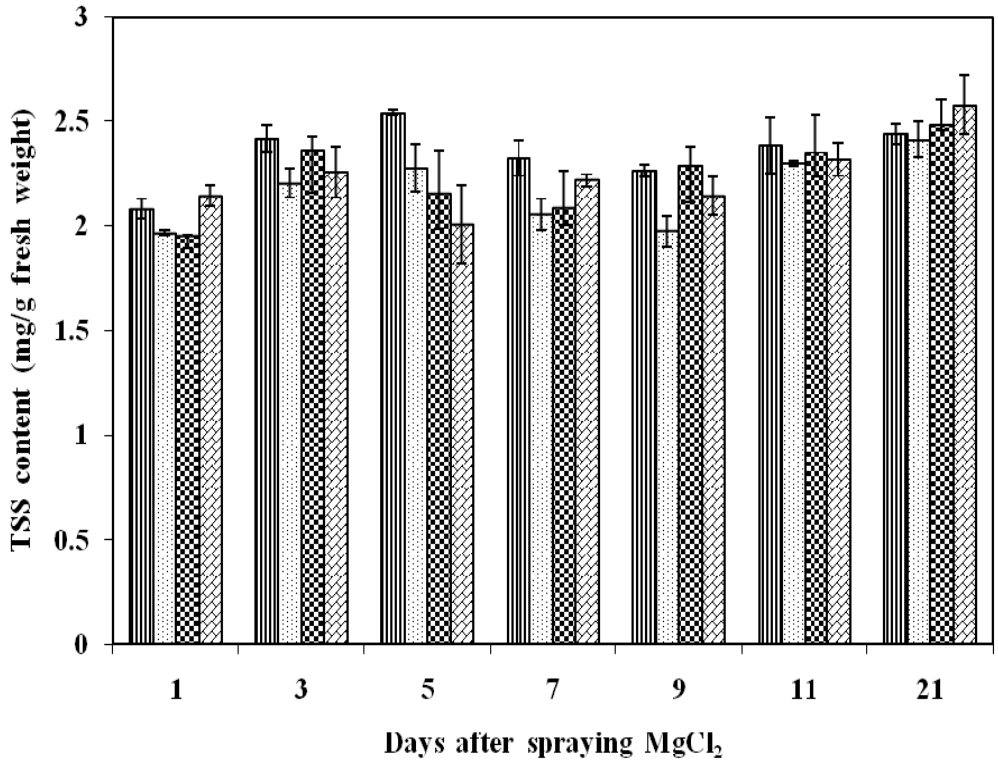
Appendix Figure B13 Changes in TSS content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



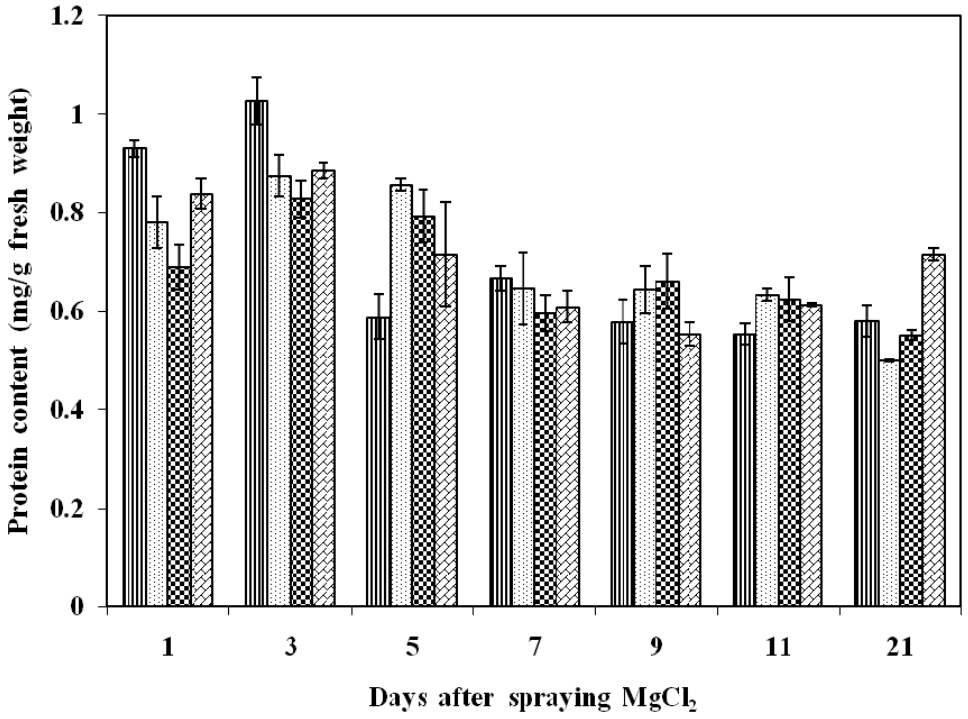
Appendix Figure B14 Changes in TSS content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



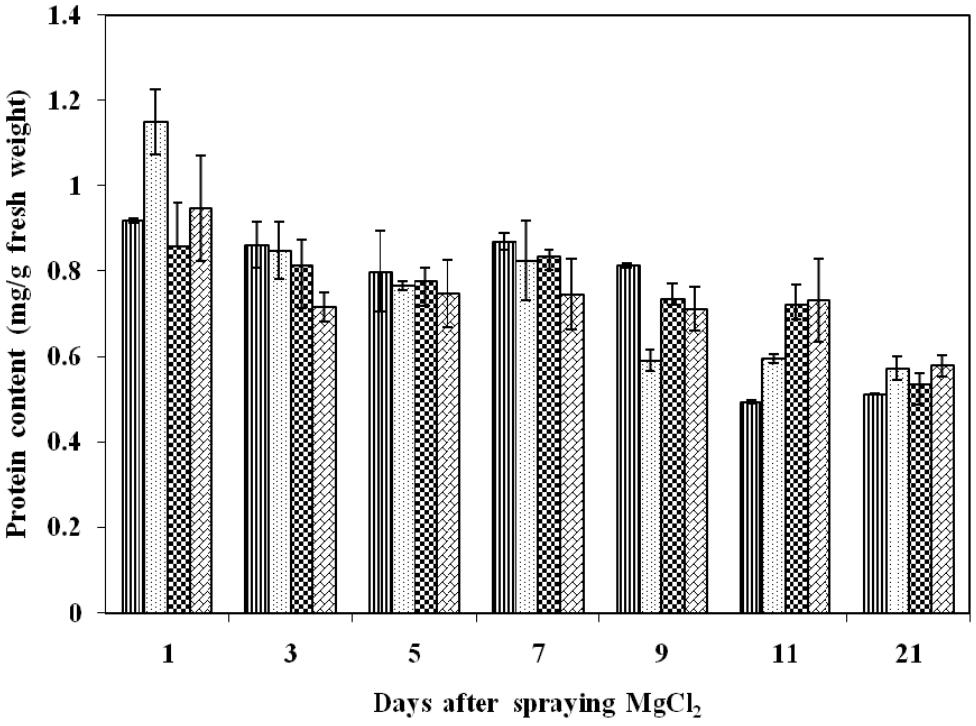
Appendix Figure B15 Changes in TSS content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



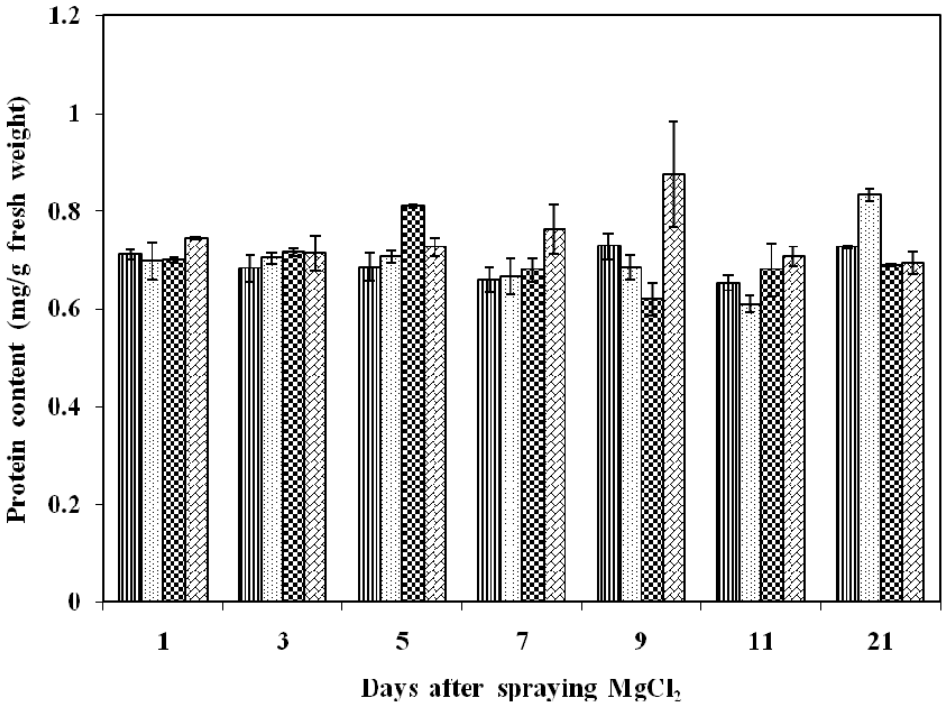
Appendix Figure B16 Changes in TSS content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations.; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



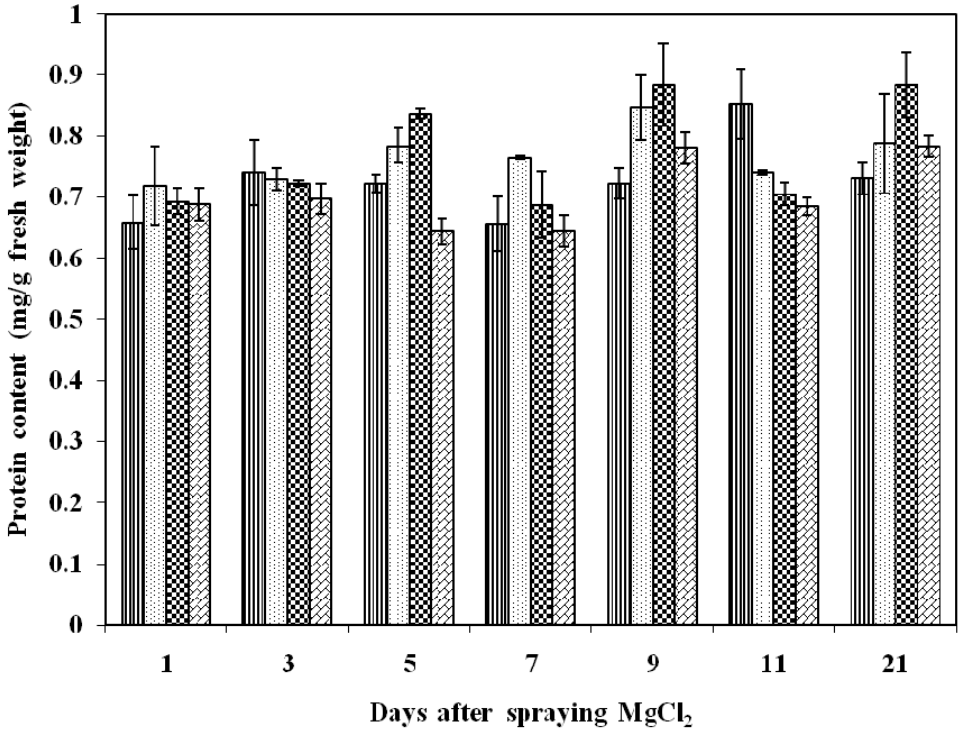
Appendix Figure B17 Changes in protein content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



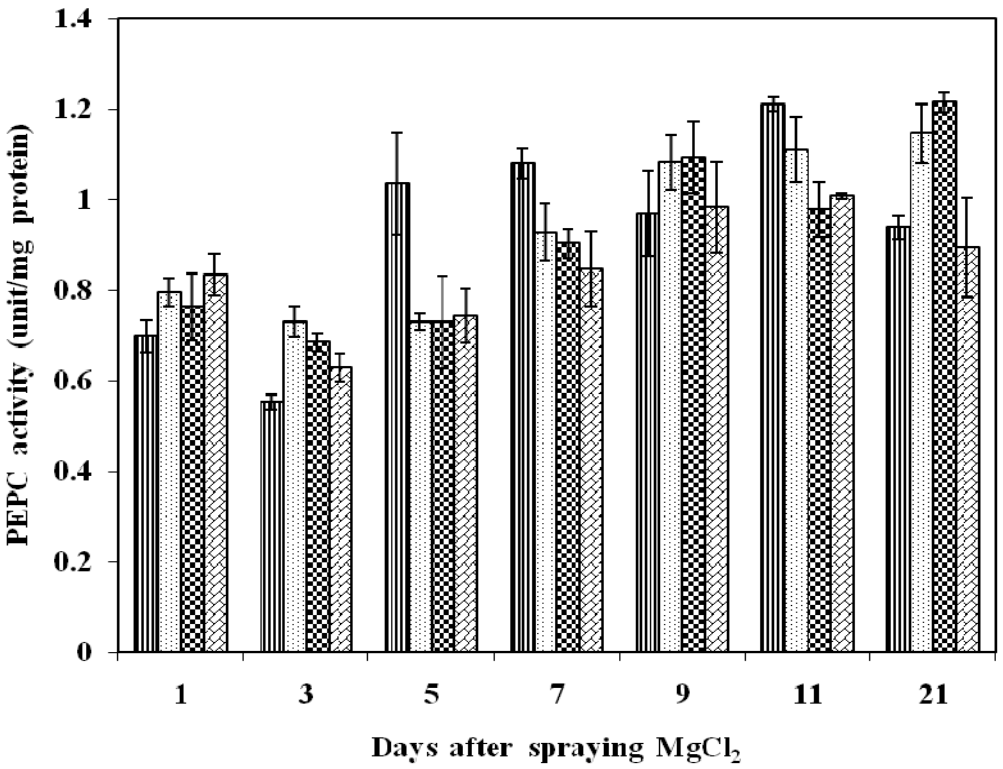
Appendix Figure B18 Changes in protein content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



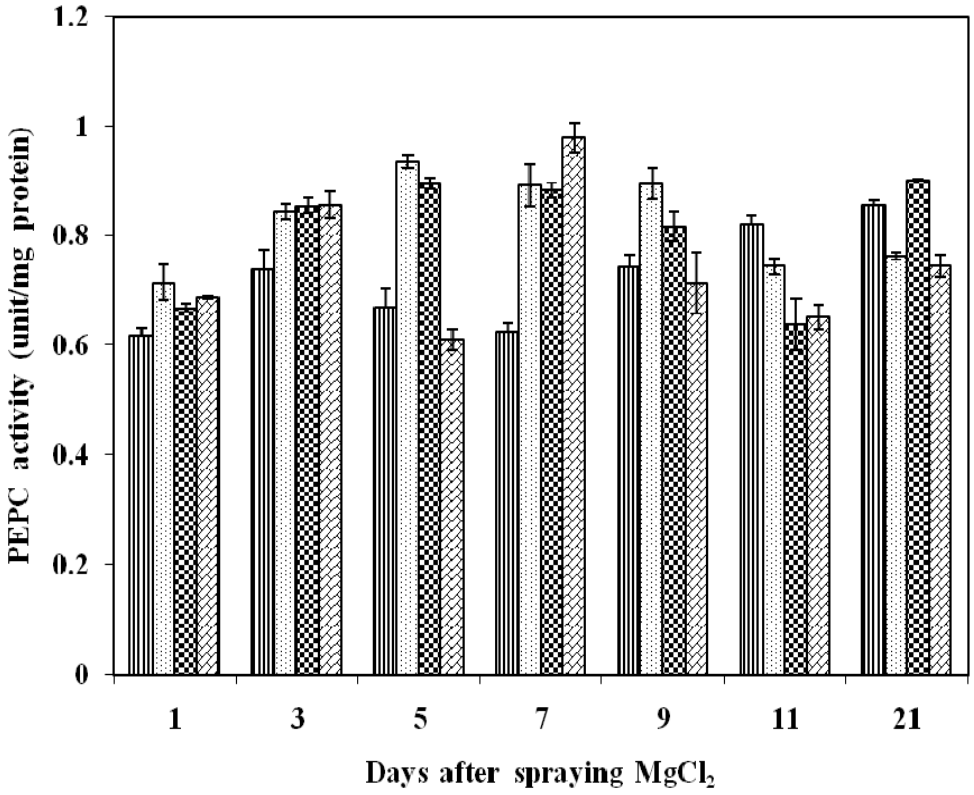
Appendix Figure B19 Changes in protein content in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



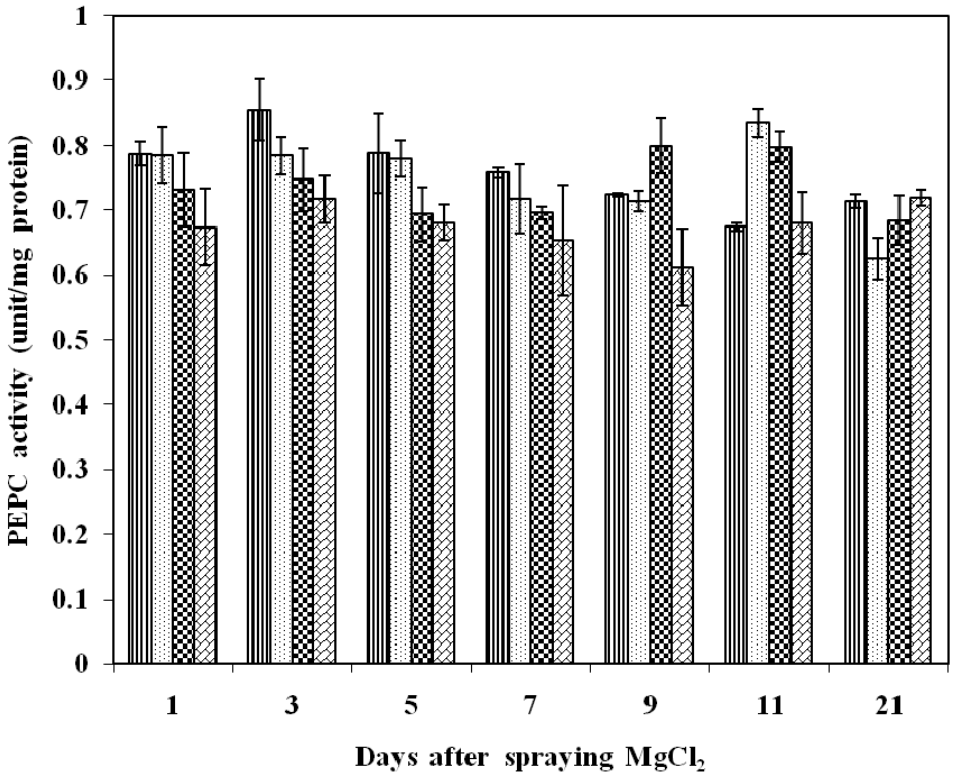
Appendix Figure B20 Changes in protein content in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



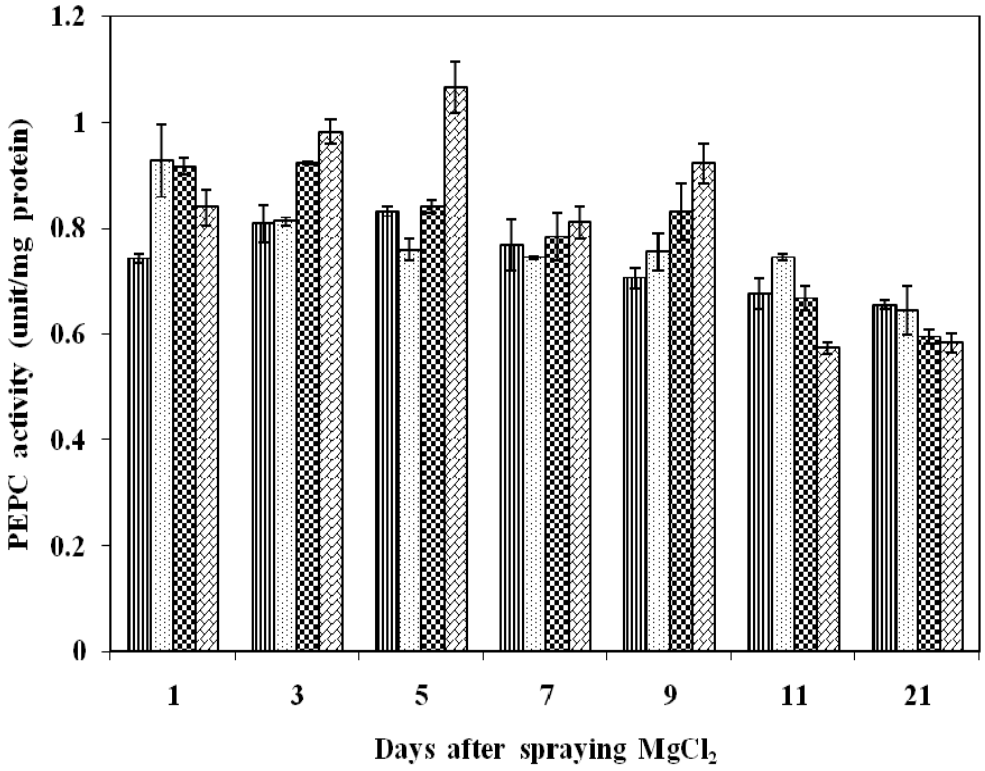
Appendix Figure B21 Changes in PEPC activity in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



Appendix Figure B22 Changes in PEPC activity in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at vegetative stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).



Appendix Figure B23 Changes in PEPC activity in the leaves of a feeder maize, Nakhon Sawan 2 (NS 2), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM ( ), 0.05 mM ( ), 0.10 mM ( ), and 0.15 mM ( ). Data represent the mean ± S.E. (n = 3).



Appendix Figure B24 Changes in PEPC activity in the leaves of a sweet maize, Hawaiian Sugar Supersweet (HSS), at reproductive stage after spraying with different $MgCl_2$ concentrations; 0.00 mM (▥), 0.05 mM (⬚), 0.10 mM (▩), and 0.15 mM (▨). Data represent the mean ± S.E. (n = 3).

Appendix C

Experimental data

Appendix C

Experimental data

Appendix Table C1 Analyses of variance for chlorophyll *a* content in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.010	4.895	0.032*
Error	8	0.002		
Total	11			
Days 3	3	0.002	0.460	0.718ns
Error	8	0.003		
Total	11			
Days 5	3	0.019	3.830	0.057 ns
Error	8	0.005		
Total	11			
Days 7	3	0.005	2.037	0.187 ns
Error	8	0.002		
Total	11			
Days 9	3	0.007	1.669	0.250 ns
Error	8	0.004		
Total	11			

Appendix Table C1 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.006	1.517	0.283 ns
Error	8	0.004		
Total	11			
Days 21	3	0.009	6.279	0.017*
Error	8	0.001		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

Appendix Table C 2 Analyses of variance for chlorophyll *a* content in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.050	6.975	0.013*
Error	8	0.007		
Total	11			
Days 3	3	0.002	0.699	0.578 ns
Error	8	0.002		
Total	11			
Days 5	3	0.017	3.878	0.056 ns
Error	8	0.004		
Total	11			
Days 7	3	0.027	10.938	0.003**
Error	8	0.002		
Total	11			
Days 9	3	0.049	9.903	0.005**
Error	8	0.005		
Total	11			

Appendix Table C2 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.005	0.790	0.533 ns
Error	8	0.006		
Total	11			
Days 21	3	0.005	2.475	0.136 ns
Error	8	0.002		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Appendix Table C3 Analyses of variance for chlorophyll *a* content in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.001	0.827	0.515^{ns}
Error	8	0.001		
Total	11			
Days 3	3	0.018	8.663	0.007**
Error	8	0.002		
Total	11			
Days 5	3	0.008	1.340	0.328^{ns}
Error	8	0.006		
Total	11			
Days 7	3	0.006	1.449	0.299^{ns}
Error	8	0.004		
Total	11			
Days 9	3	0.018	4.640	0.037*
Error	8	0.004		
Total	11			
Days 11	3	0.008	3.176	0.085^{ns}
Error	8	0.002		
Total	11			
Days 21	3	0.005	2.455	0.138^{ns}
Error	8	0.002		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Appendix Table C4 Analyses of variance for chlorophyll *a* content in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.007	3.370	0.075 ^{ns}
Error	8	0.002		
Total	11			
Days 3	3	0.006	0.709	0.573 ^{ns}
Error	8	0.008		
Total	11			
Days 5	3	0.003	2.919	0.100 ^{ns}
Error	8	0.001		
Total	11			
Days 7	3	0.010	1.997	0.193 ^{ns}
Error	8	0.005		
Total	11			
Days 9	3	0.003	1.306	0.338 ^{ns}
Error	8	0.002		
Total	11			

Appendix Table C4 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.010	8.173	0.008**
Error	8	0.001		
Total	11			
Days 21	3	0.025	7.385	0.011*
Error	8	0.003		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Appendix Table C5 Analyses of variance for chlorophyll *b* content in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.001	2.922	0.100 ^{ns}
Error	8	0.000		
Total	11			
Days 3	3	0.000	0.386	0.766 ^{ns}
Error	8	0.001		
Total	11			
Days 5	3	0.004	3.852	0.056 ^{ns}
Error	8	0.001		
Total	11			
Days 7	3	0.000	1.268	0.349 ^{ns}
Error	8	0.000		
Total	11			
Days 9	3	0.001	2.662	0.119 ^{ns}
Error	8	0.001		
Total	11			
Days 11	3	0.002	3.359	0.076 ^{ns}
Error	8	0.000		
Total	11			
Days 21	3	0.004	10.154	0.004 **
Error	8	0.000		
Total	11			

ns = non-significance

****** = significant at the 99% level of confidence

Appendix Table C6 Analyses of variance for chlorophyll *b* content in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.013	7.728	0.010*
Error	8	0.002		
Total	11			
Days 3	3	0.001	0.363	0.782 ns
Error	8	0.002		
Total	11			
Days 5	3	0.003	5.240	0.027*
Error	8	0.001		
Total	11			
Days 7	3	0.006	7.414	0.011*
Error	8	0.001		
Total	11			
Days 9	3	0.012	9.144	0.006**
Error	8	0.001		
Total	11			
Days 11	3	0.001	0.804	0.526 ns
Error	8	0.001		
Total	11			
Days 21	3	0.001	2.690	0.117 ns
Error	8	0.000		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Appendix Table C7 Analyses of variance for chlorophyll *b* content in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.003	2.676	0.118 ^{ns}
Error	8	0.001		
Total	11			
Days 3	3	0.004	6.833	0.013*
Error	8	0.001		
Total	11			
Days 5	3	0.002	1.251	0.354 ^{ns}
Error	8	0.001		
Total	11			
Days 7	3	0.002	2.632	0.122 ^{ns}
Error	8	0.001		
Total	11			
Days 9	3	0.003	4.080	0.050 ^{ns}
Error	8	0.001		
Total	11			
Days 11	3	0.001	2.437	0.140 ^{ns}
Error	8	0.000		
Total	11			
Days 21	3	0.003	14.582	0.001**
Error	8	0.000		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

****** = significant at the 99% level of confidence

Appendix Table C8 Analyses of variance for chlorophyll *b* content in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.003	2.648	0.120 ns
Error	8	0.001		
Total	11			
Days 3	3	0.001	0.613	0.625 ns
Error	8	0.001		
Total	11			
Days 5	3	0.001	3.970	0.053 ns
Error	8	0.000		
Total	11			
Days 7	3	0.003	2.494	0.134 ns
Error	8	0.001		
Total	11			
Days 9	3	0.000	1.235	0.359 ns
Error	8	0.000		
Total	11			
Days 11	3	0.003	8.030	0.009**
Error	8	0.000		
Total	11			
Days 21	3	0.004	2.393	0.144 ns
Error	8	0.002		
Total	11			

ns = non-significance

****** = significant at the 99% level of confidence

Appendix Table C9 Analyses of variance for carotenoid content in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.628	0.818	0.519 ^{ns}
Error	8	0.768		
Total	11			
Days 3	3	5.983	3.282	0.079 ^{ns}
Error	8	1.823		
Total	11			
Days 5	3	5.328	1.436	0.302 ^{ns}
Error	8	3.709		
Total	11			
Days 7	3	1.616	1.565	0.272 ^{ns}
Error	8	1.032		
Total	11			
Days 9	3	4.718	2.606	0.124 ^{ns}
Error	8	1.810		
Total	11			
Days 11	3	1.379	0.942	0.464 ^{ns}
Error	8	1464		
Total	11			
Days 21	3	3.224	3.075	0.091 ^{ns}
Error	8	1.048		
Total	11			

ns = non-significance

Appendix Table C10 Analyses of variance for carotenoid content in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	24.877	7.183	0.012*
Error	8	3.463		
Total	11			
Days 3	3	0.633	0.787	0.534 ns
Error	8	0.804		
Total	11			
Days 5	3	5.852	5.051	0.030*
Error	8	1.158		
Total	11			
Days 7	3	12.697	9.692	0.005**
Error	8	1.310		
Total	11			
Days 9	3	21.365	8.097	0.008**
Error	8	2.639		
Total	11			
Days 11	3	0.821	0.277	0.840 ns
Error	8	2.962		
Total	11			
Days 21	3	1.591	2.998	0.095 ns
Error	8	0.531		
Total	11			

ns = non-significance

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****** = significant at the 99% level of confidence

Appendix Table C11 Analyses of variance for carotenoid content in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	2.190	5.016	0.030*
Error	8	0.437		
Total	11			
Days 3	3	8.205	6.301	0.017*
Error	8	1.302		
Total	11			
Days 5	3	4.393	1.364	0.321 ns
Error	8	3.221		
Total	11			
Days 7	3	4.281	1.403	0.311 ns
Error	8	3.051		
Total	11			
Days 9	3	8.026	5.444	0.025*
Error	8	1.474		
Total	11			
Days 11	3	2.804	4.591	0.038*
Error	8	0.611		
Total	11			
Days 21	3	3.100	5.530	0.024*
Error	8	0.561		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

Appendix Table C12 Analyses of variance for carotenoid content in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	3.892	4.012	0.052 ^{ns}
Error	8	0.97		
Total	11			
Days 3	3	2.752	0.835	0.511 ^{ns}
Error	8	3.295		
Total	11			
Days 5	3	3.310	2.835	0.106 ^{ns}
Error	8	1.168		
Total	11			
Days 7	3	4.463	2.102	0.178 ^{ns}
Error	8	2.123		
Total	11			
Days 9	3	1.265	2.568	0.127 ^{ns}
Error	8	0.493		
Total	11			
Days 11	3	3.994	5.276	0.027*
Error	8	0.757		
Total	11			
Days 21	3	10.487	11.942	0.003**
Error	8	0.878		
Total	11			

ns = non-significance

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Appendix Table C13 Analyses of variance for total soluble sugar (TSS) content in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.198	48.656	0.000**
Error	8	0.004		
Total	11			
Days 3	3	0.034	2.620	0.123ns
Error	8	0.013		
Total	11			
Days 5	3	0.038	7.713	0.010*
Error	8	0.005		
Total	11			
Days 7	3	0.029	1.491	1.56ns
Error	8	0.019		
Total	11			
Days 9	3	4.171	4.202	0.046*
Error	8	0.993		
Total	11			
Days 11	3	0.025	0.920	0.474ns
Error	8	0.027		
Total	11			
Days 21	3	0.171	1.414	0.308 ns
Error	8	0.121		
Total	11			

ns = non-significance

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Appendix Table C14 Analyses of variance for TSS content in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.009	0.229	0.874 ns
Error	8	0.039		
Total	11			
Days 3	3	0.055	5.593	0.023 ns
Error	8	0.010		
Total	11			
Days 5	3	0.022	1.174	0.379 ns
Error	8	0.019		
Total	11			
Days 7	3	0.041	2.964	0.097 ns
Error	8	0.014		
Total	11			
Days 9	3	0.007	0.420	0.744 ns
Error	8	0.017		
Total	11			
Days 11	3	0.073	9.588	0.005**
Error	8	0.008		
Total	11			
Days 21	3	0.026	4.309	0.044*
Error	8	0.006		
Total	11			

ns = non-significance

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****** = significant at the 99% level of confidence

Appendix Table C15 Analyses of variance for TSS content in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.100	43.255	0.000**
Error	8	0.002		
Total	11			
Days 3	3	0.025	1.859	0.215 ns
Error	8	0.013		
Total	11			
Days 5	3	0.181	4.420	0.041*
Error	8	0.041		
Total	11			
Days 7	3	0.065	5.234	0.027*
Error	8	0.012		
Total	11			
Days 9	3	0.065	1.566	0.272ns
Error	8	0.041		
Total	11			
Days 11	3	0.033	2.234	0.162ns
Error	8	0.015		
Total	11			
Days 21	3	0.213	108.069	0.000**
Error	8	0.002		
Total	11			

ns = non-significance

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****** = significant at the 99% level of confidence

Appendix Table C16 Analyses of variance for TSS content in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.025	3.871	0.056 ^{ns}
Error	8	0.006		
Total	11			
Days 3	3	0.029	0.586	0.641 ^{ns}
Error	8	0.049		
Total	11			
Days 5	3	0.152	2.647	0.121 ^{ns}
Error	8	0.057		
Total	11			
Days 7	3	0.046	2.831	0.106 ^{ns}
Error	8	0.016		
Total	11			
Days 9	3	0.062	1.784	0.228 ^{ns}
Error	8	0.035		
Total	11			
Days 11	3	0.004	0.159	0.921 ^{ns}
Error	8	0.028		
Total	11			
Days 21	3	0.016	0.713	0.571 ^{ns}
Error	8	0.023		
Total	11			

ns = non-significance

Appendix Table C17 Analyses of variance for protein content in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.031	6.820	0.014*
Error	8	0.004		
Total	11			
Days 3	3	0.023	5.302	0.026*
Error	8	0.004		
Total	11			
Days 5	3	0.040	3.279	0.080 ns
Error	8	0.012		
Total	11			
Days 7	3	0.003	0.522	0.679 ns
Error	8	0.006		
Total	11			
Days 9	3	0.008	1.309	0.337 ns
Error	8	0.006		
Total	11			

Appendix Table C17 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.004	2.044	0.186 ns
Error	8	0.002		
Total	11			
Days 21	3	0.025	27.888	0.000**
Error	8	0.001		
Total	11			

ns = non-significance

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Appendix Table C18 Analyses of variance for protein content in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.048	2.007	0.192 ^{ns}
Error	8	0.024		
Total	11			
Days 3	3	0.013	1.471	0.294 ^{ns}
Error	8	0.009		
Total	11			
Days 5	3	0.001	0.112	0.951 ^{ns}
Error	8	0.012		
Total	11			
Days 7	3	0.008	0.667	0.596 ^{ns}
Error	8	0.012		
Total	11			
Days 9	3	0.025	7.472	0.010 *
Error	8	0.003		
Total	11			
Days 11	3	0.038	4.407	0.041 *
Error	8	0.009		
Total	11			
Days 21	3	0.003	1.981	0.195 ^{ns}
Error	8	0002		
Total	11			

ns = non-significance

***** = significant at the 95% level of confidence

Appendix Table C19 Analyses of variance for protein content in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.001	1.154	0.385 ^{ns}
Error	8	0.001		
Total	11			
Days 3	3	0.001	0.381	0.770 ^{ns}
Error	8	0.002		
Total	11			
Days 5	3	0.009	8.775	0.007**
Error	8	0.001		
Total	11			
Days 7	3	0.007	1.751	0.234 ^{ns}
Error	8	0.004		
Total	11			
Days 9	3	0.035	3.350	0.076 ^{ns}
Error	8	0.010		
Total	11			
Days 11	3	0.005	1.800	0.225 ^{ns}
Error	8	0.003		
Total	11			
Days 21	3	0.014	27.119	0.000**
Error	8	0.001		
Total	11			

ns = non-significance

****** = significant at the 99% level of confidence

Appendix Table C20 Analyses of variance for protein content in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.002	0.337	0.799 ^{ns}
Error	8	0.005		
Total	11			
Days 3	3	0.001	0.352	0.789 ^{ns}
Error	8	0.003		
Total	11			
Days 5	3	0.021	18.000	0.001**
Error	8	0.001		
Total	11			
Days 7	3	0.009	2.076	0.182 ^{ns}
Error	8	0.004		
Total	11			
Days 9	3	0.015	2.336	0.150 ^{ns}
Error	8	0.007		
Total	11			
Days 11	3	0.017	5.846	0.020*
Error	8	0.003		
Total	11			
Days 21	3	0.012	1.585	0.268 ^{ns}
Error	8	0.008		
Total	11			

ns = non-significance

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Appendix Table C21 Analyses of variance for phosphoenolpyruvate carboxylase (PEPC) activity in feeder maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.011	1.687	0.246 ^{ns}
Error	8	0.007		
Total	11			
Days 3	3	0.018	8.678	0.007 **
Error	8	0.002		
Total	11			
Days 5	3	0.068	3.386	0.075 ^{ns}
Error	8	0.020		
Total	11			
Days 7	3	0.030	2.986	0.096 ^{ns}
Error	8	0.010		
Total	11			
Days 9	3	0.013	0.583	0.643 ^{ns}
Error	8	0.022		
Total	11			

Appendix Table C21 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.033	4.958	0.031*
Error	8	0.007		
Total	11			
Days 21	3	0.073	5.697	0.022*
Error	8	0.013		
Total	11			

ns = non-significance

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Appendix Table C22 Analyses of variance for PEPC activity in sweet maize leaves after $MgCl_2$ treatment at vegetative stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.005	0.722	0.567 ns
Error	8	0.007		
Total	11			
Days 3	3	0.010	1.953	0.200 ns
Error	8	0.005		
Total	11			
Days 5	3	0.078	14.671	0.001**
Error	8	0.005		
Total	11			
Days 7	3	0.070	9.243	0.006**
Error	8	0.008		
Total	11			
Days 9	3	0.020	4.567	0.038*
Error	8	0.004		
Total	11			
Days 11	3	0.022	8.868	0.006**
Error	8	0.002		
Total	11			
Days 21	3	0.018	8.759	0.007**
Error	8	0.002		
Total	11			

ns = non-significance

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Appendix Table C23 Analyses of variance for PEPC activity in feeder maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.009	9.509	0.005**
Error	8	0.001		
Total	11			
Days 3	3	0.011	6.115	0.018*
Error	8	0.002		
Total	11			
Days 5	3	0.009	7.372	0.011*
Error	8	0.001		
Total	11			
Days 7	3	0.006	2.892	0.102 ns
Error	8	0.002		
Total	11			
Days 9	3	0.018	4.650	0.037*
Error	8	0.004		
Total	11			

Appendix Table C23 (Continued)

Source of Variation	df	MS	F-Value	Sig.
Days 11	3	0.020	8.537	0.007**
Error	8	0.002		
Total	11			
Days 21	3	0.006	15.199	0.001**
Error	8	0.000		
Total	11			

ns = non-significance

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Appendix Table C24 Analyses of variance for PEPC activity in sweet maize leaves after $MgCl_2$ treatment at reproductive stage.

Source of Variation	df	MS	F-Value	Sig.
Day1	3	0.022	4.670	0.036*
Error	8	0.005		
Total	11			
Days 3	3	0.022	16.109	0.001**
Error	8	0.001		
Total	11			
Days 5	3	0.053	24.413	0.000**
Error	8	0.002		
Total	11			
Days 7	3	0.002	0.574	0.648 ns
Error	8	0.004		
Total	11			
Days 9	3	0.027	5.886	0.020*
Error	8	0.005		
Total	11			
Days 11	3	0.015	13.111	0.002**
Error	8	0.001		
Total	11			
Days 21	3	0.004	1.913	0.206 ns
Error	8	0.002		
Total	11			

ns = non-significance

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