J. Trop. Agric. and Fd. Sc. 28(1)(2000): 23–29

Impact of water stress on reproductive development in sweet pepper (*Capsicum annuum* L.). III. Inhibition of water stress-induced ethylene production by silver thiosulphate

[Kesan sesakan air terhadap perkembangan reproduktif cili sayur (*Capsicum annuum* L.). III. Penghalangan pembebasan etilena yang teraruh oleh keadaan sesakan air dengan argentum tiosulfat]

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Key words: sweet pepper (*Capsicum annuum* L.), water stress, flower abscission, ethylene production, ethylene action inhibitor, silver thiosulphate (STS), ethylene releasing compound, 2-chloroethylphosphonic acid (CEPA)

Abstrak

Andaian bahawa etilena sebagai pengantara dalam pengguguran bunga akibat sesakan air telah disokong oleh satu percubaan bagi menyekat pengguguran bunga yang teraruh oleh etilena dengan menggunakan argentum tiosulfat (STS). Semburan STS pada daun pada kepekatan 1 mM didapati sangat berkesan untuk menyekat peningkatan pembebasan etilena endogenous dan menghalang pengguguran bunga dari pokok yang telah mengalami sesakan air dari hari keenam dan seterusnya. Selepas pokok mengalami sesakan air selama 6 hari, sebanyak 11.7% bunga sekunder telah gugur. Peratusan bunga gugur ini meningkat kepada 53.3% pada hari yang kesepuluh iaitu melebihi 8 kali ganda berbanding dengan keadaan tanpa sesakan air. Pada masa yang sama pembebasan etilena dalam bunga ini juga didapati meningkat melebihi 6 kali ganda ($p < 0.05$) daripada rawatan yang tidak mengalami sebarang sesakan air iaitu dari 0.06 kepada 0.37 ηL/g berat segar/jam. Pembebasan etilena seterusnya mencapai 0.89 ηL/g berat segar/jam pada hari kesepuluh, iaitu peningkatan sebanyak lebih kurang 3 kali ganda melebihi keadaan tanpa sesakan air. Bagaimanapun, bunga dari pokok yang mengalami sesakan air dan dirawat dengan STS tidak mengalami sebarang peningkatan pembebasan etilena endogenousnya dan tidak juga mengalami sebarang pengguguran bunga. Perbezaan dalam perkembangan bunga yang dipengaruhi oleh aplikasi STS jelas digambarkan dalam pemerhatian visual. Kesemua penemuan ini mengesahkan bahawa etilena memainkan peranan utama menggalak pengguguran bunga atau kudup cili sayur yang telah menghadapi sesakan air. Aplikasi STS pada daun sehari selepas mengenakan sesakan dan diulangi setiap 3 hari, terbukti sangat berkesan dalam menyekat pengguguran.

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Abstract
The supposition that ethylene mediates the impact of water stress on flower abscission was supported by the experiment involving the use of silver thiosulphate (STS) to block ethylene-induced abscission. Foliar application of STS at 1 mM was highly effective in blocking the increase of endogenous ethylene evolution and preventing flower abscission in severely water stressed plants from day six onwards. As much as 11.7% of the secondary flowers had abscinded 6 days after experiencing water stress and this increased to 53.3% on day 10, which accounted for more than eightfolds greater than the non-stress condition. Concurrently, there was also a sixfold increment (p <0.05) of ethylene evolution in these flowers from 0.06 to 0.37 ηL/g FW/h as compared to the non-stress condition. A further increase of the ethylene evolution to 0.89 ηL/g FW/h was observed on day 10, which was about 3 times greater than the non-stress condition. However, flowers from water stressed plants treated with STS did not experience any marked increase in their endogenous ethylene evolution which registered from 0.07 to 0.10 ηL/g FW/h neither was there any flower abscission. Comparison in the characteristics of flower development as influenced by the application of STS was clearly marked by the visual observation. These findings confirmed that ethylene has a major role in promoting flower or bud abscission in water stressed sweet pepper and that foliar application of STS one day after imposing water stress and repeated every 3 days proved highly effective in blocking abscission.

Introduction

Flower abscission induced by unfavourable treatments could not be directly related to reductions in assimilate production or dry matter partitioning to the leaves or stems (Halevy 1987; Jaafar et al. 1994, 1998), or to the changes in tissue water relations *per se* (Jaafar et al. 1994, 1998). However, ethylene is believed to be involved in mediating abscission. The involvement of endogenous and exogenous ethylene in flower abscission following the imposition of stress or the application of ethylene-generating sprays is well established (Halevy and Mayak 1981; Sexton et al. 1985; Abeles et al. 1992).

In recent studies, Jaafar et al. (1998) observed a dramatic increase in tissue ethylene production preceding flower abscission induced by the imposition of water stress. Later, Jaafar et al. (1999) confirmed that exogenous ethylene through the application of 2-chloroethylphosphonic acid (CEPA), an ethylene-releasing compound, also increased ethylene evolution from the abscinding buds and mediated the impact of water stress on flower abscission.

The action of ethylene can be competitively inhibited by various chemicals including silver nitrate (Beyer 1976) and silver thiosulphate (STS) (Veen 1983, 1986). These chemicals are thought to act by blocking binding sites for ethylene by combining with the ethylene receptor, thereby preventing the cells from responding to ethylene (Veen 1986; Abeles et al. 1992). Both chemicals are persistent and specific in their action, although the usefulness of silver nitrate has been limited by its relative immobility within plant tissues and the phytotoxicity, which is generally induced following its application at effective concentrations (Aharoni and Lieberman 1979; Veen 1983; Atta-Aly et al. 1987). In contrast, silver complexed with thiosulphate is extremely mobile within the plant, is less phytotoxic (Veen and Van De Geijn 1978) and remains active within plant tissues for extended periods (Reid et al. 1980). At higher concentrations, however, it becomes phytotoxic (Wang and Dunlap 1990). Cameron and Reid (1983) found that petal abscission in geranium seedlings (*Pelargonium hortorum* Bailey) that was exposed to drought was completely suppressed by foliar sprays with STS; whilst Mason and Miller (1991) successfully reduced the abscission of flowers treated with CEPA when they pre-treatment with STS.

Since ethylene is widely believed to be involved in mediating abscission, blocking its action with ethylene-action inhibitor may be expected to prevent or reduce this process. An experiment was conducted using STS as an inhibitor of the promotory effect of ethylene on flower abscission. The main aim of the experiment was to establish whether STS provided protection against water stress-induced flower abscission, thus to test further the role of increased endogenous ethylene production in mediating the induction of flower abscission by water stress.

Materials and methods

Propagation and seedling management

A glasshouse experiment was conducted at the University of Nottingham, UK between 10 October 1993 and 2 January 1994. Seeds of *Capsicum* hybrid variety Blue Star (Know-You Seed Company, Taiwan) were thinly sown in flat trays containing Levington F2 compost (Fisons Horticulture Ltd. Ipswich, UK). The trays were placed on a propagating bed, which provided a basal temperature of 24 ^{o}C with a glasshouse ambient temperature of 20–22 ^{o}C. When the cotyledons had fully expanded on 26 October 1993, uniform seedlings were pricked out individually into 9 cm pots containing Levington M2 potting compost and placed on benches under natural lighting conditions. When the third pair of true leaves were about 1 cm long, the mean daily temperature of the glasshouse was increased to 26 $\pm$ 3 ^{o}C with ventilation set at 29 ^{o}C. The plants received natural radiation, supplemented with 400 W high-pressure sodium lamps (SON/T) during 0500–2300 h to provide an 18-h daylength and additional irradiance of 2.5 MJ/m^2 (Photosynthetic active radiation: 400–700 ηm). Meanwhile the seedlings were watered every morning throughout the growing period to maintain optimum growth.

Treatments

Water stress treatments were imposed when secondary flowers (flowers borned at the second branching position) reached a diameter of 3.5–4.0 mm. Three treatments were imposed on secondary flowers 61 days after germination (DAG). The treatments were no water stress (NS), severe stress (SS), and severe stress plus a spray treatment with 1 mM STS (SS+STS). Method of water stress imposition followed has been explained in an earlier work by Jaafar et al. (1998). The chemical STS was applied on the first day after water stress treatment was imposed and repeated every 3 days. Since STS was effective regardless of whether it was applied to the whole plant or only to an individual developing inflorescence (Cameron and Reid 1983), about 10 mL of 1 mM STS was applied as a foliar spray every 3 days. Prior to this, a preliminary trial was carried out to predetermine the most suitable STS concentration to be used. It was found that STS spray at 5 Mn STS induced crinkling and necrotic leaves (Jaafar 1995), high probable symptoms of phytotoxicity on tissues due to strong chemical concentration. The control and severe stress treatments were sprayed with distilled water. Each treatment contained 36 plants. The experiment continued for 15 days after the treatments were imposed.

Experimental design and statistical analysis

The treated plants were arranged randomly using the table of random numbers in a Randomised Complete Block Design (RCBD), replicated 3 times. Data were analysed using the analysis of variance (ANOVA), which provided the means for all variables measured. For percentage of flower abscission in flower counts, the arc sine transformed data were used in the ANOVA. Comparison of treatment means was performed using DMRT test.

STS solution preparation

STS solution was prepared as described by Reid et al. (1980). Mixing aqueous solutions of silver nitrate ($AgNO_3$) and sodium thiosulphate ($Na_2S_2O_3.5H_2O$) should result in essentially complete formation of the STS complex, implying stability (Ghosh 1974). Stock solutions of silver nitrate (0.1 M) and sodium thiosulphate (0.1 M) (Fisons Scientific Apparatus, Loughborough, UK) were stored in clear storage bottles at room temperature (Cameron et al. 1985). STS was prepared on the day of the application by mixing known volumes of these solutions with deionised water to produce a concentration of 1.0 mM. Silver nitrate was then added slowly to sodium thiosulphate solution in the ratio 1:4 to avoid the formation of a black precipitate of silver sulphide (Ag_2S).

Flower count and ethylene measurement

Flower count was described by Jaafar (1995). Flower counts were carried out on the 6^{th} and 10^{th} day after imposition of water stress followed by application of STS to determine the treatment effects on reproductive growth, particularly on the secondary flowers. The measurements include total number of flowers produced and number abscinded. Measurement of ethylene evolution was carried out as described by Jaafar et al. (1998) to determine the influence of STS on ethylene production and flower abscission.

Results

Flower abscission

A very highly significant flower abscission (p <0.001) of 11.7% was observed 6 days after imposing the treatments, in plants that were subjected to severe stress (*Table 1*). By the 10^{th} day after treatment began, abscission had increased very markedly to 53.3% in the severely stressed plants (p <0.001), which accounted for more than eightfolds greater than that abscised under non-stress condition (6.4%). In contrast, flowers from severely stressed plants treated with 1 mM STS spray exhibited complete flower retention, suggesting that STS treatment was effective in preventing water stress-induced flower abscission.

Characteristics of flower development as influenced by the treatments are well depicted in *Plates 1a, 1b* and *1c*. Clear flower retention by STS spray in severely stressed plants is exhibited in *Plate 1c*. In contrast, a relatively high percentage of flowers in the severely stressed plants were seen to have abscinded (*Plate 1b*).

Rate of ethylene evolution from flowers

Ethylene evolution from the flowers of severely stressed plants was significantly greater than in the other treatments (*Table 2*). At a time when 11.7% of the flowers had abscinded, ethylene evolution in the severely stressed plants registered a significantly higher value of about 0.37 ηL/g FW/h (p <0.05), a rate that was about 6 times higher than in the non-stress plants. However, when severely stressed plants were treated with STS, there was no equivalent increase in ethylene evolution indicating that STS is effective in restraining the increase in ethylene evolution preceding a severe stress condition.

Table 1. Inhibitory effects of silver thiosulphate on flower abscission (n=18)

Treatment	Flower abscission (%) Days after start of treatment	
	6	10
Non-stress	0.0 b	6.4 b
Severe stress	11.7 a	53.3 a
Severe stress + STS	0.0 b	0.0 c

Mean values with different letters within each column are significantly different (p <0.001) according to DMRT

Plate 1a. A non-stressed plant at 80 days old showing normal reproductive and vegetative growth and development. Arrows show the position of normally developed secondary flowers in under non-stress condition

Plate 1b. A severely stressed plant at 80 days old showing high percentage of secondary flower abscission but with normal vegetative growth and development as in non-stress plant. Arrows show the position of the abscinded secondary flowers

By day 10, ethylene evolution had increased even further in the severely stressed plants, but this effect was again effectively blocked by STS (SS+STS: 0.10 *vs* SS: 0.89 ηL/g FW/h; p <0.001). A small increase in ethylene production between days 6 and 10 was also observed in the NS control treatment, possibly reflecting an inherent increase associated with natural ageing of the flowers. An effective blocking of ethylene production in young flowers due to foliar spray of 1 mM of STS seemed to slow down flower development as observed in *Plate 1c*.

Plate 1c. A severely stressed plant treated with silver thiosulphate at 80 days old showing effective retention of its secondary flowers from stress-induced flower abscission and with normal vegetative growth. Arrows show the position of well retained secondary flowers

Table 2. Inhibitory effects of silver thiosulphate on ethylene evolution from detached flowers (n=18)

Treatment	Ethylene evolution (ηL/g FW/h)	
	Days after start of treatment	
	6	10
Non-stress	0.06 b	0.29 b
Severe stress	0.37 a	0.89 a
Severe stress + STS	0.07 b	0.10 c

Mean values with different letters within each column are significantly different (p <0.05) according to DMRT

Discussion

The results obtained strongly suggest that ethylene may mediate the impact of water stress on flower abscission, a supposition supported by the experiments involving the use of STS to block ethylene-induced abscission. These confirmed that ethylene has a major role in promoting flower or bud abscission in water stressed sweet pepper and that foliar application of STS one day after imposing water stress proved highly effective in blocking abscission. Although ethylene evolution increased significantly in severely stressed plants, no similar promotion of ethylene evolution was observed in plants treated with STS. On the other hand application of STS seemed to delay flower development.

Many previous studies have also observed the protective influence of STS application against stress-induced flower abscission. However, most of these works concentrated on stresses other than water stress. For instance, Cameron and Reid (1981) showed that foliar application of STS produced 80–90% retention of flowers and buds in zygocactus plants stressed by exposure to ethylene or exposure to 26 °C and darkness for 4 weeks after application; these treatments would otherwise have induced complete abscission of buds, flowers and leaflets. Cameron and Reid (1983) also demonstrated a marked effect of STS in preventing the abscission of floral organs and flowers in potted flowering plants. For example, petal abscission from geranium flowers (*Pelargonium hortorum* Bailey) exposed to continuous light at 25 °C was completely suppressed by a 0.5 mM foliar spray of STS, while a similar treatment reduced flower drop from 83% to 22% in *Calceolaria herbeohybrida* Voss plants exposed to 4 days of drought in darkness at 25 °C. The protective influence of STS against stress-induced flower abscission was also observed when easter lily was sprayed with ethephon following treatment with STS and then exposed to a 92% reduction in irradiance for 14 days (Mason and Miller 1991). The effectiveness of the STS spray persisted for about 2 weeks after treatment.

Similar total protection against abscission was also achieved in plants sprayed with STS to run off and kept for 7–28 days before exposing the flowers and buds to ethylene or high temperature. Its impact in reducing, preventing or increasing abscission however depends on the concentration applied (Cameron and Reid 1981, 1983; Dostal et al. 1991). Application of 4 mM STS on zygocactus plants (*Schlumbergera truncata*) for example produced occasional phytotoxic symptoms of blistering of the leaves which eventually subsided to form dark depressions, whereas at concentrations lower than 2 mM provided only partial protection against ethylene and stress-induced abscission (Cameron and Reid 1981).

These results suggest that the STS applications have great potential as a means of reducing water stress-induced abscission in flowering plants, including sweet pepper. This occurs when the action of ethylene is competitively inhibited by STS (Veen 1983, 1986) thus preventing the cells from responding to ethylene (Veen 1986; Abeles et al. 1992) as indicated by lowering of the endogenous ethylene evolution. Since the application of exogenous ethylene in the form of 2-chloroethylphosphonic acid (CEPA) mimicked the effects of water stress by inducing increased flower abscission and increased ethylene evolution preceding abscission (Jaafar et al. 1999), the effectiveness of STS application in blocking the sites for ethylene thus preventing or reducing flower abscission can be further confirmed by application of STS against CEPA-induced flower absicssion.

Acknowledgement
This research was sponsored by the Malaysian Agricultural Research and Development Institute (MARDI).

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Accepted for publication on 17 July 2000