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Effects of Soil Moisture Stress at Different Growth Stages on Vitamin C, Capsaicin and β-Carotene Contents of Chilli (*Capsicum annuum* L.) Fruits and their Impact on Yield

S. Mahendran and D.C. Bandara[1]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *A study was conducted to investigate the effects of soil moisture stress on the vitamin C, capsaicin, and β-carotene contents of chilli fruits var. 'Arunalu'. Two moisture stress cycles, each of 15 days duration were imposed as the treatments at various stages of growth. A re-watering at every 5th day for a period of 15 days in between the stress cycles was practised. Moisture stress reduced the vitamin C content of the fruits. The reduction in vitamin C was greater on the 15th day of measurement from the commencement of each stress cycle than on the 5th day of stress. The vitamin C content did not vary much throughout the crop growth except during the late vegetative stage where the vitamin C values of the tender pods were low. Several days were required for a complete recovery of vitamin C. Increased leaf temperature on account of stress would have decreased the vitamin C synthesis in the fruits. Re-watering reduced the leaf temperature. Single and repeated stress cycles had similar effects on the vitamin C content of the fruits. Moisture stress increased the capsaicin content of the fruits. This may either be due to increased synthesis or reduced water content. Increased capsaicin did not return to normal several days after re-watering. Moisture stress showed slight but immediate increase in the β-carotene content, which is responsible for the redness of the fruits. However, a drastic increase was observed in β-carotene several days after re-watering the plants which were previously subjected to moisture stress during the pod maturing stage. The late vegetative stage was the most critical stage for moisture stress. The yield was drastically reduced during this stage with considerable reduction in vitamin C and β-carotene contents of the fruits. A slight increase in the capsaicin content was observed at this stage.*

INTRODUCTION

The quality of dry chilli is of significant importance and is based on a high degree of pungency, bright red colour, good flavour and high vitamin C content. Pungency is produced by capsaicin and other vanillyl amides and the red colour of the ripe fruits is due to carotenoid pigments of which β-carotene is the most important (Purseglove *et al.*, 1981).

The fruits of most capsicum species contain significant quantities of vitamin C and this can be as high as 150–180 mg 100 g $^{-1}$. Mozafar (1994) reported that colourful vegetables contain high amount of ascorbic acid. Ishikawa *et al.* (1997) have reported that deep green cultivars of chilli contain high amounts of ascorbic acid. As in the case with

1 Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

certain other constituents of the fruit, the vitamin C content increases gradually with maturity, reaches a maximum at the fully red-ripe stage and decreases thereafter (Kitagawa, 1973). Chilli pods with a bright red colour command higher prices. Deep red fruits tend to retain their colour longer than those which are of a light shade. Although the cultivar has a dominant influence on the quality determinant properties, the environment in which it grows also has a significant impact on quality characters (Purseglove *et al.*, 1981).

When chilli plants are subjected to moisture stress during the podding and pod maturing stages, the effect of stress not only reflects on the morphological and physiological characteristics of chilli, but also on the quality. Rahman *et al.* (1978) reported that ascorbic acid content of chilli fruits decline with increasing moisture stress. Oxidative deterioration of vitamin C in tomato fruits increases proportionately with an increase in moisture stress (Davies *et al.*, 1991). Whether prolonged moisture stress reduces the vitamin C content of chilli fruits and the extent to what stress affects vitamin C content needs to be investigated.

In addition, how and to what extent moisture stress affects the pungency as indicated by capsaicin content and red colour as indicated by β-carotene content of chilli fruits are under question. Quagliotti (1971) observed that pungency was influenced when plants were stressed for moisture. High temperature and moisture stress at the time of maturity increased the pungency in chilli fruits (Lindsey and Bosland, 1995). The most important controllable factors affecting the retention of β-carotene of Romanian paprika varieties are moisture and temperature; deterioration occurring more rapidly at high moisture stress and high temperature (Purseglove *et al.*, 1981).

The present study was conducted with the objective of understanding the effect of rapid (water withheld completely at once) moisture stress at various growth stages on the vitamin C, capsaicin and β-carotene contents of chilli fruits.

MATERIALS AND METHODS

Uniform seedlings of chilli var. 'Arunalu' was raised in the nursery and were transplanted in a field (Reddish Brown Earth soil) at the Field Crops Research and Development Institute, Maha Illuppallama which is situated at 138 m elevation a.m.s.l. with a latitude of 8°5' North located in the North Central Province of Sri Lanka. The climate is warm (33–35°C) with an average annual rainfall of approximately 1500 mm.

The experiment was arranged in a Randomized Complete Block Design with 6 treatments and 4 replications (Fig. 1). Treatment No. 1 (T_1) where no moisture stress was experienced by plants served as the control. Different stress treatments had moisture stress of 15 day period in 2 different cycles with a re-watering of 15 days in between the stress at different stages of growth. Moisture stress treatment was applied by withholding water completely.

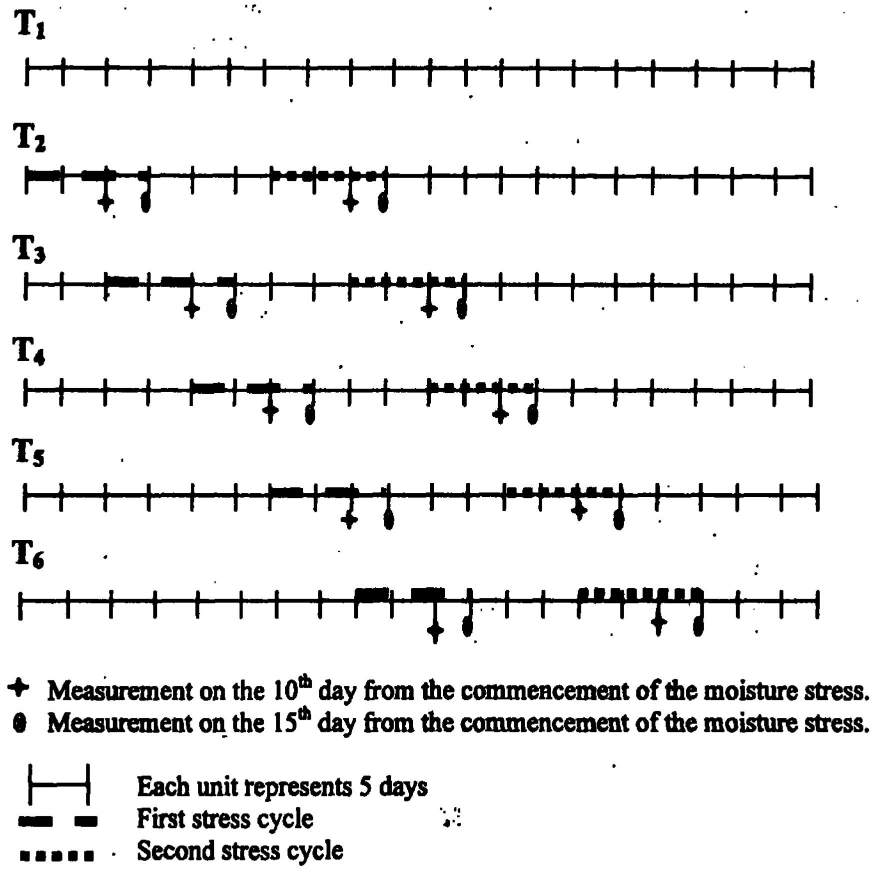

Fig. 1. Diagrammatic representation of the manner in which stress treatments were imposed at various stages of growth.
[Note: T_1 = Control-Regular watering at 5 days interval at field capacity, T_2 = 15 days stress at late vegetative and podding stages, T_3 = 15 days stress at flowering and pod maturing stages, T_4 = 15 days stress at pod setting and pod maturing stages, T_5 = 15 days stress at podding and fruit ripening stages, T_6 = 15 days stress at pod maturing and fruit ripening stages].

Determination of vitamin C content

The green chilli pods were randomly harvested from treatments subjected to moisture stress of 15 days during the late vegetative, flowering, pod setting, podding, pod maturing and fruit ripening stages of the crop. The pods were harvested from the above stages of the crop on the 15th day from the commencement of their respective stress cycles.

In chilli, unlike other field crops the vegetative phase overlaps the reproductive phase. After certain period of vegetative growth, few plants started bearing flowers while the majority of them were still in the vegetative phase. Some of these flowers produced tender pods before the rest of the crop reached the flowering stage. When 50% of the plants reach flowering the crop is assumed to have attained the flowering stage. The tender pods produced from the plants were harvested for vitamin C determination during the vegetative stage of the crop. A few plants at the flowering stage had certain amount of pods and the vitamin C content of these pods was determined while the rest of the crop was still in the flowering stage. This is how the pods were collected during the vegetative and flowering stages of the crop for the determination of vitamin C. The vitamin C content was estimated using standard methods of analysis (AOAC, 1993).

Estimation of capsaicin and β-carotene

Matured red chilli fruits were randomly collected from the plants when they experienced the 2nd stress cycle during the fruit ripening stage (T_6). The fruits were collected on the 15th day from the commencement of the stress and were analysed for capsaicin (Hoffman *et al.*, 1983) and β-carotene (AOAC, 1993) using HPLC technique.

Fruit Yield

Ripened fruits were harvested in 6 pickings from the plants that were subjected to moisture stress treatments at various growth stages. The fruits were oven dried at 105°C for 48 h and their dry weight was recorded.

RESULTS AND DISCUSSION

Yield

Moisture stress reduced the yield of chilli (Fig. 2). The reduction was highest (approximately 50% compared to the control) when the stress was imposed during the late vegetative stage. The crop which underwent moisture stress during the flowering, pod setting, podding and pod maturing stages produced significantly lower yield than the control. There was no significant difference in the yield of chilli which was subjected to stress during the podding and pod maturing stages.

There were significant differences between treatments in the vitamin C contents of the matured green pods at various stages of growth (Table 1). In the treatments where the plants experienced the 1st stress cycle during the late vegetative (T_2), flowering (T_3), pod setting (T_4), podding (T_5) and pod maturing (T_6) stages, the vitamin C content of the pods on the 15th day of measurement from the commencement of the stress was significantly lower than their respective control values. The vitamin C content on the 5th day of measurement from the commencement of the stress during the flowering, pod setting, podding and pod maturing stages were also significantly lower than their respective control values.

There was no complete recovery in the vitamin C content of the pods on the 10th day of re-watering the plants which previously underwent the 1st stress cycle during the late vegetative, flowering, pod setting and podding stages of the crop. In the treatment where the plants experienced the 1st stress cycle during the podding stage the vitamin C content

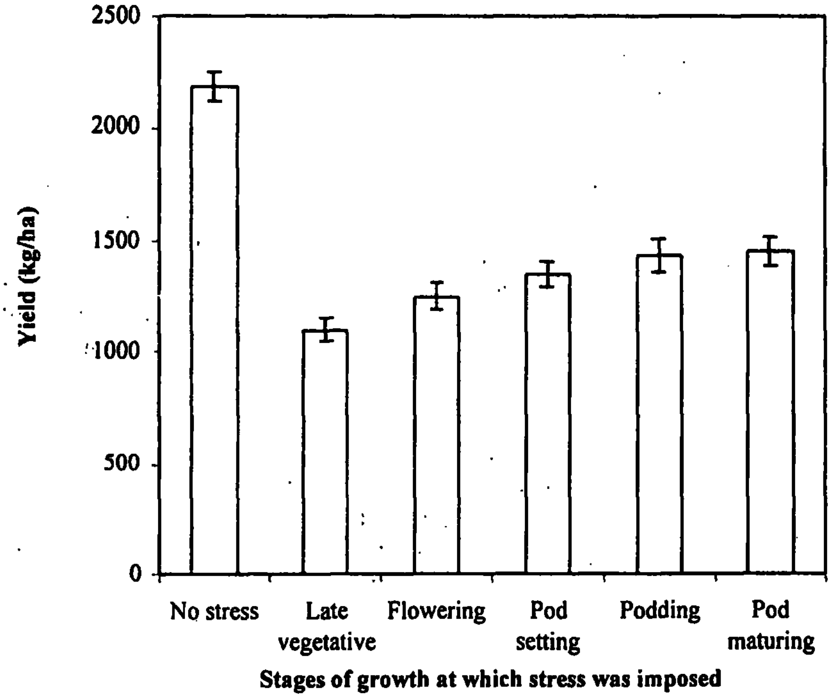

Fig. 2. **Effect of soil moisture stress at various stages of growth on the yield of chilli var. 'Arunalu'.**

Table 1. **Effect of soil moisture stress at various stages of growth on the vitamin C content of chilli pods.**

Treatments	Vitamin C content (mg 100 g^{-1})							
	Late vegetative	Flowering	Pod setting	Podding	Pod maturing 1	Pod maturing 2	Fruit ripening 1	Fruit ripening 2
T_1	59.3 a	117.2 a	115.9 a	116.9 a	117.4 a	116.8 a	117.3 a	117.6 a
T_2	41.1 c	100.7 c	104.9 b	88.6 d	100.9 c	115.3 a	116.0 a	116.5 a
T_3	52.0 b	89.3 d	100.3 c	102.7 b	90.3 d	100.9 c	116.4 a	115.8 a
T_4	60.0 a	105.8 b	91.5 d	98.9 c	105.9 b	89.9 d	101.8 c	115.5 a
T_5	59.7 a	117.5 a	106.9 b	90.7 d	101.2 c	105.6 b	89.6 d	102.2 b
T_6	60.1 a	116.8 a	116.1 a	104.8 b	91.8 d	102.5 c	105.6 b	88.1 c

Values in the same column followed by the same letter do not differ significantly ($P<0.05$).
Values are the means of 30 pods in 3 replicates.

on the 15th day of measurement from the commencement of the stress was 90.7 mg 100 g^{-1}. The plants which experienced the 1st stress cycle during the late vegetative stage received the 2nd stress cycle after a period of 15 days re-watering. The vitamin C content of the pods on the 15th day of measurement from the commencement of the 2nd stress cycle was 88.6 mg 100 g^{-1}. There was no significant difference in the vitamin C content between the above 2 treatments.

The plants which experienced the 1st stress cycle during the podding stage were re-watered after the stress treatment. The vitamin C content of the pods when measured on the 10th day of re-watering was 101.2 mg 100 g^{-1}. The plants which experienced the 1st stress cycle during the late vegetative stage were subjected to the 2nd stress cycle after a period of 15 days re-watering. The vitamin C content on the 10th day of re-watering after the 2nd stress cycle was 100.9 mg 100 g^{-1}. There was no significant difference in the vitamin C content of the pods between the above 2 treatments.

In the treatment where the plants of the pod maturing stage received the 1st stress cycle, the vitamin C content of the pods on the 15th day of measurement from the commencement of the stress was 91.8 mg 100 g^{-1}. The plants which experienced the 1st stress cycle during the flowering stage received the 2nd stress cycle after a period of 15 days re-watering. The vitamin C content on the 15th day of measurement from the commencement of the 2nd stress cycle was 90.3 mg 100 g^{-1}. There was no significant difference in the vitamin C content of the pods between the above 2 treatments.

The plants which were exposed to the 1st stress cycle during the pod maturing stage were re-watered after the stress treatment. The vitamin C content of the pods on the 10th day of re-watering was 102.5 mg 100 g^{-1}. The plants which received the 1st stress cycle during the flowering stage were subjected to the 2nd stress cycle after a period of 15 days re-watering. The vitamin C content on the 10th day of re-watering after the 2nd stress cycle was 100.9 mg 100 g^{-1}. There was no significant difference in the vitamin C content of the pods between the above 2 treatments. A complete recovery in the vitamin C content was observed on the 20th, 30th and 40th day after re-watering the plants which previously experienced the 2nd stress cycle during the podding and pod maturing stages.

There were significant differences between treatments in the capsaicin (Fig. 3) and β-carotene (Fig. 4) contents of the fruits when the plants were subjected to the 2nd stress cycle during the fruit ripening stage (T_6).

In the treatment where the plants of the fruit ripening stage (T_6) were exposed to the 2nd stress cycle, the capsaicin content on the 15th day of measurement from the commencement of the stress was significantly higher (5.11 mg g^{-1} dry wt.) than the control (3.86 mg g^{-1} dry wt.). There were no significant differences in the capsaicin content of the fruits on the 10th (4.60 mg g^{-1} dry wt.) and 20th (4.58 mg g^{-1} dry wt.) day after re-watering the plants which previously underwent the 2nd stress cycle during the pod maturing (T_4) and fruit ripening (T_5) stages. These values however, were significantly lower than the value for the plants which underwent 15 days stress during the fruit ripening stage. The capsaicin contents of 4.14 mg g^{-1} dry wt. and 4.04 mg g^{-1} dry wt. were obtained on the 30th and 40th day after re-watering the plants which previously underwent the 2nd stress cycle during the podding and pod maturing stages.

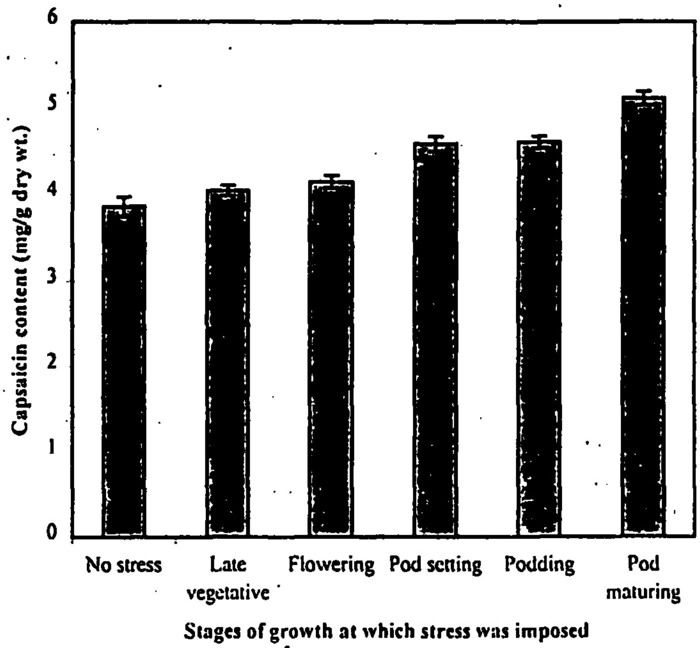

Fig. 3. Effect of soil moisture stress on the capsaicin content of chilli fruits.

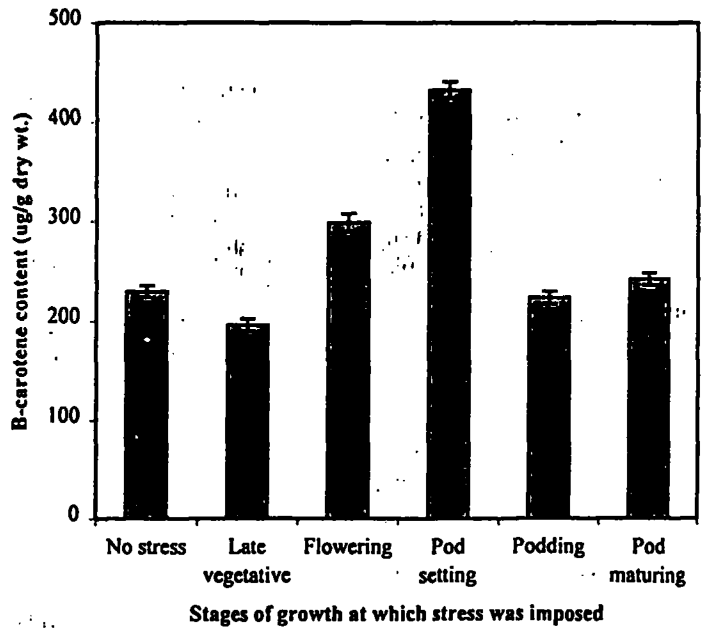

Fig. 4. Effect of soil moisture stress on the β-carotene content of chilli fruits.

In the treatment where the plants of the fruit ripening stage (T_6) experienced the 2nd stress cycle, the β-carotene content of the fruits on the 15th day of measurement from the commencement of the stress was 242.1 μg g^{-1} dry wt. when compared to 229.5 μg g^{-1} dry wt. of the control plants. There was a steep increase in the β-carotene content of the fruits 20 and 30 days after re-watering the plants which previously underwent the 2nd stress cycle during the pod maturing stage. A peak β-carotene content of 432.6 μg g^{-1} dry wt. was observed 20 days after re-watering the plants which previously experienced the 2nd stress cycle during the pod maturing stage. The plants stressed at the late vegetative stage had the lowest β-carotene content (196.2 μg g^{-1} dry wt) when measured several days after re-watering.

From the above observations it can be said that moisture stress reduced the vitamin C content of the fruits and the extent of reduction is positively related to the degree of stress. The vitamin C is very sensitive to changes in environmental conditions. It gets oxidized very rapidly when exposed to high temperatures (Davies *et al.*, 1991). The proposed route for vitamin C (L-ascorbic acid) synthesis commences from D-glucose (Counsell and Hornig, 1981). When plants experience moisture stress, stomata close and is followed by a decline in the CO_2 fixation. A reduction in D-glucose synthesis would have occurred during periods of stress which in turn would have lead to reduced vitamin C production. Moisture stress would have reduced the substrate concentration for vitamin C synthesis. Prolonged stress would have lead to greater decline in the substrate concentration. Reduction in the substrate would have been due to reduced photosynthetic rate. The vitamin C content of the pods did not vary much throughout the crop growth except during the late vegetative stage where the vitamin C content of the tender pods was low. Low vitamin C content in the tender pods may be due to the immaturity stage of the pods.

Another possibility that vitamin C content would have been reduced in the fruits of stressed plants may be due to the increased leaf temperature. An average leaf temperature of 36.8°C was recorded on the 15th day of measurement from the commencement of each stress cycle. As stated before, temperature affects the rate of destruction of vitamin C (Davies *et al.*, 1991). The leaf temperature progressively builds up as a consequence of moisture stress. Hence, with the development of a rapid stress such as the one experienced by the plants, a substantially high leaf temperature would have been built up and contributed towards the reduction of vitamin C. Re-watering decreased the leaf temperature (32°C) and thereby would have reduced the oxidative deterioration of vitamin C. Re-watering would have helped to sustain the vitamin C content in the fruits.

A delayed recovery in the vitamin C content after re-watering may be due to delayed D-glucose synthesis for L-ascorbic acid production. Re-watering makes the stomata to re-open and increases CO_2 fixation. Hence, an increase in D-glucose synthesis occurs which enables the synthesis of vitamin C in the fruits.

Non significant difference in the vitamin C contents of the pods on the 15th day of the 1st and the 2nd stress cycles belonging to the T_5, T_2 and T_6, T_3 treatments indicates that the 1st stress cycle had no after-effects on the synthesis of vitamin C on the 2nd stress cycle. There was also no acclimatization in the synthesis of vitamin C in the plants belonging to the above treatments. Reduced vitamin C content on account of stress would have been compensated by a re-synthesis during periods of re-watering. A complete recovery in vitamin C would have occurred within a period of 15 days re-watering after the stress treatment. This would have been due to increased substrate production during the period of re-watering.

Moisture stress increased the capsaicin content of the fruits. This increase may either be due to increased synthesis of capsaicin or reduced water content in the cells as a result of removal of water from the cells. When plants experienced moisture stress, the pods also would have sensed the moisture deficit effects. Whether moisture stress actually increases the capsaicin synthesis needs to be investigated. Re-watering reduces the capsaicin content either due to dilution or direct inhibition on the synthesis. The presence of substantial quantity of capsaicin content several days after re-watering may either be due to a slow process of break down of the increased capsaicin content or reduced solubility of capsaicin to added water.

Moisture stress did not show immediate effect on the β-carotene content of the fruits. Increase in the β-carotene content of the fruits was noticed several days after re-watering the plants which experienced moisture stress during the pod maturing stage. The transition in colour of the fruit from green to red usually takes place only a few days, during which time the colour is brownish owing to the co-occurrence of chlorophyll and the red pigments. Three phases are now recognized as occurring during the 'after-ripening' of chilli pods: during the initial period of about 2 weeks, there is a very rapid increase in the pigment contents, principally of β-carotene (Purseglove *et al.*, 1981). Moisture stress during the pod maturing stage would have temporarily suspended the 1st phase of rapid increase in the pigment content. Re-watering for several days would have lead to a sharp rise in the β-carotene synthesis. Moisture stress at the late vegetative stage substantially reduced the β-carotene content of the fruits. The reason for this needs to be investigated.

Significant reduction in the yield of chilli during the time the crop experienced moisture stress at the late vegetative stage indicates the critical stage of the crop. As pointed out by Techawongstien *et al.* (1992) plant growth and yield were more appreciably affected by stress at the vegetative stage than at the mature stages in chilli. The yield reduction in the plants treated at the late vegetative stage was due to the decrease in the fruit number. The reduction in the fruit number by prolonged water stress at the late vegetative stage may have considerably affected flower bud formation and development in chilli, presumably due to the poor development of the vegetative parts in the treated plants at this stage. The plants which experienced the soil moisture stress during the vegetative stage were stunted in appearance with an average height of 23 cm. The leaves were severely wilted and showed wrinkled appearance. The surface of the leaves was thin, soft and leathery. The canopy size of 17.6 cm length and 17 cm breadth was noticed when the stress was imposed during the vegetative stage. The canopy size of the control plants was 33.4 cm length and 30.6 cm breadth during the vegetative stage.

When the plants were exposed to moisture stress at the flowering stage, a severe drop in flowering (an average of 1.8 g dry weight of flowers $plant^{-1}$) was noticed. The flowers of the stressed plants were smaller (an average of 0.7 cm in length) than those of the control (an average of 1.6 cm in length) ones. Moisture stress reduces cell division and thereby affects leaf area development (Techawongstien *et al.*, 1992). The leaf area of

302.2 cm² plant⁻¹ was obtained when stress was imposed at late vegetative stage compared to the control value of 703.7 cm² plant⁻¹. Reduction in leaf area reduces the photosynthetic capacity of the leaves. This results in low carbohydrate production for the future pod formation in chilli.

The relationship between the yield and the vitamin C contents of the fruits is shown in Fig. 5. In the regularly watered plants with the yield of 2183 kg ha^{-1}, the average vitamin C content ranged from 115.9 to 117.6 mg 100 g^{-1}. The capsaicin and the β-carotene contents of the ripe fruits for this treatment are 3.86 mg and 229.5 μg g^{-1} dry wt., respectively. In the treatment where the plants of the late vegetative stage experienced 15 days moisture stress, the yield was reduced to 1099 kg ha^{-1} with the vitamin C content of 41.1 mg 100 g^{-1} in the tender fruits. The values of 4.04 mg and 196.2 μg g^{-1} dry wt. were obtained for capsaicin and β-carotene respectively, under the same treatment.

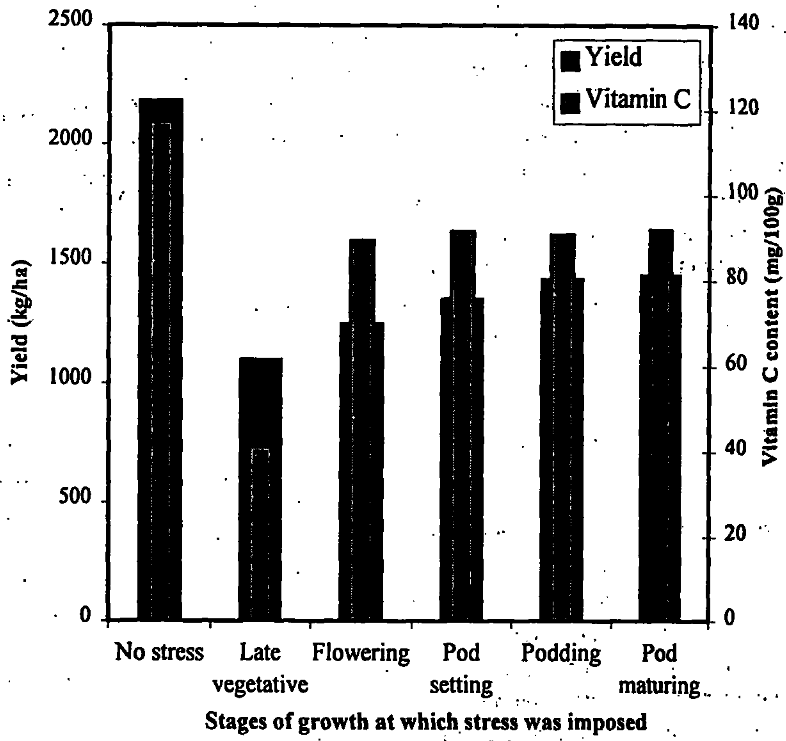

Fig. 5. The relationship between yield and vitamin C content of chilli fruits at various growth stages.

When the plants of the pod setting stage were subjected to 15 days stress, the vitamin C content was 91.5 mg 100 g^{-1} with the yield of 1351 kg ha^{-1}. At this stage the β-carotene content reached a peak value of 432.6 mg g^{-1} dry wt. Moisture stress of 15 days at the pod maturing stage reduced the vitamin C and β-carotene contents to 91.8 mg and 242.1 μg g^{-1} dry wt respectively, with the highest capsaicin content of 5.11 mg g^{-1} dry wt. The yield during this time was 1452 kg ha^{-1}. The above stage of growth were deliberately chosen to show how soil moisture can cause peak changes in the quantity and quality of chilli compared to the rest of the stages of growth. From the above observations it can be said that the late vegetative stage is the most critical stage for moisture stress compared to the other stages of the crop. The stress at this stage drastically reduced the yield with considerable reductions in the vitamin C and β-carotene contents of the fruits. There was an increase in the capsaicin content at this stage compared to the regularly watered plants. Moisture stress at the pod setting stage although significantly reduced the yield, the quality of chilli with respect to vitamin C, capsaicin and β-carotene contents was increased.

CONCLUSIONS

The above study determined to what extent moisture stress affects the vitamin C, capsaicin and β-carotene contents of the fruits and thereby the overall quality of fruits. Long term moisture deficits during the late vegetative, flowering, pod setting, podding, pod maturing and fruit ripening stages delayed the recovery of vitamin C synthesis and subsequently affected the yield. A similar trend was noticed in the reduction and recovery of vitamin C throughout the growth of the crop. Moisture stress increased the pungency of the fruits. Increased pungency slowly deteriorates after re-watering. Moisture stress had little immediate effect on the β-carotene content. Delayed effects of moisture stress on β-carotene is greater than the immediate effect. It can be concluded that moisture stress at the late vegetative stage caused highest yield reduction with reduced quality compared to the other stages of growth.

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