Proline Content in *Brassica* under High Temperature Stress

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Received May 31, 1995

Abstract

Proline content in different cultivars of cabbage, Chinese cabbage and their hybrids under high temperature stress was determined to identify differences in heat tolerance. Proline content in the leaves of the interspecific hybrids between cabbage cultivar (cv.) 'Yoshin' (*Brassica oleracea* var. *capitata* L.) and Chinese cabbage cv. 'Kenshin' (*B. campestris* var. *pekinensis* L.), and between Chinese kale cv. 'Sen-yo shirobana' (*B. oleracea* var. *alboglabra Bailey*) and 'Kenshin' was intermediate between that of their parents at 35℃. At 25℃ and 35℃, the proline contents were higher in stalks and floral buds than in leaves, and decreased drastically at 35℃. A negative correlation was found between pollen viability and the rate of decrease of the proline content in the floral buds. Proline contents were reduced by ca. 40% and pollen viability by 51%, when the temperature increased from 25℃ to 35℃. The increase in the proline content was 1.2, 1.6 and 1.8 times in the leaves of heat-tolerant cabbage cv. 'Yoshin', Chinese cabbage cv. 'Kenshin' and Chinese kale cv. 'Full white', respectively. In contrast, the increase in the proline content in the heat-sensitive cabbage cv. 'YR Kinshun', Chinese cabbage cv.'Chihiri 70' and Chinese kale cv.'Large leaf' was 3.5, 17.6 and 3.6-fold, respectively. The results obtained suggest that proline accumulation may be used as a criterion for selection of heat-tolerant genotypes in *Brassica*.

Additional key words: *Brassica oleracea, B. campestris*, heat tolerance, hybrids, proline, reproductive stage

INTRODUCTION

Free proline is known to accumulate under stress conditions such as high temperature, drought, salinity and waterlogging in tomato, cowpea, bean and barley (1, 2, 3, 4). Proline is, therefore, considered to be a useful component for

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evaluating the tolerance of a crop to high temperature (1). Proline accumulation is faster in sensitive cultivars than in tolerant ones regardless of stress conditions. The accumulation of proline is one of the best characterized osmoregulatory response (5). In some tissues, the proline level increased as much as 100-fold in response to stress (6). The accumulation of proline results from an increased conversion of glutamate to pyrroline-5-carboxylate, then to proline (7, 8).

High temperature stress caused male sterility in tomato (9), rice (10), wheat (11) and cowpea (2). The factors responsible for the low pollen fertility have not been elucidated. However, it is assumed that there is a positive correlation between the pollen fertility and proline content in floral organs. Kuo et al. (1) reported that tomato plants exposed to high temperatures showed low proline contents in anthers and pollen, and high contents in leaves. There are few studies on the effect of high temperature stress during the reproductive stage on the translocation of proline in *Brassica* from leaves to floral organs. Besides, a large number of heat-tolerant hybrids of *Brassica* have been developed for cultivation in the tropics and their tolerance to high temperature must be evaluated. The objectives of this study were to: 1) determine the proline levels in *Brassica* at different temperatures to identify heat-tolerant or sensitive cultivars; 2) determine the proline contents in different organs of *Brassica* plants during reproductive growth at high temperatures; and 3) determine whether proline accumulation plays an important role in pollen viability under heat stress.

On the present report the proline content in somatic and sexual hybrids between *Brassica oleracea* L. and *B. campestris* L. was also examined.

MATERIALS AND METHODS

In the first experiment, one somatic hybrid, two sexual hybrids and their parents were used as plant materials. The somatic hybrid was derived from cabbage (*Brassica oleracea* var.*capitata* L.) cv. 'Yoshin' and Chinese cabbage (*B. campestris* var. *pekinensis* L.) cv. 'Kenshin' (Yoshin + Kenshin), and the sexual hybrids from 'Yoshin' and 'Kenshin' (Yoshin×Kenshin) and Chinese kale (*B. oleracea* var. *alboglabra* Bailey) cv. 'Sen-yo shirobana' and 'Kenshin' (Sen-yo shirobana x Kenshin). The sexual interspecific hybrids were developed through in vitro ovule culture and their chromosomes were doubled by 0.3% (w/v) colchicine treatment (12, 13).

In the second experiment, 4 cultivars each of cabbage and Chinese cabbage, or 3 cultivars of Chinese kale were used for proline determination in leaves of 35-day-old seedlings. At least one heat-sensitive cultivar was included in each species for the study. The objective was to screen cultivars for heat tolerance at an early stage through proline determination in leaf. In both experiments, three plants from each cultivar or hybrid were randomly selected for each treatment. Only the 5th leaf from the top was used for proline determination and all the samples were collected at 9 A.M.

Plant culture. Seeds were sown in vermiculite and one week after germination, seedlings were transplanted into pots 12cm in diameter, filled with a soil : compost (3:1) mixture. Fertilizer was applied at the rate of 0.5 g NPK (14-14-14), 1 g single super-phosphate and 2g magnesium carbonate per kg of soil mixture. The seedlings were grown in a glasshouse under natural day conditions, namely under 25/20 ± 2℃ and 35/30 ± 2℃ day/night temperature regimes.

Pollen viability. Pollen viability was examined by treatment with 1% (W/V) acetocarmine and stainable grains were considered to be viable. In each genotype 1000 pollen grains were examined. All the experiments were arranged in a completely randomized design with three replications and data were analyzed by Duncan's multiple range test at 5% level of significance.

Proline determination. Leaves, stalks and floral buds weighing 0.5 g were collected from the hybrids and their parents in the first experiment, whereas, only leaf samples (0.5 g) in the second experiment. The samples were homogenized in 10 ml of 3% (v/v) sulfosalicylic acid. Two ml of the supernatant was reacted with 2 ml acid-ninhydrin and 2 ml glacial acetic acid in a test tube for 1 h at 100℃, and the reaction was terminated by transferring the test tubes into an ice bath. The reaction mixture was extracted with 4 ml of toluene, then 2 ml of the toluene phase was used for absorbance reading at 520 nm. Proline content was expressed on a fresh weight basis from a standard curve, using standard L-proline, according to the method of Bates et al. (14).

RESULTS

Proline contents in the leaves were significantly high in 'Yoshin' and 'Sen-yo shirobana' at 25℃ but only in 'Sen-yo shirobana' at 35℃ (Table 1). The proline contents of the interspecific hybrids was lower than those of their parents at 25℃, but intermediate value was obtained at 35℃, and the content was the lowest in the somatic hybrid among the hybrids.

In the stalks, proline content in 'Sen-yo shirobana' (9.12 μ mol/g FW) was significantly higher than in the other materials and that in a hybrid between 'Sen-yo shirobana' × 'Kenshin' at 25℃ was the lowest (4.60 μ mol/g FW). Proline content of this hybrid was similar to that of 'Kenshin' at 25℃, while at 35℃ that of the somatic hybrid between 'Yoshin' + 'Kenshin' was the highest (5.57 μ mol/g FW). The lowest proline content (3.18 μ mol/g FW) in stalks at 35℃ was recorded in 'Kenshin' (Table 1). In the hybrid between 'Sen-yo shirobana' × 'Kenshin' at 35℃ the content was 3.90 μ mol/g FW, a value intermediate between those of the parents.

In the floral buds, the highest proline content was found in 'Sen-yo shirobana' (12.43 μ mol/g FW) and the lowest in the hybrid between 'Yoshin' × 'Kenshin' (8.23 μ mol/g FW) at 25℃. At 35℃, the proline contents in the floral buds were reduced drastically, in all the genotypes (Table 1). The highest proline contents in the floral buds at 35℃ were detected in the hybrid between 'Sen-yo shirobana' × 'Kenshin' (8.91 μ mol/g FW), and the lowest in 'Kenshin' (3.79 μ mol/g FW).

Pollen viability was the highest in 'Sen-yo shirobana' (88.0%) at 25℃. All the hybrids and the parents were highly viable (>80%) at 25℃. However, the viability decreased by 26% to a value of 73% at 35℃. Pollen viability at 35℃ was highest in 'Sen-yo shirobana'(65.0%) and lowest in the hybrid between 'Sen-yo shirobana' × 'Kenshin' (21.7%). On the average, 52% of the pollen viability

Table 1. Proline content in the leaves, stalks and floral buds, and pollen viability of the interspecific hybrids and their parents in *Brassica* during reproductive growth.

Cultivar/ Hybrid	Proline content (μ mol/g FW)						Pollen viability (%)	
	Leaves		Stalk		Floral buds			
	25℃	35℃	25℃	35℃	25℃	35℃	25℃	35℃
Yoshin	1.50a[a)]	2.00b	-[b)]	-	-	-	-	-
Kenshin	0.37b	0.53d	4.62d	3.18e	9.18c	3.79d	83.6b	59.2b
Sen-yo shirobana	1.30a	3.09a	9.12a	4.28c	12.43a	4.27c	88.0a	65.0a
Yoshin + Kenshin	0.39b	1.37c	6.09c	5.57a	9.23c	6.43b	83.7b	25.3d
Yoshin × Kenshin	0.33b	1.94b	7.13b	5.23b	8.23d	6.57b	82.2c	32.9c
Sen-yo shirobana × Kenshin	0.38b	2.00b	4.60d	3.90d	10.71b	8.91a	79.5d	21.7e

a) In a column, means followed by the same letter are not significantly different at the 5% level

b) Yoshin did not produce flowers at 25℃ as it requires vernalization for 3-5 weeks at the 7-8 leaf stage for flowering.

decreased under high temperature stress. There was a negative correlation between the proline content in the floral buds from 25 to 35℃ and the pollen viability at 35℃ (Fig.1).

Proline contents in the leaves of 35-day-old plants of cabbage, Chinese cabbage and Chinese kale grown at 25℃ and 35℃ are shown in Fig. 2. At 25℃, the lowest proline content was found in 'YR Kinshun' (0.34 μ mol/g FW) and the highest in 'Kinkei No. 201' (2.77 μ mol/g FW). A similar trend was observed at 35℃ except for the lowest value in 'Yoshin'. In Chinese cabbage, the proline content was the lowest in 'No. 11' (0.24 μ mol/g FW) and the highest in 'Chihiri 70'(0.47 μ mol/g FW) at 25℃. However, at 35℃, the lowest proline contents were observed in 'Kenshin' (0.55 μ mol/g FW) and 'No. 11' (0.56 μ mol/g FW) highest in 'Chihiri 70' (8.56 μ mol/g FW) with an intermediate level in 'Kensui' (2.05 μ mol/g FW). Among the three cultivars of Chinese kale, both at 25℃ and 35℃, the lowest proline contents were found in 'Sen-yo shirobana' (1.18 and 2.57 μ mol/g FW at 25℃ and 35℃, respectively) while the highest contents in 'Large leaf' (2.25 and 8.07 μ mol/g FW at 25℃ and 35℃, respectively). Proline content in 'Full white' was 2.93 μ mol/g FW at 35℃.

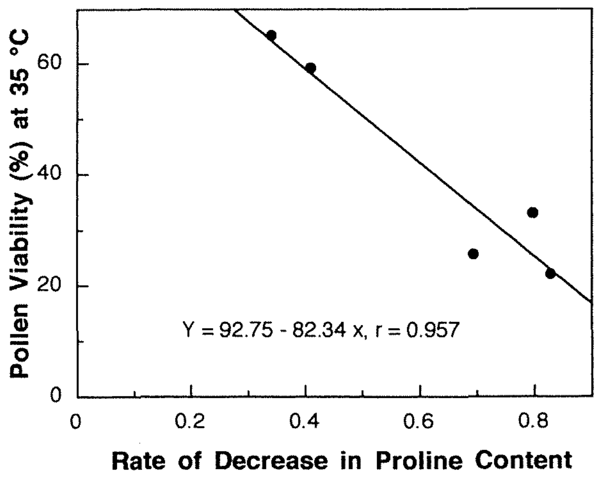

Fig. 1. Correlation between rate of decrease in proline content in the floral buds from 25℃ to 35℃ and pollen viability in *Brassica* at 35℃.

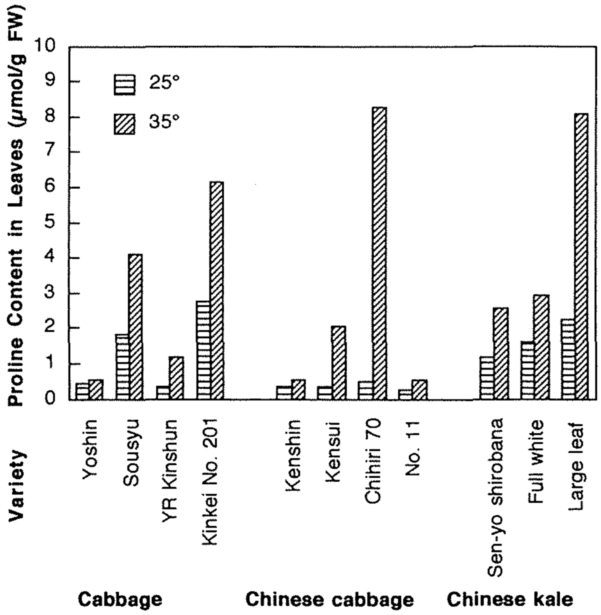

Fig. 2. Proline content in the leaves of heat-tolerant and sensitive cultivars of cabbage, Chinese cabbage and Chinese kale at 25℃ and 35℃.

DISCUSSION

Proline accumulation in the leaves and floral buds under high temperature stress has been reported in tomato (1). Proline content in the leaves was lower than in floral buds. However, high temperature increased the proline content in the leaves. Our results also showed a similar trend, as the proline content was higher in the stalks than in the leaves at both 25℃ and 35℃. However, the proline content in the leaves increased at 35℃ while in the stalks it decreased (Table 1). In the floral buds, the proline content was high at 25℃, but, it was reduced on an average by ca. 40% at 35℃, which ultimately affected the pollen viability. The results are in agreement with the those of Mutters et al. (2), who reported that the mean proline content of pollen in cowpea was 41 mg/g FW under an optimal temperature regime and much less (21 mg/g FW) under a hot regime (reduction by 48.8%). Similar results were reported in maize by Palfi et al.(15) who considered that a large amount of proline is necessary for pollen development and pollen tube growth. Proline content in the floral buds may be used as one of the criteria to evaluate heat tolerance in Brassica. Kuo et al. (1) found similar results in tomato and reported that a high proline content in the floral buds may be necessary to withstand high temperatures. Our results (Fig. 1) showed that there was a highly negative correlation between the rate of decrease in the proline content in floral buds from 25 to 35℃ and pollen viability at 35℃. Britikov et al. (16) also found a correlation between pollen fertility and proline content.

Proline content in the leaves of young cabbage, Chinese cabbage and Chinese kale varied considerably. The significant increase in the proline content at 35℃ in 'YR Kinshun' (3.5-fold), 'Chihiri 70' (17.6-fold), and 'Large leaf' (3.6-fold) for each variety suggests that they are heat-sensitive. On the contrary, 'Yoshin' (1.2-fold), 'Kenshin' (1.6-fold) and 'Full white' (1.8-fold), in which the increase was low, were heat-tolerant. Hanson et al. (4) demonstrated that proline accumulation in the leaves of barley was effective in the selection of drought-tolerant progenies.

The results obtained suggest that there was a negative correlation between the proline content in the floral buds and pollen viability in the somatic and sexual interspecific hybrids between *B. oleracea* and *B. campestris* and their parents. Proline content in the leaves increased with the increase in temperature, and since the increase was considerable (2.2- to 17.6-fold) in the heat-sensitive cultivars, it is sufficient that the proline content may be used as a selection criterion for tolerance to high temperature.

ACKNOWLEDGEMENT

The authors thank Dr. T. Yamashita, Director, JIRCAS Okinawa Subtropical Station, Okinawa, Japan for his critical comments and suggestions in reviewing the manuscript.

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高温ストレス下におけるアブラナ科作物のプロリン含量について

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摘　要

高温ストレス下での，キャベツ（*Brassica oleracea* var. *capitata* L.），ハクサイ（*B. campestris* var. *pekinensis* L.），カイラン（*B. oleracea* var. *alboglabra* Bailey）の数品種及びキャベツとハクサイ，カイランとハクサイの種間雑種のプロリン含量を測定比較した。キャベツ品種“葉深”とハクサイ品種“捲心”との，及びカイラン品種“尖葉白花”とハクサイ品種“捲心”との種間雑種の，35℃高温下におけるプロリン含量は，それぞれの親のプロリン含量値のほぼ中間的な値を示した。25℃及び35℃高温，二つの温度条件下で，供試各品種の花芽部，茎軸部，葉身部のプロリン含量を比較した結果，いずれの温度条件下においても花芽部及び茎軸部のプロリン含量は葉身部より大きな値を示した。又，二つの温度条件下（25℃及び35℃）における花芽部及び茎軸部のプロリン含量を測定比較した結果，35℃高温下で，急激なプロリン含量の減少が確認された。更に35℃高温下においては，花芽部のプロリン含量減少率と花粉活性との間に負の相関が認められた。一方，温度を25℃から35℃に上昇させたときの，供試各品種の平均プロリン含量は9.96から5.99 μ mol/g生重に減少し，その減少率は約40%と顕著であった。又，同条件下（25℃から35℃への上昇）では，平均51%の花粉活性の減退が観察された。高温耐性品種，キャベツ“葉深”，ハクサイ“捲心”及びカイラン“Full white”の各葉身部プロリン含量増加率は，それぞれ1.2，1.6及び1.8倍を示した。又，これらと対照的に，高温感受性品種，キャベツ“ＹＲ金春”，ハクサイ“チヒリ70”及びカイラン“Large leaf”では，それぞれ3.5，17.6，及び3.6倍と，高い葉身部プロリン含量増加率を示した。これらの結果は，プロリン代謝特性が，アブラナ科作物高温耐性品種の選抜指標として大きな意味を持つことを示唆している。

キーワード：キャベツ，ハクサイ，高温耐性，雑種，プロリン，生殖生長期