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中文摘要

本研究分析三種結球白菜品種相對耐熱品種‘亞蔬一號’及‘桃園亞蔬二號’與相對不耐熱品種‘瑞農 720’，於不同溫度(25, 30, 35, 40 °C)不同處理(0, 6, 12, 48 及 72 小時)下觀察植株受高溫前後、植株的生長、外部形態及內部抗氧化酵素活性、葉綠素螢光、相對含水量及電解質滲漏等生理的變化，評估酵素活性可否成為蔬菜品質與產量之生理指標，並將此酵素選殖出來。以 actin 為 internal control，利用 real-time PCR 分析在高溫逆境下抗氧化酵素 mRNA 之相對表現量。利用 RACE 法擴增其抗氧化酵素基因 cDNA full length 末端，經選殖入載體並定序後，將其序列經 NCBI 網站以 blastn 比對此 cDNA full length 序列，以確定此段基因。並使用南方墨點法確認其 copy number。

結果顯示，三品種之葉片葉綠素螢光及相對含水量因溫度提高而對植物造成逆境，而以葉綠素螢光 Fv/Fm 較有鑑別效果，但電解質滲漏對結球白菜耐熱性鑑定較不敏感。APX 活性於三品種高溫處理之後隨時間點升高，‘RN720’於高溫時 MDA 上升，表示受到較多逆境傷害而不耐熱，反之‘ASVEG2’沒有升高而為耐熱品種。另外，MDA 含量及 APX 之活性於品種間有顯著差異，但 SOD 及 CAT 之活性於品種間無顯著差異。瑞農 720 之 APX mRNA 相對表現量在 35°C 和 40 °C 6 小時後表現量開始增加，而桃園亞蔬二號於 35°C 12 小時 mRNA 相對表現量大幅增加。經 RACE 擴增出之 APX cDNA 全長為 1056bp，其序列與油菜、甘藍及蘿蔔之 APX 序列都有 95% 以上的相似度。

關鍵字: 結球白菜、熱逆境、抗壞血酸過氧化酶、即時聚合鏈反應、快速增幅 cDNA 末端

ABSTRACT

The objectives of this work were to study the changes of antioxidative system in Chinese cabbage under 25, 30, 35, and 40 °C for 0, 3, 6, 12, 48, and 72 hours, and identify any antioxidative enzyme linked to heat-tolerance traits under heat stress. Heat-tolerant varieties “AV1” and “ASVEG2” and heat-susceptibility variety “RN720” were used to measure the antioxidative enzymes, chlorophyll fluorescence, turgor pressure and ion leakage under the growth chamber. The response of target gene expressions and regulations under the stress were determined using real-time PCR. Actin mRNA was used as internal control. A full-length target cDNA was amplified using RACE technique followed by cloning it into the vector for sequencing, and comparing it with any known sequences in the NCBI-blastn. Genomic Southern hybridization was carried out to prove the existence of the target DNA in the Chinese cabbage.

The result shows that the turgor pressure, ion leakage, and CAT and SOD activities were not able to significantly identify the differences between genotypes. However, genotypes under heat stress could be clearly determined by Fv/Fm value. The increased APX activity and decrease MDA content provide the Chinese cabbage plants with increased heat tolerance. The increased levels of APX transcripts were detected at 6 h of 35 and 40°C in RN720, as well as at 12 h 35°C in ASVEG2. The completion of a full length APX cDNA clone was 1056bp. The sequence of Chinese cabbage APX gene had greater than 95% homology to that from the rape seed, cabbage and radish APX gene.

Keywords: Chinese cabbage, heat stress, ascorbate peroxidase, real-time PCR, RACE.