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EXPRESSION OF β-CAROTENE IN ADVANCED PROGENIES DERIVED FROM DIFFERENT BACKCROSSES OF THE HIGH-YIELDING RICE VARIETIES TO THE TRANSGENIC GOLDEN RICE LINES

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ABSTRACT

Using a backcross scheme, we have transferred the trait expressing β-carotene in the japonica transgenic lines to the three Vietnamese high-yielding varieties of indica type. The contents of carotenoid and its components including β-carotene of the progenies of BC3F3, BC3F4 and BC3F5 were measured. The results showed that the expression of β-carotene depended on the backcross. The backcrosses with AS996 and OM1490 as the recurrent parents showed higher expression of β-carotene in the progenies than the backcross with OM2031, indicating the genetic background of the recurrent parent affected the expression level of β-carotene in the progenies. In the BC3F5, a number of promising lines showing high expression level of β-carotene were identified and selected. The results of this study revealed the possibility to develop high-yielding varieties containing high content of β-carotene.

Key words: β-carotene, backcross, carotenoid, golden rice, transformation

INTRODUCTION

Rice is the staple food of the Asia, particularly in the developing countries. Rice provides much energy in the diet, but it is extremely poor in micronutrients like iron and totally lacks vitamin A. Human diseases due to vitamin A deficiency prevail in developing countries affecting millions of people and causing permanent blindness and impairment of the immune system. A survey in Vietnam showed that 30% of children below 5 year old had low vitamin A level in the serum and one of three women had low vitamin A level in the breast milk (Nhan Dan, 2007). Therefore, the enrichment of rice for higher nutrition values would be helpful in reducing malnutrition in the poor who depends on rice as the major food daily.

In recent years, the advances in plant biotechnology have resulted in the production of the transgenic rice containing β-carotene (pro-vitamin A) called as golden rice (Ye et al., 2000; Tran Thi Cuc Hoa et al., 2003). This opens up a new direction in developing the rice varieties locally adapted and rich in pro-vitamin A, so that such varieties will be more applicable in production. Following this direction we have transferred the golden rice trait from the transgenic lines derived from Taipei 309- a japonica variety to the Vietnamese high-yielding varieties (indica) by backcrossing. In this paper, we report the expression of β-carotene in backcross progenies derived from different backcrosses of the high-yielding rice varieties to the transgenic golden rice lines. The results indicated that it is possible to develop a high-yielding and adapted varieties containing high content of pro-vitamin A.

MATERIALS AND METHODS

Rice varieties

The rice varieties and transgenic golden rice lines are from the japonica variety Taipei 309 (T3 generation). Among the four transgenic lines, one was produced by using the vector pCarNew (Tran Thi Cuc Hoa et al., 2004) (Fig. 1A) and the other three were produced by using the vector pFun3 (Fig 1B) co-transformed with the vector pDXS (Tran Thi Cuc Hoa et al., 2004, Al-Balibi et al., 2006) (Fig 1C). Both the vector pCarNew and pFun3 contained the genes encoding for the formation of β-carotene (*psy and crtI*). The vector pFun3 was a modified version of the vector CarNew in which the *crtI* gene was replaced by the synthetic *crtI* gene with optimized codon. The vector pDXS contained the gene *dxs* encoding for the enzyme deoxyxylulose phosphate synthase,

linking primary carbon metabolism to prenyllipid biosynthesis. The T0 transgenic plants produced by transformation were identified by Southern analysis (Fig. 2) to advance to T1, T2, and T3.

Three high-yielding rice varieties, AS 996, OM1490 and OM2031 were used for backcrossing to the above transgenic lines. These varieties were popular and widely adapted to the ecological conditions of the Mekong Delta of Vietnam.

Southern blot analysis

Genomic DNA was isolated from frozen rice leaves following the method by McCouch et al. (1988). Ten micrograms of genomic DNA were digested with *Hin*d III and *Eco*RI. Southern blot analysis was carried out following standard protocols (Sambrook et al; 1989)

Backcrossing

The high-yielding varieties were used as the recurrent parents and the transgenic lines were the donor parents. The F1 seeds derived from crossing the recurrent parent with the donor parent which expressed dark yellow colour were selected to produced F_2 plants. The first backcross (BC_1) was done by crossing the F_2 plant with the recurrent parent and selection and backcrossing was the same as to produce BC_2 and up to BC_3. From BC_3 progenies were advanced by self pollination up to BC_3F_5.

Analysis of total carotenoid and β-carotene

Rice grains were dehulled, polished and grinded to fine powder. The total carotenoid was analyzed by spectrophotometer (Smartspec 3000). The components of carotenoid including β-carotene were measured by High Performance Liquid Chromatography (HPLC) following the protocol described by Tran Thi Cuc Hoa. et al. (2008).

Table 1. Parentage of backcrosses of high-yielding varieties to transgenic golden rice.

Backcross No./Name	High-yielding varieties (recurrent parent)	Transgenic line (donor parent)	Vector used to produce transgenic line
1 (Car/AS996)	AS996	(E2-14b F/D/TP PN:2)-5-3/4	pCarNew
2 (Fun3/AS996)	AS996	(E1-14C/TP PN:2)-9-1/4	Fun3+ pDXS
3 (Fun3/OM1490)	OM1490	(E2-14b F/D/TP PN:1)-4-4/4	Fun3+ pDXS
4 (Fun3/OM2301)	OM2301	(E2-14b F/D/TP PN:2)-5-2/4	Fun3+ pDXS

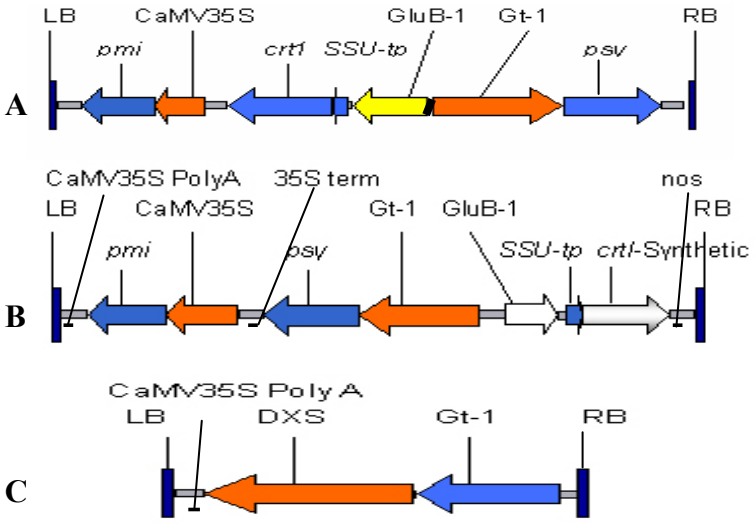

Fig. 1. Schemes of vector inserts of pCarNew (A) pFun3 (B) and pDXS (C)

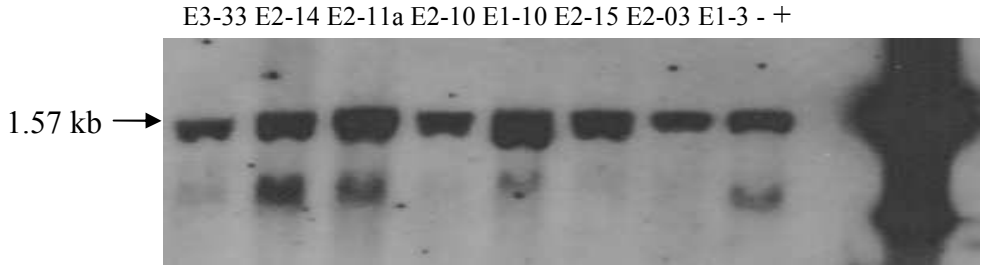

Fig. 2. Southern blot analysis of T0 transgenic plants (Taipei 309)

DNA was digested with *Hin*dIII and *Eco*RI. The expected transgene band size for the gene *psy* was shown as 1.57 kb
-: Non-transformed plant, +: Plasmid, M: Marker

RESULTS AND DISCUSSION

In our backcrossing scheme, the selection of F_1 seeds based on visual expression of dark yellow color to advance to F2 plants for backcrossing to the recurrent parent appeared to be efficient. The selection of self-pollinated progenies (BC_3F_3, BC_3F_4, BC_3F_5) was based on high expression of β-carotene among the lines and among individuals within the lines.

The expression of β-carotene in progenies derived from different backcrosses is presented in Fig. 3A (BC_3F_3), Fig. 3B (BC_3F_4) and Fig. 3C (BC_3F_5)

In BC3F3, the progenies showed better expression of β-carotene belonged to the backcross using AS996 as the recurrent parent and the transgenic line transformed by the vector pCarNew as the donor parent (Car/AS996). These progenies had contents of β-carotene ranging from 1.8 to 7.5 μg/g, which was better than the progenies derived from other backcrosses.

In BC_3F_4, all the progenies derived from 4 backcrosses had higher levels expression of β-carotene than those of BC3F3, particularly some individuals reached contents of β-carotene above 20 μg/g (Car/AS 996 in Fig. 3B).

In BC_3F_5, the progenies of the backcross Car/AS996 remained the superiority, but the progenies of the backcross Fun3/OM1490 showed a significant improvement as compared to BC_3F_4 (Fig. 3C).

The results of this study revealed that the genetic background of the recurrent parents influenced the expression of the progenies derived from backcrossing. The effect of different transgenic lines used as the donor parent was not clear because the backcross Car/AS996 and pFun3/AS996 showed no significant difference of β-carotene in their progenies.

The selection process applied in this study helped to identify the progenies showing best performance after each generation through selection among lines and within lines, hence at last it is possible to select the best lines applicable to transfer to production. In our study, individual plants of BC_3F_5 were measured for total carotenoid and its components (Fig. 4) and we have identified a number of lines showing high expression level of β-carotene. The two lines presented in Fig. 5 and Fig. 6 as an example. The line Fun3/AS996.1.1.1.1.1.2.8.4 (Fig. 4) had a total carotenoid of 13.2 μg/g and β-carotene of 7.6 μg/g (Fig. 5). The line Fun3/OM1490.1.1.1.1.3.3.1.28 (Fig. 4) had a total carotenoid of 17.9 μg/g and β-carotene of 13.3 μg/g (Fig. 6), and its kernels expressed a dark yellow color as shown in Fig. 7.

The results of this study present the success in developing high-yielding rice varieties of indica type contained β-carotene by backcrossing the transgenic japonica golden lines opening up a practical application of golden rice in production.

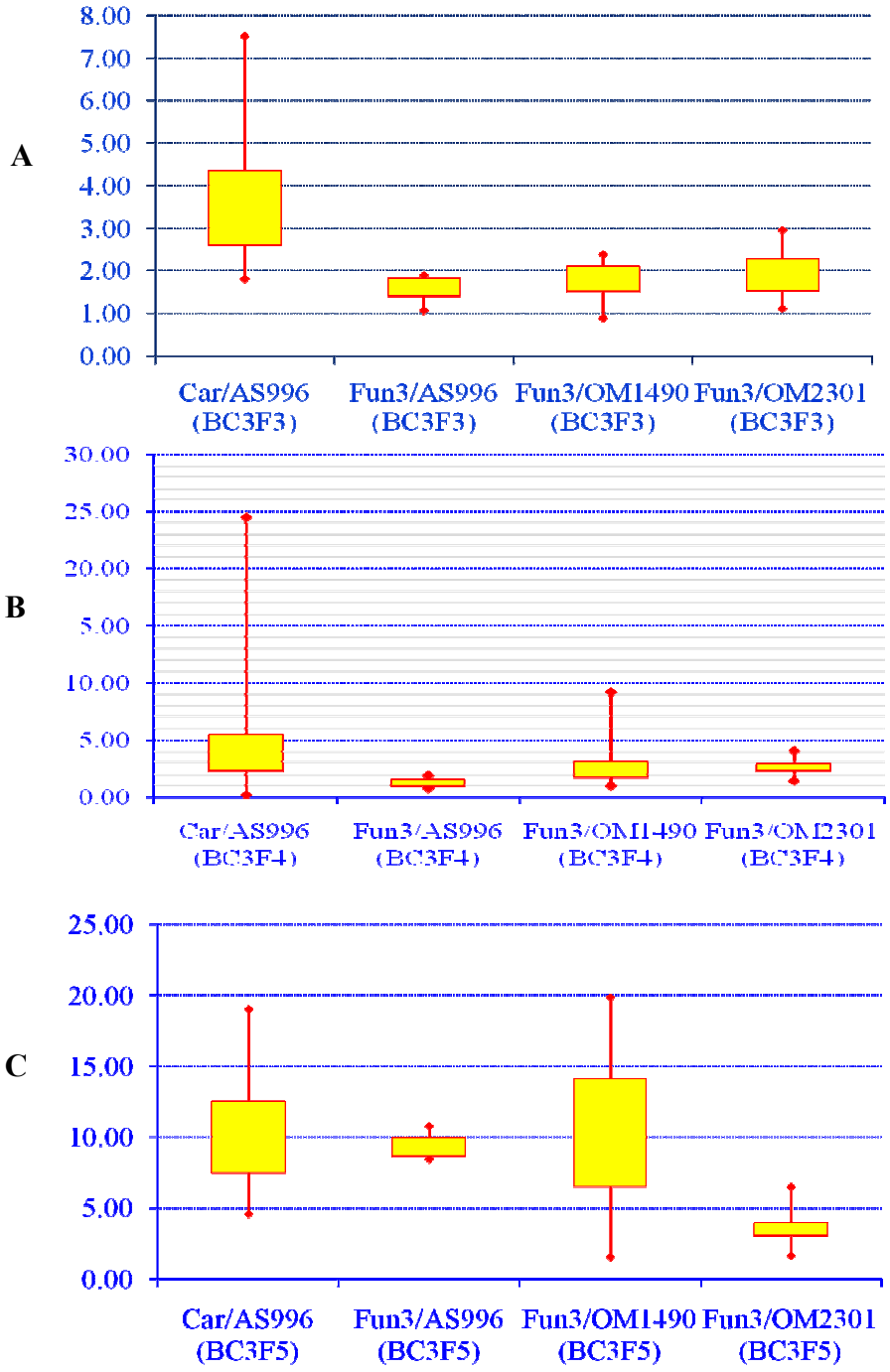

Fig 3. β-carotene of the progenies of BC_3F_3, BC_3F_4 and BC_3F_5 derived from 4 backcrosses

Bar (rectangular frame) represents 75% of BC individuals that have carotenoid content equivalent to the level of carotenoid indicated on the vertical axis.

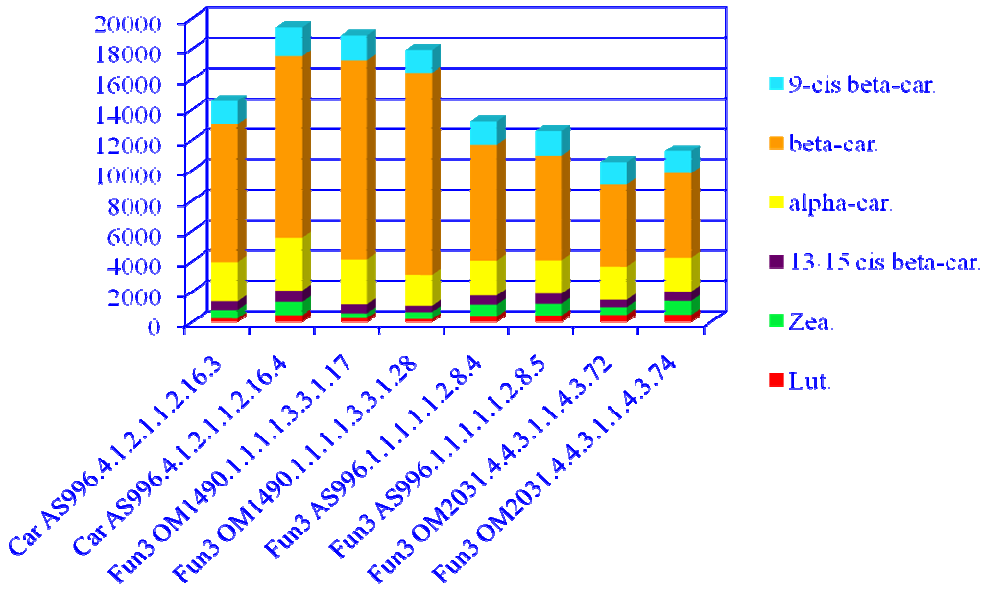

Fig. 4. HPLC analysis of some promising high-yielding rice lines from BC_3F_5 progenies derived from four backcrosses

Car: pCarnew, Fun3: pFun3 + pDXS (co-transformation)

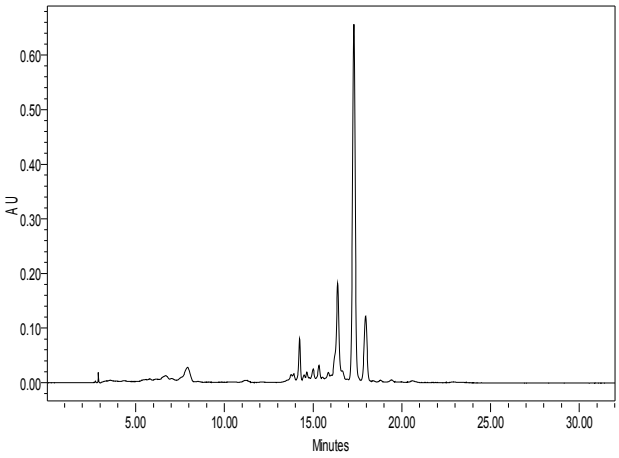

Fig 5. Carotenoid analysis (ng/g) of the selected BC3F5 line Fun3/AS996.1.1.1.1.1.2.8.4: lutein: 343.76, zeaxanthin: 786.589, 13/15 cis β-carotene: 610.52, α-carotene: 2288.53, **β-carotene: 7646.07**, 9-cis β-carotene: 1547.58, total carotenoid: 13223.06

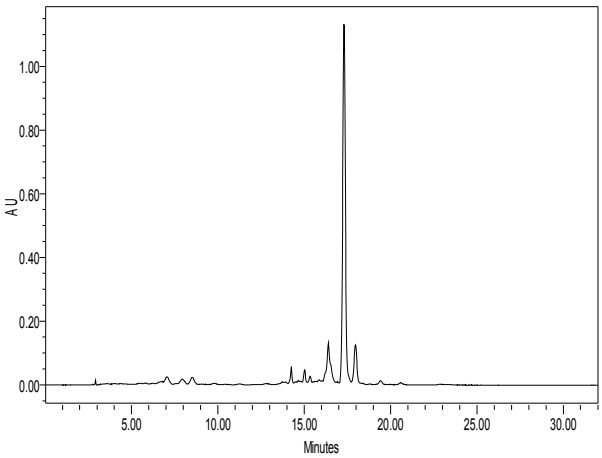

Fig 6. Carotenoid analysis (ng/g) of the selected BC3F5 line Fun3/OM1490.1.1.1.1.3.3.1.28 lutein: 183.825, zeaxanthin: 452.23, 13/15 cis β-carotene: 422.61 α-carotene: 2011.29 **β-carotene: 13329.8**9, 9-cis β-carotene: 1523.592, total carotenoid: 17923.45

Fig 7.Rice kernels of the BC_3F_5 line Fun3/OM1490.1.1.1.1.3.3.1.28 expressed dark yellow color in contrast to the normal variety (OM1490) with white

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Biểu hiện β-Carotene trong các dòng lúa tạo ra bằng hồi giao các giong lúa cao sản với dòng lúa vàng biến đổi gen

Bằng áp dụng hệ thống lai hồi giao, đặc tính lúa vàng chứa β-carotene của các dòng lúa joponica biến đổi gen được chuyển vào 4 giống lúa cao sản Việt Nam thuộc nhóm indica. Hàm lượng carotenoid và các thành phần của carotenoid trong đó β-carotene của các dòng BC3F3, BC3F4 và BC3F5 được phân tích và định lượng. Kết quả cho thấy mức độ biểu hiện của β-carotene ở các dòng lúa tạo từ lai hồi giao tùy thuộc vào tổ hợp lai giao hồi giao. Tổ hợp hồi giao với giống lúa AS996 và OM1490 cho mức độ biểu hiện β-carotene cao hơn so với tổ hợp hồi giao với giống lúa OM2301, chứng tỏ đặc tính di truyền của giống hồi giao ảnh hưởng đến sự biểu hiện của β-carotene ở các dòng tạo ra bằng hồi giao. Ở thế hệ BC_3F_5 một số dòng chứa hàm lượng β-carotene đã được xác định. Kết quả nghiên cứu cho thấy khả năng tạo ra các giống lúa cao sản biến đổi gen chứa β-carotene.

Từ khóa:: β-carotene, carotenoid, chuyển nạp gen, hồi giao, lúa vàng