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Section: Agricultural and Food Biotechnology

Genetic Manipulation of Xanthophyll Cycle on New Plant Type (NPT) Rice to Enhance Its Resistance towards High Light Stress

Sugiyono [#)] ***and Peter Horton*** [*)]

[#)] Faculty of Biology, Jenderal Soedirman University, Purwokerto 53123; [*)] Robert Hill Institute for Photosynthesis Research, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN, UK;

ABSTRACT

The objective of this study was to genetically manipulate the carotenoid metabolism and xanthophyll cycle in New Plant Type (NPT) rice with a view to enhance its resistance towards high light stress. A Crtl gene was introduced into NPT rice (IR 65600-42-5-2) using Agrobacterium strain EHA 101 driven by a 35S promoter (pBECKS$_{2000}$.6::crtl), and has demonstrated for the first time a genetic manipulation of carotenoid biosynthesis in NPT rice. It was found that all Crtl-expressing plants were normal and fertile, and the Crtl gene was inserted into the genome and inherited by the progeny. Carotenoid Analysis of T0 of the Crtl-plants showed that there were dramatic increases in the violaxanthin content and the xanthophyll cycle pool size, although they were not reproduced in the T1 plants. The zeaxanthin contents of T1 plants were almost 50 % higher than that of the control plants and reflected in the higher de-epoxidation state of the xanthophyll cycle pool. These transgenic plants had a more active de-epoxidation reaction, as confirmed by the result of ΔA_{505} measurement, and led to a faster formation of NPQ. It was also observed that Crtl-plants have higher qE, which is the major contributor of NPQ.

Keywords: Crtl, NPT rice, transgenic rice, xanthophyll cycle

A. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops (Biswas, *et al.*, 1998; Liu *et al.*, 1992), which accounts for 35 – 60 % of the calories consumed by 3 billion Asians (Khush, 1997). The population of rice consumers is increasing at the rate of 1.8 % annually (Khush, 1997). If the current level of provision of rice is to be maintained, it will be necessary to increase rice yields by 300 million tonnes p.a. (Elliott, 1995), to achieve the production of 850 million tons, required to feed the additional rice consumers (Borlaug, 1997; Khush, 1997).

In order to meet the world's increasing demand for rice, the International Rice Research Institute (IRRI) has set up "IRRI's strategic plan", which highlighted the rice ideotype called "New Plant Type". The most important characteristic of this NPT rice is a yield potential of 13 – 15 tons/ha, although so far this has not been achieved during field testing, due to an incomplete grain filling problem (Peng *et al.*, 1993).

Yield potential is defined as the grain yield obtained when water, nutrients, or pests do not limit growth. The driving forces in crop yield formation are both a source of carbohydrates and a sink (spikelets) for these carbohydrates (Kropff *et al.*, 1993). The source for grain production is determined by three components: 1) amount of stem reserves allocated to the grains, 2) rate of dry matter production during the grain-filling period, and 3) length of the grain-filling period or growth duration. It is, therefore clear that manipulation of photosynthesis will play a very important role in solving the grain-filling problem for rice production. Increasing the net photosynthetic rate means increasing the source strength for rice grain filling.

Photosynthesis is a process that depends on light as the energy source. The absorption of energy above the prevailing requirement of the plant can lead to photoinhibition and photo-oxidative damage to the plant. The efficient use of solar energy in photosynthesis depends upon the ability of an organism to safely dissipate excess energy (Demmig-Adams and Adams, 1996). Whenever the utilisation and dissipation of energy through photosynthesis, in combination with the photoprotective processes, are insufficient for dealing with the absorbed light, the photosynthetic apparatus may be damaged. Thus, light stress results not from high light *per se*, but rather from an excess of absorbed light beyond that utilised in photosynthesis, which can arise when the ratio of photon flux density (PFD) to photosynthesis is high, due to either the increase of PFD or the decrease of photosynthesis at a constant PFD (Demmig-Adams and Adams, 1992).

Several processes most probably contribute to the survival of plants when they are exposed to excess excitation, which include: the movement of chloroplasts and whole

leaves; leaves may develop greater surface reflectance; and the absorption of excess light within the leaf by screening compounds/pigments other than chlorophyll (Demmig-Adams and Adams, 1992; Demmig-Adams and Adams, 1996b). The harmless and controlled thermal dissipation of excessive energy directly within the photochemical system that involves the de-epoxidised state of the xanthophyll cycle, is one of the most recent discoveries (Demmig-Adams and Adams, 1992; Gilmore *et al.*, 1994). Furthermore, immediate and rapid scavenging of reactive molecules at the site where they are photo-produced and the repair and *de novo* synthesis of oxidised target molecules are also essential for protection (Asada *et al.*, 1998).

In addition to the fact that carotenoids are the most widely distributed class of natural pigments, they also have very important functions: firstly, they act as absorbers of light between 400 – 600 nm for photosynthesis. Secondly, they provide protection against photo-oxidation. Thirdly, they act as 'scaffolding' which plays an essential role in the assembly and stability of LHCII. Furthermore, the carotenoids of the xanthophyll cycle have been implicated in the photoprotective de-excitation of chlorophyll in the light-harvesting pigment bed when light is excessive (Demmig-Adams and Adams, 1992; Kuhlbrandt *et al.*, 1994; Adams and Demmig-Adams, 1995; Cunningham and Gantt, 1999; DellaPenna, 1999; Yamamoto *et al.*, 1999).

The xanthophyll cycle, also known as the violaxanthin cycle, consists of light-dependent inter conversions of three xanthophylls in a cyclic reaction involving a de-epoxidation sequence from the diepoxide violaxanthin via the monoepoxide antheraxanthin to the epoxide-free form zeaxanthin, and an epoxidation sequence in the reverse direction (Figure 1). This xanthophyll cycle is present in thylakoid membranes, with the de-epoxidation occurring on the lumenal side and the epoxidation on the stromal side, in all higher plants, ferns and mosses (Demmig-Adams and Adams, 1992; Demmig-Adams and Adams, 1996; Demmig-Adams *et al.*, 1999; Horton *et al.*, 1999; Yamamoto *et al.*, 1999).

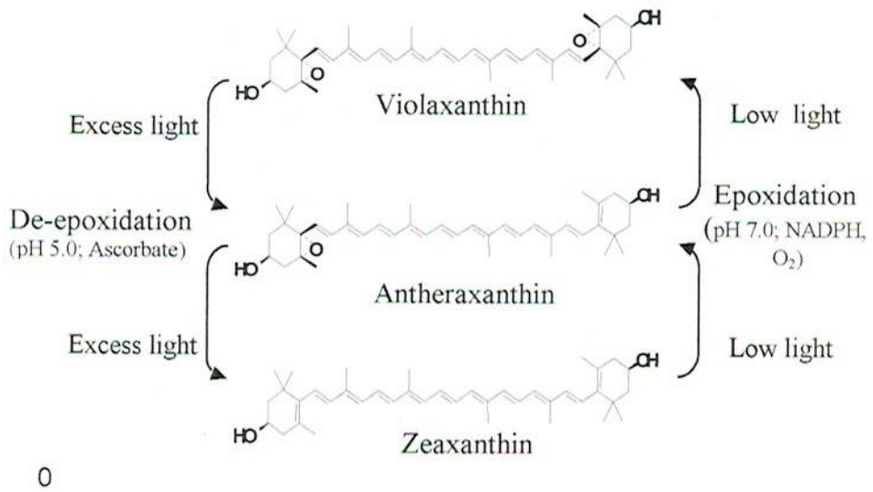


Figure 1. A scheme for the xanthophyll cycle, showing the influence of excess or limiting light and the enzymes involve.

Violaxanthin begins to be converted to antheraxanthin and zeaxanthin at the PFD at which photosynthesis cannot use all of the excitation energy. In other words, the accumulation of zeaxanthin occurs under conditions of excess light and the reconversion of zeaxanthin to violaxanthin occurs upon return to non-excessive light levels (Adams and Demmig-Adams, 1995; Demmig-Adams and Adams, 1992; Demmig-Adams and Adams, 1996; Demmig-Adams *et al.*, 1999).

The de-epoxidation of violaxanthin to zeaxanthin is a step-wise removal of the 5-6 epoxide, catalysed by the enzyme violaxanthin de-epoxidase. This reaction sequence requires a low thylakoid lumen pH, reduced ascorbate and the availability of violaxanthin to the de-epoxidase (Adams and Demmig-Adams, 1995; Demmig-Adams and Adams, 1992; Gilmore and Yamamoto, 1992; Gilmore *et al.*, 1994; Yamamoto *et al.*, 1999). On the other hand, the epoxidation of zeaxanthin to violaxanthin requires O_2, NADPH and has a higher pH optimum than the de-epoxidation reactions (at a pH around 7.5). It occurs in the dark or under dim light after the induction of de-epoxidation and is slow relative to the

forward de-epoxidation (Adams and Demmig-Adams, 1995; Demmig-Adams and Adams, 1992; Gilmore and Yamamoto, 1992; Gilmore *et al.*, 1994; Yamamoto *et al.*, 1999).

B. Materials and methods

General procedure. All transformation and tissue culture works were carried out in a Laminar Air Flow Cabinet under strictly aseptic conditions. All media and equipment used were autoclaved for at least 30 min at 121 °C, 15 lbs/in^2.

The consruct. A co-integrative plasmid of *Agrobacterium* strain EHA101(pBECKS$_{2000}$.6::CrtI) was used during this experiment. This plasmid harboured The coding region of the phytoene desaturase gene (*CrtI*) from *Erwinia uredovora* attached to the transit peptide (*tp*) sequence of the pea rubisco small sub unit, CaMV (cauliflower mosaic virus) *35S* promoter and terminator sequences, the gus A gene with a monocotyledonous chimeric intron from the maize catalase gene as visual marker gene, *npt II* and *hph* genes as selectable marker genes (for detail of the vector construction see Sugiyono, 2001; Sugiyono *et al.*, 2002).

Agrobacterium-mediated transformation of NPT rice. Scutellum-derived-calli of a NPT rice (IR65600–42–5–2) were infected with the *Agrobacterium* EHA101 (pBECKS$_{2000}$.6::CrtI) under fully optimised conditions. The hygromicyn-tolerant-calli were tested for successful transformation by assay of the expression of gus A gene as described in Jefferson, (1987). The putative-transformed-calli were then regenerated and transferred to soil. For the detail of *Agrobacterium*-mediated transformation of NPT rice see Sugiyono, 2001 and Sugiyono *et al.*, 2001.

Carotenoid analysis. Leaf samples were taken from greenhouse-grown plants using cork borer no 4 (1.131 cm in diameter), and stored directly in liquid nitrogen. Leaf samples were extracted with ethanol and diethyl ether, filtered and dried under a steady stream of

N_2. Pigment content and composition were determined using HPLC (Waters) with a 5 μm spherisorb ODS2 column (250 x 4.6 mm; Fischer Scientific Ltd). The data were analysed using Waters Millenium 2010 Chromatography Manager software. The carotenoid content was calculated by integrating the chromatogram at peak wavelengths, 447 nm for lutein, antheraxanthin and *cis* isomers of β-carotene, 455 nm for zeaxanthin, β-carotene and chlorophyll b, 441 nm for neoxanthin, 437 nm for violaxanthin and 431 nm for chlorophyll a. Data for the chlorophylls were adjusted by the values of 1.512 for chlorophyll b and 1.99 for chlorophyll a. The content of each carotenoid was expressed as a percentage of the total carotenoid and the de-epoxidation state (DES) was calculated as Z + (1/2A) / Z + A + V where Z, A, and V are the contents of zeaxanthin, antheraxanthin and violaxanthin, respectively (as described in Ruban *et al.*, 1994).

Absorbance changes measurement. Measurement of the ΔA_{505} was carried out according to Yamamoto *et al.*, (1972) and Ruban *et al.*, (1993). Absorbance changes were measured in an SLM DW2000 spectrophotometer. Leaf pieces (3.0 x 1.5 cm) were inserted in a 1-cm^2 cuvette at 45 ° to the DW2000 light path. An optic fibre, at 90 °, delivered actinic light and the fluorescence measuring beam and collected fluorescence emission. The photomultiplier was protected by a Corning 4-96 filter and an OCL1 Cyan T400–570 mirror. The red actinic light from a 250-W tungsten halogen lamp at 300 μmol PAR $m^{-2}s^{-1}$ was defined by a Corning 5–58 filter. The instrument slit width was 5 nm and the wavelengths used were 505 nm and 565 nm.

C. Results and discussion

Growth of the transgenic plants. All transgenic plants grew normally, showing no symptoms of the dwarfism or albinism. Moreover, all transgenic and tissue culture-derived control plants flowered and set seeds which indicated that sterility did not occur in this work. In contrast, 46 % of the transgenic plants were found to be sterile after particle-

bombardment-mediated transformation of the japonica rice (TP 309) (Burkhardt *et al.*, 1997). The normal growth of the transgenic plants suggests that there were no metabolic disturbances in the plant. Violaxanthin and neoxanthin have long been thought as the precursors of the biosynthesis of the growth regulator ABA (Zeevart and Creelman, 1988; Cunningham and Gantt, 1998). There is evidence to suggest that phytoene desaturase catalyses the regulated step in ABA biosynthesis in many organisms (Hable *et al.*, 1998). In addition, alteration of gibberelin levels was found in previous work on transgenic potato over-expressing phytoene synthase (Fray *et al.*, 1995). Romer *et al.*, (2000) also found no observable morphological difference in transgenic tomato expressing the *Crtl* gene. A normal vegetative phenotype and fertile plants were also found in the work of Ye *et al.*, 2000, who tested 10 plants expressing four introduced carotenoid biosynthesis genes.

Carotenoid content. The phytoene desaturase (*Crtl*) gene produces an enzyme which catalyses the four sequential desaturations of phytoene to form lycopene (Cunningham and Gantt, 1998). Carotenoid composition of primary transformant are shown in Figure 2. In Figure 2.a, it can be seen that *Crtl* expression resulted a very significant increase in violaxanthin content and xanthophyll cycle pool size (Figure 2.b). Two transgenic lines (Lc.12.4 and Lc.12.13.1) in particular, had a remarkable increases in violaxanthin content and xanthophyll cycle pool size, up to 90 % higher than those of the control plant. A significant increase of violaxanthin as a result of *Crtl* introduction was also found in tobacco (Misawa *et al.*, 1994) and in tomato (Romer *et al.*, 2000).

There was also a decrease in the content of β-carotene of the transgenic plants (Figure 2.c). The two lines which had a very high content of violaxanthin and xanthophyll cycle pool size (Lc.12.4 and Lc.12.13.1) showed a dramatic reduction in β-carotene content. Expression of *Crtl* gene in transgenic tobacco also resulted in up to a 4 % increase in the β-carotene content (Misawa *et al.*, 1994). A significant increase in β-carotene was also observed in the leaves of *Crtl* tomato, and this increased reached 3.5-

fold in the fruit (Romer *et al.*, 2000). In contrast, it was found here that the expression of *Crtl* in rice was associated with a reduction of the β-carotene content in the leaf. This suggests that the increases in violaxanthin and neoxanthin contents in rice were at the expense of β-carotene. The total carotenoid content of the *Crtl* plants was also significantly reduced as evidenced from the decrease of the carotenoid:chlorophyll of the *Crtl* expressing plants (Figure 2.d).

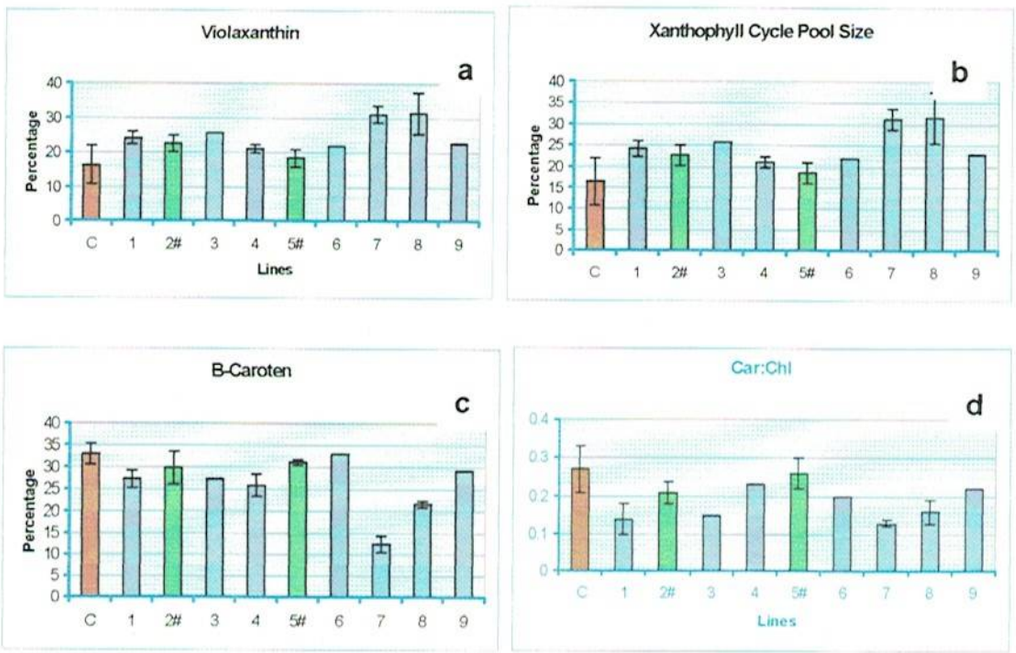


Figure 2. Carotenoid compositions of the control plant and the primary transformants as described in the text; The brown bar is the control plant, the blue bars are the gus A + *Crtl* expressing transgenic plants, the green bars are the gus A positive and *Crtl* (-) transgenic plants; Carotenoid was calculated as the percentage of the total carotenoids. Each bar represents the mean of five replicates from five different plants and the error bars represent the standard error of the means; (a) Violaxanthin; (b) Xanthophyll Cycle Pool Size; (c) β-Carotene; (d) car:chl ratio.

The introduction of *Crtl* gene into tobacco did not change the total carotenoid content of the transgenic plants (Misawa, *et al.*, 1994). However, in the experiments described here, it was found that the total carotenoid was significantly reduced, as

indicated by the decrease in carotenoid:chlorophyll. This finding is consistent with Romer *et al.*, (2000), who also observed a decrease of Car:chl in *Crtl* tomato. It is not clear why an increase in phytoene desaturase activity should lead to a decrease in carotenoid synthesis.

The results of the carotenoid analysis of T1 plants are presented in Figures 3 a-g. It can be seen that there was no significant difference between the control and the transgenic plants in the levels of antheraxanthin (Figure 3.a), carotenoid: chlorophyll ratio (Figure 3.b) and β-carotene content (Figure 3.c),. Moreover, Figure 3.d shows that the violaxanthin contents of the transgenic lines was only slightly higher than that of the control plant. The high violaxanthin content of line Lc12.13.1 in the T0 generation apparently was not maintained in the T1 generations. The carotenoid analysis also revealed that the xanthophyll cycle pool size of the transgenic plants was also only slightly higher than those of the control plant (Figure 3.e).

Although the levels of violaxanthin pool in the transgenic plants were not significantly different, the zeaxanthin content of the transgenic plants after illumination under saturating light in the laboratory were remarkably different and up to 50 % higher than that of the control plants (Figure 3.f.). The high zeaxanthin content of the transgenic plants was reflected in the increase in the de-epoxidation state (Figure 3.g). In addition, it was also found that Lc.12.1 and Lc.12.13.1 lines, which had the highest violaxanthin contents among the transgenic plants, also had the highest zeaxanthin levels.

This high content of zeaxanthin was reflected in the higher de-epoxidation state of the xanthophyll cycle pool. This indicates that these transgenic plants had a more active de-epoxidation reaction, which would result in a more rapid conversion from violaxanthin to zeaxanthin. Measurement of ΔA_{505} confirmed that these plants converted violaxanthin into zeaxanthin more rapidly.

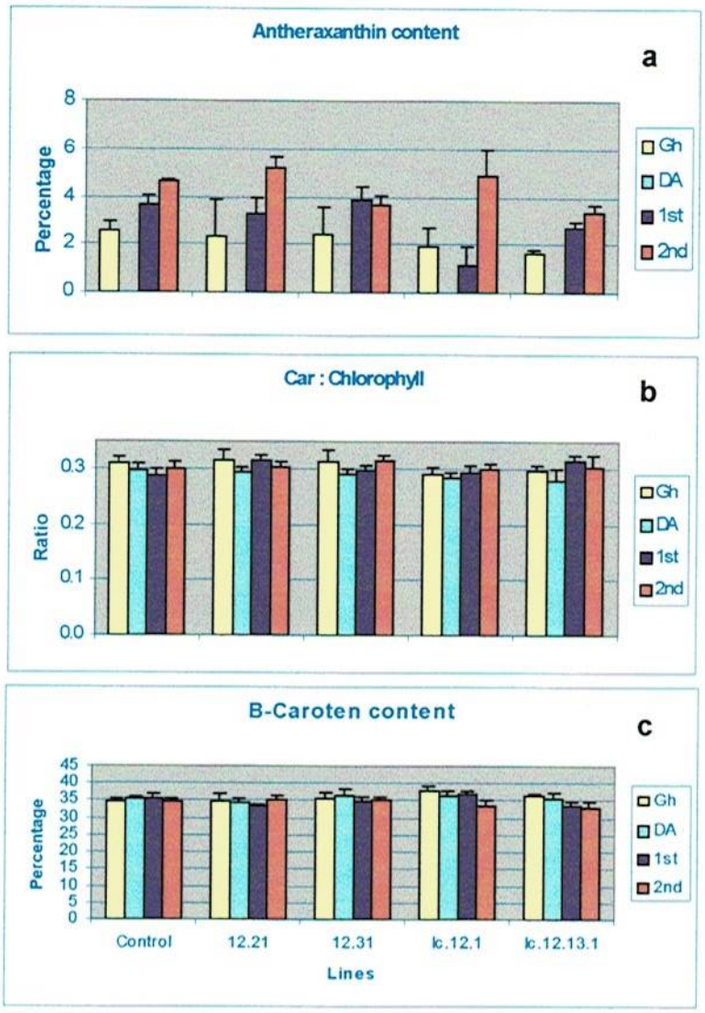
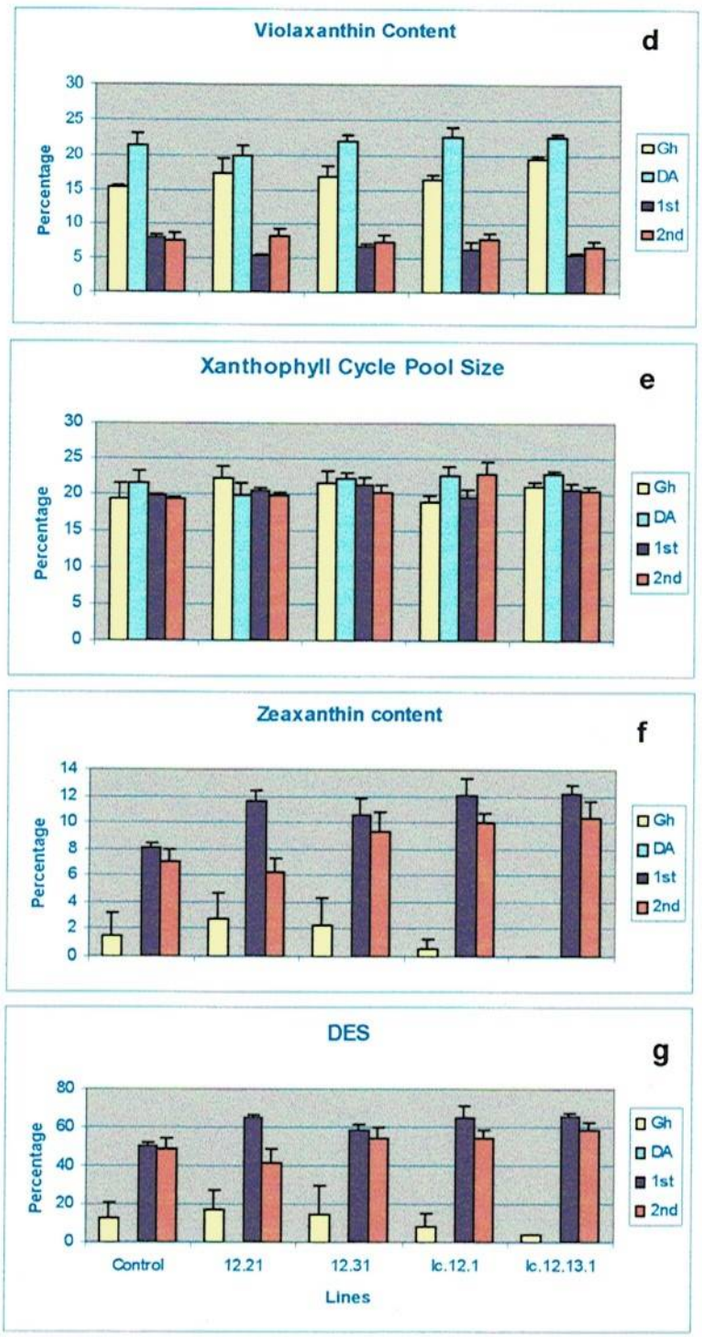


Figure 3. Carotenoid compositions of control and T1 generation of NPT rice, grown in the soil system. Carotenoid was calculated as the percentage of the total carotenoids. Each bar represents the mean of five replicates from five different plants and the error bars represent the standard error of the means; (Gh): Leaf samples were taken from the greenhouse condition; (DA): Leaf samples were taken after dark adaptation; (1^{st} and 2^{nd}): Leaf samples were taken after the first and second periods of saturating light, respectively; (a) antheraxanthin; (b) carotenoids:chlorophyll, (c) β-Carotene; (d) violaxanthin; (e) xanthophyll cycle pool size (f) zeaxanthin; (g) de-epoxidation state (DES).


Violaxanthin Content
d
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25
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Xanthophyll Cycle Pool Size
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25
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15
10
5
0
Gh
DA
1st
2nd
Zeaxanthin content
f
Percentage
14
12
10
8
6
4
2
0
Gh
DA
1st
2nd
DES
g
Percentage
80
60
40
20
0
Gh
DA
1st
2nd
Control
12.21
12.31
lc.12.1
lc.12.13.1
Lines

Figure 3. d-g.

The high zeaxanthin content of the *Crtl* plants may arise for several reasons. Firstly, the introduction of the *Crtl* gene may have produced a "signal" which stimulates the de-epoxidase to convert the violaxanthin to zeaxanthin. Higher de-epoxidation states are a symptom of stress and it is possible that expression of *Crtl* or gus A gene has in some way interfered with normal photosynthesis. Secondly, expression of *Crtl* may have increased the level of violaxanthin de-epoxidase enzyme, which leads to more rapid formation of zeaxanthin. Thirdly, the introduction of the *Crtl* gene may have resulted in the violaxanthin becoming more accessible to the de-epoxidase. This could arise from an alteration in thylakoid organisation affecting violaxanthin distribution. At present the available data does not allow the clear determination of which of these explanations is correct.

Absorbance changes analysis (ΔA_{505}). The higher zeaxanthin contents and DES of some of the transgenic lines may result from altered xanthophyll cycle activity. To test this possibility, the kinetics of zeaxanthin formation were measured using the absorbance change at 505 nm (ΔA_{505}). It can be seen that after illumination there were 505 nm absorbance changes that saturated after around 5 min (Figure 4). The transgenic lines had larger and more rapid absorbance changes than the control plant. Two lines (Lc.12.1 and Lc.12.13.1) which had the highest violaxanthin and zeaxanthin contents showed the largest absorbance changes. It was also found that the transgenic lines, which have higher violaxanthin contents, had faster conversion rates and shorter times to reach the maximum extent of conversion than the control plants.

Fluorescence analysis. The differences in violaxanthin de-epoxidation kinetics were expected to lead to altered kinetics of chlorophyll fluorescence quenching. Fluorescence measurement were carried out on both control and transgenic. The results of some fluorescence parameters measured showed that there was no difference in Fv/Fm between the control and transgenic lines. The expected value of 0.8 was found

and indicated that the plants used in the measurement were in a healthy unstressed condition. There was no difference in the maximum level of non-photochemical quenching (qN), although there was a slight increase in qE in the transgenic plants. It was also found that the transgenic lines had higher qP and slightly higher ϕPSII than the control plants.

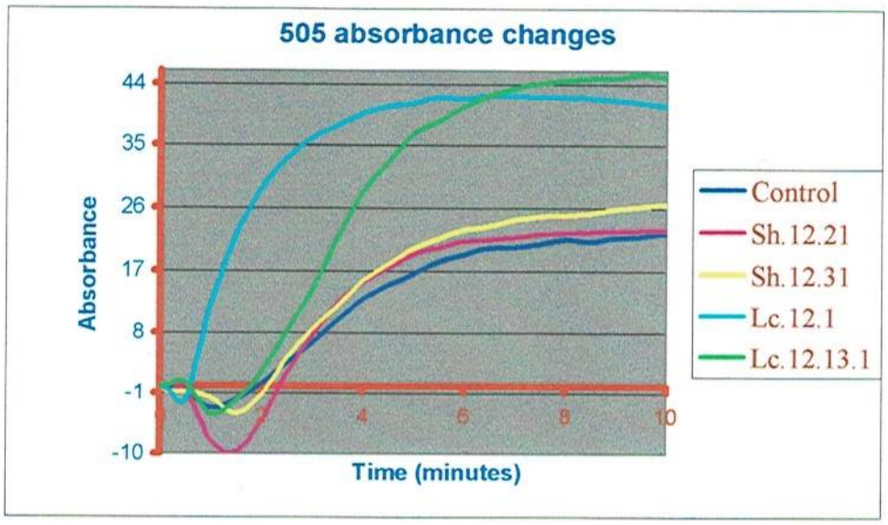

Figure 4. Absorbance at 505 nm – 565 nm (ΔA_{505}) on 10 minutes illumination with 300 μmol PAR m-2s-1 of the control and the T1 plants.

The induction of non-photochemical quenching in the transgenic plants was different to that of the control plants. The amplitude of the initial fast phase was greater in the transgenic plants, although the total amplitude was unchanged. The rate of formation of NPQ was also faster in transgenic lines.

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2nd Circular

IBC

The 3rd Indonesian Biotechnology Conference 2004

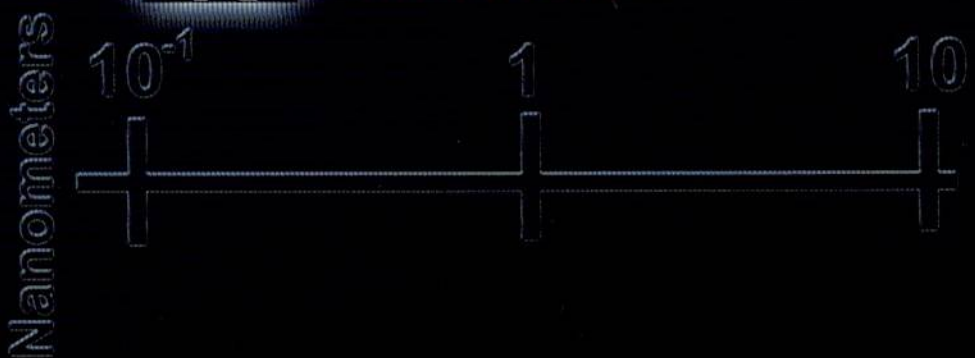
An International Conference and Exhibition

INNA Grand Bali Beach Hotel, Sanur, Bali
December 1-3rd, 2004

"Recent Advances in Biotechnology for Human Health and Food Sustainability"

Hosted by :

Konsorsium Bioteknologi Indonesia



Message from the Organizing Committee

First of all, we would like to apologize for delaying the 3rd Indonesian Biotechnology Conference 2004 (IBC 2004) from 7- 9th October to 1 – 3rd December 2004. The IBC 2004 will be held at the INNA Grand Bali Beach Hotel, Sanur, Bali, with the theme of "Recent Advances in Biotechnology for Human Health Food Sustainability".

IBC 2004 which is organized and hosted by the Indonesian Biotechnology Consortium, will have a scientific conference and an exhibition of biotechnology related products and technologies. The Conference is expected to be an ideal opportunity to promote scientific communication among scientists, industries, government employees and workers in the field of biotechnology, and to commercialize proven technology to private sectors. Several topics will be discussed during the Conference, including

1. Agricultural and Food Biotechnology,
2. Marine and Fishery Biotechnology,
3. Medical, Pharmaceutical, and Nutraceutical Biotechnology,
4. Industrial and Environmental Biotechnology,
5. Bio-Energy.

The discussion of the topics will be focused on the aspects of Genomic-Proteomic, Genetic Engineering, Bioprocess, and Bioinformatics.

The Conference will be organized into three main sessions. First, the Plenary Session, at which more than twenty invited Senior Scientists from Indonesia, Singapore, Japan, Germany, Australia, the Netherlands and the USA, will present their outstanding scientific papers on different aspects of biotechnology. The second is the Symposium at parallel session, and the third is the Contributing Paper Sessions, which will also be held in parallel according to the main topics. The Conference will also have Poster Sessions.

The Indonesian Biotechnology Consortium wishes to invite scientists from Research Institutions, Universities, Professional Organizations, and Companies, to participate actively in the exciting program of IBC 2004. We do wish you and your associates could join this event as Conference participants or exhibitors.

We look forward to welcoming you at IBC 2004 in Bali.

Important Conference Schedules

Conference and Exhibitions dates	December 1st - 3rd, 2004
Deadline for abstract submission	October 1st 2004
Deadline for complete manuscript submission	December 1st 2004
Notification of abstract acceptance	October 20th 2004
Registration deadline for discounted Registration-fee	November 10th2004
No reimbursement of fees after this deadline	November 10th2004

IBC2004 Secretariates :

IBC2004 Organizing Committee

Office of the Vice Rector for International Affair (PR-IV)
Udayana University, Kampus Bukit Jimbaran
Denpasar, Bali, INDONESIA.
Phone : (62-361) 704625; (62-811) 387389; (62-818) 380205
Facs. : (62-361) 704625 ; (62-341) 560011
E-mail : IBC2004@brawijaya.ac.id

Conference Programs

Tuesday, November 30th, 2004

08.30 am – 07.00 pm : Conference Registration and Exhibition
07.00 pm – 09.00 pm : Welcoming Party and KBI Meeting

Wednesday, December 1st, 2004

08.00 am – 09.00 am : Conference Registration
09.00 am – 10.00 am : Opening Ceremony
10.00 am – 10.30 am : Coffee Break
10.30 am – 11.00 am : Plenary Session I : Chairman, the Indonesian Biotech Consortium
11.00 am – 11.30 am : Plenary Session II : Genomic -Proteomic
11.30 am – 12.00 am : Plenary Session III : Bioinformatics
12.00 am – 01.30 pm : Lunch
01.30 pm – 02.00 pm : Plenary Session IV : Genetic Engineering
02.00 pm – 02.30 pm : Plenary Session V : Bioprocess
02.30 pm – 03.00 pm : Plenary Session VI : Economical Aspect of Biotechnology
03.00 pm – 03.30 pm : Coffee Break
03.30 pm – 04.15 pm : Parallel Session I
04.15 pm – 05.00 pm : Parallel Session II
07.00 pm – 10.00 pm : Dinner and Cultural Performances

Thursday, December 2nd, 2004

08.30 am – 09.00 am : Plenary Session VII : Genomic-Proteomic
09.00 am – 09.30 am : Plenary Session VIII : Bioinformatics
09.30 am – 10.00 am : Plenary Session IX : Genetic Engineering
10.00 am - 10.30 am : Coffee Break
10.30 am – 11.15 am : Plenary Session X : Bioprocess
11.00 am – 11.30 am : Plenary Session XI : Genomic-Proteomic
11.30 am – 12.00 am : Plenary Session XII : Bioinformatics
12.00 am – 01.30 pm : Lunch
01.30 pm – 02.15 pm : Parallel Session III
02.15 pm – 03.00 pm : Parallel Session IV
03.00 pm – 03.30 pm : Coffee Break
03.30 pm – 04.15 pm : Parallel Session V
04.15 pm – 05.00 pm : Parallel Session VI

Friday, December 3rd, 2004

08.30 am – 09.00 am : Plenary Session XIII : Genetic Engineering
09.00 am – 09.30 am : Plenary Session XIV : Bioprocess
09.30 am – 10.00 am : Plenary Session XV : Genomic-Proteomic
10.00 am - 10.30 am : Coffee Break
10.30 am – 11.15 am : Parallel Session VII
11.15 am – 12.00 am : Parallel Session VIII
12.00 am – 02.00 pm : Friday Pray and Lunch
02.00 pm – 03.00 pm : Parallel Session
03.00 pm – 03.30 pm : Coffee Break
03.30 pm – 04.00 pm : Plenary Session XVI : Bioinformatics
04.00 pm – 04.30 pm : Plenary Session XVII : Genetic Engineering
04.30 pm – 05.00 pm : Plenary Session XVIII : Bioprocess
05.00 pm – 05.15 pm : Announcement of the best Presentation and Poster Awards
05.15 pm – 05.30 pm : Closing Ceremony

Invited Speakers:

1. Anthony Gendall, Dr. (La Trobe University, Australia)
2. Biswarup Mukhopadhay, Prof. (Virginia Technology, USA)
3. Djoko Santosa, Dr. (Central Res. Inst. For Biotechnology of Estate Crops, Indonesia)
4. Harianto Solichin (PT. NIKKO Securities)
5. Hideaki Kandori, Prof. (Nagoya Institute of Technology, Japan)
6. Hideaki Nojiri, Ph.D. (University of Tokyo, Japan)
7. Hiroshi Sano, Ph.D. (AsiaSEED, Japan)
8. Hubb Lofler, Dr. (The Netherlands)
9. K. Kawakita, Prof. (Nagoya University, Japan)
10. Ken-ichi Arai, Prof. (The Tokyo Metropolitan Institute of Medical Sciences, Japan)
11. Kenzo Iwao, Prof. Dr. (Nagoya Institute of Technology, Japan)
12. Mazayuki Nozue, Prof. (Shinzu University, Japan)
13. Mineo Kojima, Prof. (Shinshu University, Japan)
14. Nengah Sujaya, Ph.D. (Udayana University, Indonesia)
15. Nobuyuki Uozumi, Prof. (Nagoya, University, Japan)
16. Naomichi Nishio, Prof. Dr. (Hiroshima University, Japan)
17. P. Patrick Cleary, Prof. (University of Minnesota, Minneapolis, USA)
18. Sangkot Marzuki, Prof. (Eijkman Research Institute, Indonesia)
19. Sekiguchi, Prof. (Shinshu University, Japan)
20. Shinji Tuyumu, Prof. (Shizuoka University, Japan)
21. Shugo Nakamura, Ph.D. (Tokyo University, Japan)
22. Tan Tin Wee, Prof. (Singapore National University, Singapore)
23. Tim Hirst, Prof. (Sydney University, Australia)
24. Toshio Omori, Prof. (Shibaura Institute of Technology, Tokyo, Japan)
25. Yasunobu Matsumoto, Prof. Dr. (Tokyo University, Japan)
26. Yuko Saiki, Ph.D. (University of Tokyo, Japan)
27. Winfried Storhas, Prof. (Mannheim University of Applied Sciences, Germany)

Steering Committee IBC 2004

Chairman : A. Syaifuddin Noer, Ph.D.

Member :

- Prof. Ir. Joedoro Soedarsono, Ph.D.
- Prof. dr. Abdul Salam M. Sofro, Ph.D.
- Prof. Dr. Ir. Wisjnuprapto
- Ir. Basuki, M.Sc.
- Dr. Ir. Koesnandar, MEng.
- Dr. Ir. A. Machmud Thohari, DEA.
- Dr. Ir. A. Dimyati, MSc.
- Dr. Usep Sutisna
- Dr. Wahono Soemaryono, APU
- Prof. Ir. I.G.P. Wirawan, Ph.D
- Debbie Sofie Retnoningrum, Ph.D
- Ir. Edwan Kardena, Ph.D.

Organizing Committee IBC 2004

- Chairman : Prof. Ir. I.G.P. Wirawan, Ph.D
- Secretary : Prof. Ir. Liliek Sulistyowati, Ph.D.
- Treasurer : Ir. I.M. Sritamin, MS.
- Member :
  - Prof. Dr.dr. Djanggan Sargowo, Sp.PD, SpJP (K)
  - Prof. Dr. dr. I. Ketut Suastike, SpPD,KE.
  - Ir. Bambang Sugiharto, Ph.D.
  - Ir. I.N. Wijaya, Ph.D.
  - Ir. I.N. Arya, PhD.