PHOTOSYNTHETICA 46 (3): 363-369, 2008

Effect of gamma radiation on mutant induction of *Fagopyrum dibotrys* Hara

C.F. JIA and A.L. LI*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences &Peking Union Medical College, 100094, Beijing, P.R. China

Abstract

Agronomic traits, photosynthetic pigments, gas exchange, and chlorophyll (Chl) fluorescence parameters of red stem buckwheat (*Fagopyrum dibotrys* Hara) mutants induced by γ-radiation were compared with green control at seedling stage. Plant height, number of first-class branches, and rhizome biomass were inhibited significantly ($p<0.01$). Chl *a*, Chl *b*, and Chl *a+b* contents decreased with elevated dose of γ-rays, while increasing carotenoid content indicated that buckwheat was capable of adjusting to the radiation damage. Decrease in net photosynthetic rate was the result of both stomatal and non-stomatal limitations. Fluorescence parameters, such as F_0, F_m, F_v/F_m, F_v/F_0, Φ_{PS2}, electron transport rate, and photochemical quenching declined significantly ($p<0.01$) as compared with control due to photoinhibition, while non-photochemical quenching increased to enhance thermal dissipation. Lower parameters implied that leaf tissue was damaged significantly by high dose of γ-radiation and therefore leaf senescence was accelerated.

Additional key words: agronomic traits; buckwheat; carotenoids; chlorophyll; ^{60}Co; gas exchange; quenching; stomatal and non-stomatal limitations.

Introduction

Radiation mutagenesis has gained rapid development since discovery of high mutant frequency in *Drosophila melanogaster* by X-rays (Muller 1927). A large number of energy rays is frequently applied in mutant breeding, such as X-, β-, and γ-rays, neutrons, and protons. Gamma rays belong to the most efficient tools to create mutants in plants, with the advantages of convenient operation, short cycle, and high mutation quantity (Datta *et al*. 2001, Rajadurai 2001, Naito *et al*. 2005, Sato *et al*. 2006, Eroglu *et al*. 2007, Selvi *et al*. 2007).

Buckwheat [*Fagopyrum dibotrys* (D. Don.) Hara], an erect perennial herb of Polygonaceae, is one of the national key conservative wild plants in China (the first group), sanctified by the State Council of China in 1999. The rhizome of buckwheat is a traditional folk medicine for treatment of lung abscess, dysentery, rheumatism, and tumefaction. Buckwheat propagates mainly by asexual mode because of self-incompatibility, inhibiting the development of new cultivars through crossing. So it becomes urgent to create new genotypes to expand the genetic diversity of buckwheat for improving germplasm resources.

Chlorophyll (Chl) fluorescence as a rapid and non-invasive indicator of photochemical quantum yield and photoinhibition of photosynthetic system in plants has been applied widely in stress responses, mutant selection, and crop monitoring (Papageorgiou 1975). The PAM fluorometer has made great contribution to selection and mutant breeding (Schreiber 1983, Niyogi *et al*. 1998, Varotto *et al*. 2000).

Quenching analysis of fluorescence is an efficient way to test cold and water tolerance in maize hybrid breeding (Fracheboud *et al*. 1999, O'Neill *et al*. 2006). *Oryza sativa* breeding was monitored by actual photochemical efficiency of photosystem 2, Φ_{PS2} and electron transport rate, ETR (Murchie *et al*. 1999).

Received 6 February 2008, *accepted* 30 April 2008.

*Corresponding author; fax: +86-10-62896810, e-mail: alli@implad.ac.cn

Abbreviations: Chl – chlorophyll; Car – carotenoids; PS2 – photosystem 2; ETR – electron transport rate; F_0 – minimum fluorescence; F_m – maximum fluorescence; F_v – variable fluorescence; F_v/F_m – maximum photochemical efficiency; LHCP – light-harvesting chlorophyll-protein complexes; PS – photosystem; q_P, NPQ – coefficients of photochemical and non-photochemical quenching; Φ_{PS2} – actual photochemical efficiency of PS2.

Acknowledgements: We express our sincere thanks to Drs. Song and Xiong for critical reading of the manuscript. Comments from two anonymous reviewers and editorial changes provided by the editors of Photosynthetica were helpful in revising earlier version of the paper.

Eight cultivars of *Pisum sativum* were classified using fluorescence induction curves into three classes according to their resistance against drought stress: resistant, intermediate, and sensitive (Maldonado-Rodriguez *et al*. 2003). Fluorescence screening in offsprings of rice hybrids and cotton heterosis was employed by Chinese researchers (Tang *et al*. 2005, Wang *et al*. 2005, Zhang *et al*. 2006).

To our knowledge, only few researches of photosynthesis characteristics of medicinal plants mutated by γ-rays were reported. In order to analyze the variance of new phenotype of buckwheat and interpret the adaptation mechanism to radiation, evaluation of plant growth, pigment contents, photosynthetic gas exchange, and Chl fluorescence parameters was performed in red mutant and green control seedlings by exposure to different dosage of γ-rays. Such investigations should help in new cultivar selection and improve buckwheat breeding.

Materials and methods

Plants: Experiment was carried out at the research station, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College (39°47'N, 116°25'E, altitude 50 m), belonging to temperate continental climate, average temperature 8–12 °C with annual precipitation of 0.6 m. The irradiated cv. of buckwheat Jiangsull with green stem has been introduced in Beijing region long time ago. Young rhizomes of perennial buckwheat were cut into segments with 6–8 shoots and exposed to radiation at rate of 2.05 Gy min^{-1} by ^{60}Co in γ-chamber, Institute for Application of Atomic Energy, Chinese Academy of Agricultural Sciences, Beijing in April 2006. Segments were planted immediately in field after radiation at row and individual space of 0.6×0.4 m. M_1 rhizomes of buckwheat were harvested in autumn and replanted in field next year. Red stem buckwheat mutants were selected from 5, 10, 15, and 20 Gy of radiation treatment, respectively, and compared with the green control in agronomic traits, pigment contents, gas exchange, and Chl fluorescence parameters.

Agronomic traits: Plant height and number of first-class branches were measured in the field after plantlets emergence in May. Young and robust branches of plantlets were prepared for cutting in June, harvested and dried in a 50 °C oven for rhizome biomass evaluation in October.

Gas exchange: In the third mature leaf on the upper robust branches net photosynthetic rate (P_N), stomatal conductance for water (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were determined in field by a portable infrared photosynthesis system *LI-6400* (*LI-COR*, Lincoln, USA) at 09:30–11:30 on a sunny day of May in open-circuit mode. Photosynthetic photon flux density (PPFD) and leaf temperature were maintained at 800 μmol m^{-2} s^{-1} and 25 °C, respectively. Water use efficiency (WUE) was calculated according to Penuelas *et al*. (1998).

Chl fluorescence was measured by the *LI-6400* system with a fluorescence chamber (*LI-COR*, Lincoln, USA) on the same leaf as gas exchange. F_0 and F_m were determined in predawn after a night dark adaptation, and F_v/F_m was calculated ($F_v = F_m - F_0$). Samples were continuously irradiated by "actinic light" varying with the outdoor conditions for quenching measurement. Φ_{PS2} and ETR (Genty *et al*. 1989), q_P (Bilger and Schreiber 1986), and NPQ (Bilger and Björkman 1990) were calculated.

Photosynthetic pigments were determined in the same leaf just after gas exchange and fluorescence measurements. 0.2 g leaf discs (0.01 m diameter) were ground in 80 % acetone and the homogenate was centrifuged at 1 000×*g* for 5 min. Absorbance of supernatant was determined at 663, 646, and 470 nm by a spectrophotometer *UV-2550* (*Shimadzu*, Kyoto, Japan). Chl and carotenoid (Car) contents were calculated according to Lichtenthaler (1987).

Statistical analyses were performed in *Excel* and *SAS V8* (*Statistical Analysis System*), differences between means of treatments were compared by ANOVA and LSD test at the 95 % level ($p<0.05$). Each data point was mean of three replications ($n = 3$) except for the rhizome biomass measurement ($n = 10$). Correlation analysis was done among the photosynthetic parameters and between these parameters and rhizome biomass of buckwheat cuttings.

Results

Agronomic traits: Weak emergence, less branches, red stem and petiole, and smooth leaf with red veins were typical for mutants in comparison with control rough leaves with green veins. Plant growth of red mutants was inhibited by γ-radiation significantly as compared with green control ($p<0.01$). Number of first-class branches, plant height, and rhizome biomass were reduced correspondingly; especially the latter presented negative correlation to γ-ray dose. Plant height in low dose (5 or 10 Gy) decreased significantly compared to high dose ($p<0.01$), respectively, but number of first-class branches and rhizome biomass did not show significant differences (Table 1).

Table 1. Agronomic traits of red buckwheat seedlings affected by γ-radiation. *Different letters* within parameter indicate significant differences for $p<0.01$.

γ-ray dose [Gy]	Plant height [m]	Number of branches	Rhizome biomass [g]
0	0.72±0.03 a	16.7±1.76 a	64.48±4.82 a
5	0.50±0.01 b	8.33±0.88 b	54.73±2.45 ab
10	0.49±0.01 b	8.00±1.00 b	52.46±3.05 ab
15	0.41±0.01 c	8.33±1.86 b	49.37±3.65 b
20	0.39±0.01 c	7.00±0.58 b	47.63±3.32 b

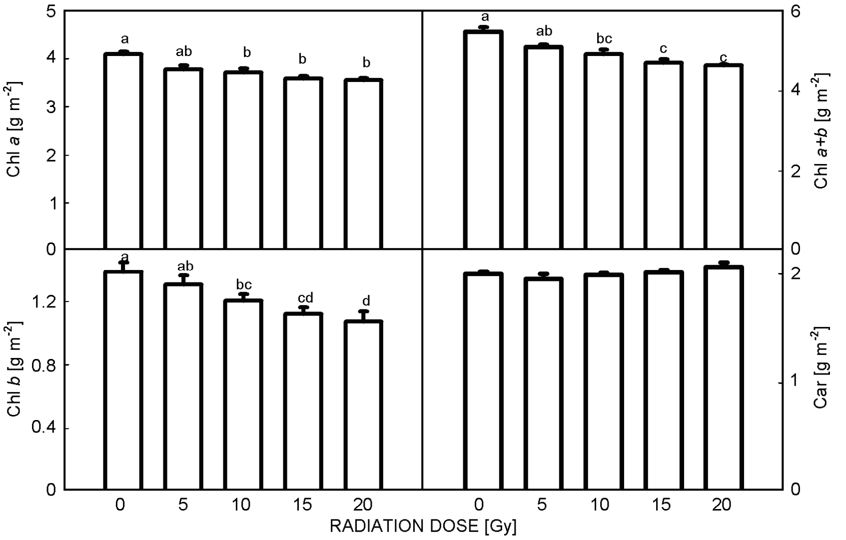

Fig. 1. Contents of chlorophyll (Chl) *a*, *b*, and *a*+*b* and carotenoids (Car) of red buckwheat seedlings. *Different letters* within parameter indicate significant differences for $p<0.01$.

Table 2. Gas exchange parameters of red buckwheat seedlings affected by γ-radiation. *Different letters* within parameter indicate significant differences for $p<0.01$.

γ-ray dose [Gy]	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mol(H$_2$O) m^{-2} s^{-1}]	C_i [μmol(CO$_2$) m^{-2} s^{-1}]	E [μmol(H$_2$O) m^{-2} s^{-1}]	WUE
0	20.50±0.55 a	1.061±0.040	303.70±1.20 b	4.80±0.32	4.27±0.35 a
5	16.10±0.49 b	1.023±0.110	316.00±4.93 a	5.04±0.48	3.19±0.21 b
10	15.80±0.54 b	0.998±0.040	312.70±3.53 ab	5.07±0.13	3.12±0.04 b
15	15.70±0.67 b	0.984±0.040	310.70±1.76 ab	5.10±0.09	3.08±0.14 b
20	15.30±0.62 b	0.930±0.080	307.30±3.28 ab	5.13±0.14	2.98±0.04 b

Photosynthetic pigments: With increasing γ-ray dose, contents of Chl *a* and Chl *b* were lower in red mutants as compared with control ($p<0.01$) (Fig. 1). Car content decreased at low dose, but increased with enhancing γ-ray dose.

Gas exchange: P_N and WUE of red mutants were reduced as compared with control ($p<0.01$), while C_i and E were enhanced. E increased but C_i and g_s decreased in correspondence with γ-radiation (Table 2). There was no significant difference in gas exchange parameters among radiation treatments.

Chl fluorescence: F_0, F_m, F_v/F_m, F_v/F_0, Φ_{PS2}, and q_P of red mutants declined significantly as compared with control ($p<0.01$). ETR was without difference, but NPQ value increased with increasing γ-ray dose (Fig. 2). F_m, F_v/F_m, and F_v/F_0 in low dose were significantly higher than in high dose ($p<0.01$), F_0 and ETR differed between doses ($p<0.05$), but there was no difference in Φ_{PS2}, q_P, and NPQ.

Correlation among photosynthetic parameters and rhizome biomass: There were significant positive correlations between Chl *a* content and P_N, F_0, F_v/F_m ($p<0.05$), between Chl *b* content and F_0, F_v/F_m ($p<0.01$), between Chl *a*+*b* and F_0, F_v/F_m ($p<0.01$), respectively. Car content was negative correlated with F_v/F_m and F_0 ($p<0.01$).

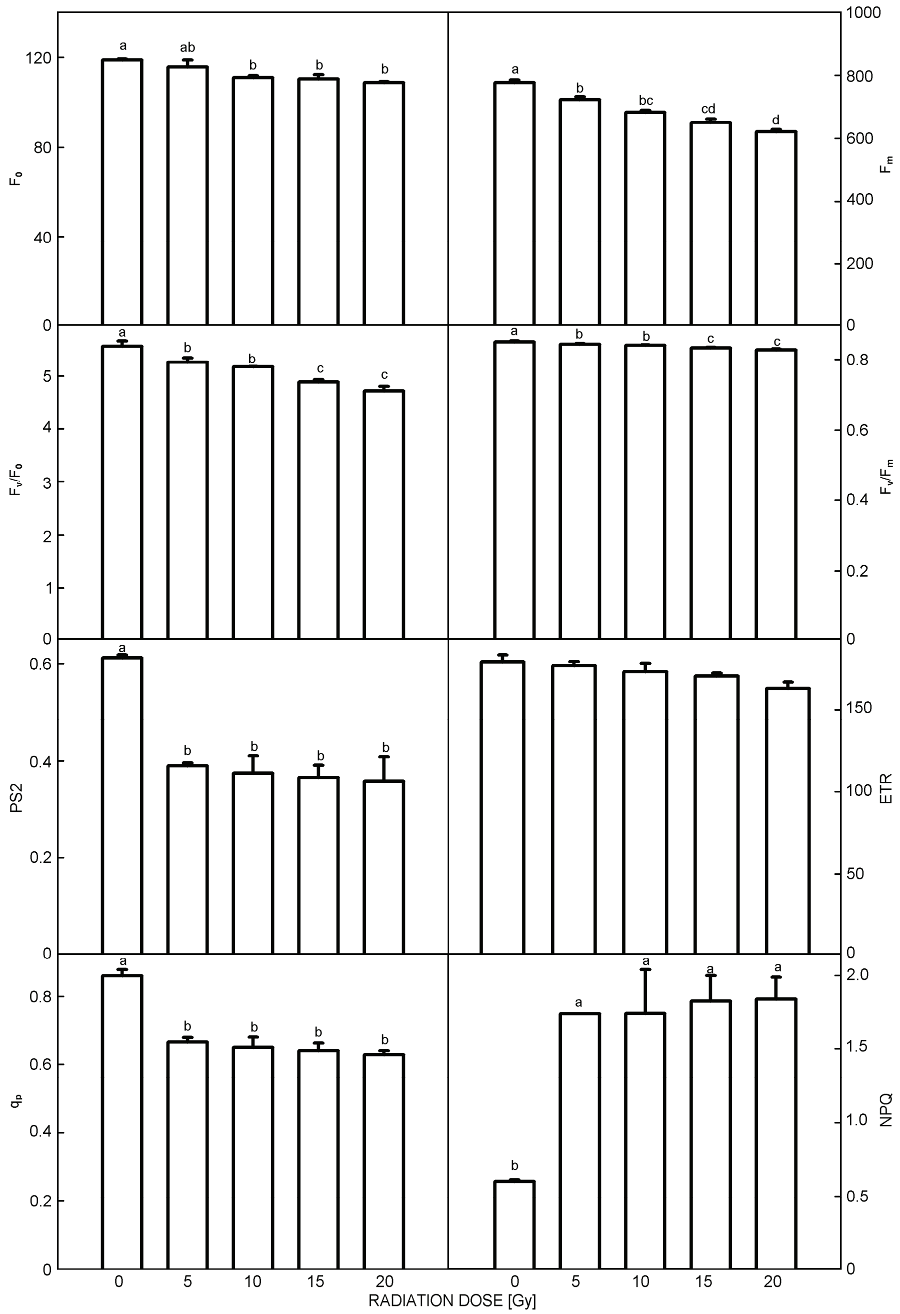

Fig. 2. Chlorophyll fluorescence parameters F_0, F_m, F_v/F_m, F_v/F_0, PS2, ETR, q_P, and NPQ of red buckwheat seedlings. *Different letters* within parameter indicate significant differences for $p<0.01$.

Table 3. Correlation coefficients (r_{xy}) among photosynthetic parameters and rhizome biomass of red buckwheat cuttings ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

Parameter	P_N	F_0	F_v/F_m	Rhizome biomass
Chl *a*	0.9471^{*}	0.9433^{*}	0.9371^{*}	0.9979^{***}
Chl *b*	0.8014	0.9720^{**}	0.9861^{**}	0.9418^{*}
Chl *a*+*b*	0.9081^{*}	0.9649^{**}	0.9677^{**}	0.9914^{**}
Car	–0.1605	–0.5960	–0.6913	–0.4271
Rhizome biomass	0.9541*	0.9476^{*}	0.9378^{*}	–

Moreover, P_N was determined mainly by Chl *a* content, while F_0 and F_v/F_m by Chl *b* content. Hence lower photosynthetic parameters might be induced by reducing Chl content by γ-radiation (Table 3).

There were significant positive correlations among Chl *a* ($p<0.001$), Chl *a+b* ($p<0.01$), Chl *b*, P_N, F_0, F_v/F_m ($p<0.05$), and rhizome biomass of buckwheat cuttings except for Car content. Factors arrayed according to their contribution to rhizome biomass were Chl *a*>Chl *a+b*> P_N>F_0>Chl *b*>F_v/F_m>Car. Chl *a* content showed the most important correlation with rhizome biomass of buckwheat cuttings ($r = 0.9981$).

Discussion

Agronomic traits, pigments contents, gas exchange, and Chl fluorescence of buckwheat were significantly affected by stem phenotype (green and red). All parameters of red mutants were reduced significantly by γ-radiation compared with control.

Sharp reduction in growth of red stem buckwheat mutants, as reversed obviously to increased dry mass of soybean (Maghraby 1987) and secondary metabolite of *Lithospermum erythrorhizon* (Chung *et al*. 2006), may be the cause of slow cell division, lower hormone synthesis, abnormal nutrimental transportation, and metabolic disorders by apical meristem damage under γ-radiation (Celso and Maria 1992, Okamoto and Tatara 1995).

Loss of Chl contents indicated that Chl accumulation was inhibited by γ-radiation, which improved activities of chlorophyllase, promoted degradation of Chl, and ultimately reduced photosynthetic activity of plants (Chowdhury and Johri 2003). Moreover, excessive accumulation of active oxygen caused the damage of chloroplast membrane and accelerated the decomposition of Chl (Takeuchi *et al*. 1996). Chl *a* was more sensitive to oxygen than Chl *b*. However, damage of Chl *b* was more serious in this experiment; this implied that LHCP of PS2 was destroyed severely by γ-radiation, because Chl *b* is present in LHCP of plant chloroplasts (Brown *et al*.1975, Thornber 1975).

Though photosynthetic efficiency and biomass were reduced significantly, plants could acclimate to the variable environment through protective mechanism against over-destruction of the PS2 reaction centres and excessive loss of photosynthetic productivity. LHCP had decreased Chl content through increasing Chl *a/b* (Anderson and Aro 1994). Lower Chl content and higher Chl *a/b*, as a radiation-protection mechanism, might reduce the capture of solar energy and protect the photosynthetic organization against risk of oxidation damage by irradiation. At the same time, membrane lipid peroxidation could be prevented by higher Car content; Car function both as photosynthetic pigments and endogenous antioxidants, through absorbing surplus energy and quenching active oxygen in addition to protecting Chl by absorption of photon energy (Sakaki *et al*. 1983, Fukuzawa *et al*. 1998). Furthermore, plants adapt to environment more widely through high photon energy use efficiency by increasing the proportion of Chl *a* in reaction centres involved in photosynthesis.

Photosynthetic capacity of buckwheat fell down, while in *Capsicum annuum* it was accelerated by low dose of γ-radiation (Lee *et al*. 2002). Whether stomatal or non-stomatal limitation is the critical factor in reducing P_N could be judged by the changing patterns of both C_i and g_s (Farquhar and Sharkey 1982). If both C_i and P_N decreased with a lowering of g_s, the reduction of P_N was caused mainly by stomatal limitation, otherwise by non-stomatal limitation when C_i increased or was steadily accompanied by low P_N and g_s. According to the above theory, the photoinhibition of buckwheat leaves was synthetically affected by stomatal and non-stomatal limitation. On one hand, stomata closure and g_s reduction due to rapid transpiration and excessive loss of water restricted the CO_2 amounts that entered plant leaves through lower C_i, which finally inhibited photosynthesis by a lack of photosynthetic substrate. On the other hand, P_N still decreased with higher C_i owing to lower g_s, as a result of limited carboxylation efficiency and CO_2 assimilation due to the excessive accumulation of active oxygen in mesophyll cells by irradiation (Caemmerer and Farquhar 1981). Moreover, energy surplus of PS2 and damage of reaction centre might result from the imbalance of PS2 photochemical activity and photosynthetic electron demand for limited CO_2 assimilation (Mittler 2002, Colom and Vazzana 2003).

F_0 represents the basal emission of Chl fluorescence when redox components of photosystem are fully oxidized (Ierna 2007). F_v/F_m is a useful ratio proportional to the quantum yield of PS2 photochemistry and exhibits a high correlation with the quantum yield of net photosynthesis, whose descending value represents the photoinhibition of leaf by stress (Butler and Kitajima 1975, Demmig *et al*. 1987). F_0 and F_v/F_m are practical means to discriminate between phenotypes in buckwheat under field conditions. F_0, F_m, F_v/F_m, F_v/F_0, Φ_{PS2}, ETR, and q_P decrease in red stem buckwheat mutants indicated an increasing fraction of closed PS2 reaction centres, which was protected by enhancing thermal dissipation of excess excitation energy *via* non-radiative mechanisms due to xanthophyll cycle and inhibition of CO_2 fixation (Demmig *et al*. 1987, Guidi *et al*. 1997).

In summary, we found that the strategy of Chl fluorescence was efficient in buckwheat affected by γ-radiation. Leaf tissue was damaged significantly by high dose of radiation and therefore leaf senescence was accelerated. These results not only provide a link between Chl fluorescence and radioactive breeding, but also advocate the importance of photosynthetic pigment contents in plant defence responses.

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