Characterization and mapping of novel chlorophyll deficient mutant genes in durum wheat

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The yellow-green leaf mutant has a non-lethal chlorophyll-deficient mutation that can be exploited in photosynthesis and plant development research. A novel yellow-green mutant derived from Triticum durum var. Cappelli displays a yellow-green leaf color from the seedling stage to the mature stage. Examination of the mutant chloroplasts with transmission electron microscopy revealed that the shape of chloroplast changed, grana stacks in the stroma were highly variable in size and disorganized. The pigment content, including chlorophyll \(a\), chlorophyll \(b\), total chlorophyll and carotene, was decreased in the mutant. In contrast, the chla/chlb ratio of the mutants was increased in comparison with the normal green leaves. We also found a reduction in the photosynthetic rate, fluorescence kinetic parameters and yield-related agronomic traits of the mutant. A genetic analysis revealed that two nuclear recessive genes controlled the expression of this trait. The genes were designated \(ygld1\) and \(ygld2\). Two molecular markers co-segregated with these genes. \(ygld1\) co-segregated with the SSR marker wmc110 on chromosome 5AL and \(ygld2\) co-segregated with the SSR marker wmc28 on chromosome SBL. These results will contribute to the gene cloning and the understanding of the mechanisms underlying chlorophyll metabolism and chloroplast development in wheat.

Key Words: durum wheat, yellow-green leaf mutant, genetic mapping, agronomic traits.

Introduction

Chlorophyll (Chl) is a vital biomolecule that sustains the life processes of all plants. Chl plays a critical role in photosynthesis by absorbing light and transferring light energy to the reaction centers of the photosynthetic system. Thus, Chl is essential for plant development and agricultural production (Eckhardt et al. 2004, Flood et al. 2011). The phenotypes of leaf color mutants are varied and are affected by different genetic and environment factors. Among the numerous leaf color mutants, the yellow-green leaf color (chlorina) mutant is a special phenotype. The physiology and genetic mechanisms of this mutant are distinct from those of the other leaf color mutants. Yellow-green leaf color mutants have been identified in many high er plants, such as Arabidopsis thaliana (Jarvis et al. 2000, Liu et al. 2003), rice (Jung et al. 2003, Moon et al. 2008), barley (Bellemare et al. 1982, Preiss and Thornber 1995), maize (Asakura et al. 2008, Mei et al. 1998), sunflower (Mashkina and Gus’kov 2002) and wheat (Falbel et al. 1996, Giardi et al. 1995, Hui et al. 2012). The effects of these mutations on chloroplast development, photosynthesis, chlorophyll \(b\) accumulation and the levels of light-harvesting chlorophyll \(a/b\) proteins (Kosuge et al. 2011) have been examined. However, the rate of natural mutation is an estimated \(10^{-5}\)–\(10^{-9}\) in higher plants and is thus notably low. Artificial methods have been applied to obtain these precious mutant resources. Radiation, for example, is an effective way to induce various mutations in higher plants (Morita et al. 2009).

To date, several yellow-green leaf mutant genes have been mapped and cloned. \(ygl1\) is a yellow-green leaf mutant gene in rice. The \(YGL1\) gene encodes an enzyme required for Chl \(a\) biosynthesis. A point mutation (Pro-198 to Ser) in the \(YGL1\) gene reduces Chl synthase activity (Wu et al. 2007). \(chl1\) and \(chl9\), which have been isolated from chromosome 3 of two rice chlorina mutants, encode the ChlD and ChlII subunits of Mg-chelatase, respectively. These two genes play an important role in chloroplast development by modulating MgProto (Zhang et al. 2006). In wheat, the homoelogous chlorina loci have been mapped onto the homoelogous group 7 chromosomes. These loci include the \(cn-A1\) locus on chromosome 7A, the \(cn-B1\) locus on the 7B and the \(cn-D1\) on chromosome 7D. These mutations reduce the expression of the light-harvesting Chl \(a/b\) complex II (Klindworth et al. 1995, Watanabe and Koval 2003).

In this study, we characterized the yellow-green leaf mutant \(ygl1\) in durum wheat and mapped the mutated genes of the \(F_{2,3}\) populations with SSR markers. The mutations affecting the agronomic traits were also investigated.
Materials and Methods

Plant materials

The ygld (yellow-green leaf durum) mutant was introduced from Italy, derived from Triticum durum var. Cappelli treated by gamma radiation (Tomarchio et al. 1983). The mutant displays yellow-green leaves throughout development. The F$_{2:3}$ segregation populations were used for genetic analysis and mapping. These populations were made by crossing the T. durum cultivar Langdon with the normal green-leaf plant and the ygld mutant.

Pigment content and fluorescence kinetic parameters

The content of Chl (chlorophyll) and Cars (carotenoid) was measured using a DU 800 UV/Vis Spectrophotometer (Beckman Coulter) according to the method detailed by Lichtenthaler (1987). The fluorescence kinetic parameters were measured using the Hansatech Fluorescence Monitoring System-FMS-2. Each experiment was repeated three times.

Transmission electron microscopy analysis

The wild-type and ygld mutant leaf samples were collected from 1-week- and 4-week-old plants. All plants were grown under a controlled environment with the same light intensity, temperature and living conditions. First, the leaf sections which were cut to about 5 mm in length from fresh leaves, were quickly fixed in a solution of 2% glutaraldehyde. Next, the sections were fixed in a solution of 1% OsO$_4$ and the samples were stained with uranyl acetate and dehydrated in ethanol. The thin sections were embedded in Spurr’s medium. Finally, the samples were sliced to 50 nm in thickness, and stained again then examined using a JEOL 100 CX electron microscope.

Agronomic trait analysis

The agronomic traits of the F$_{2:3}$ populations were examined. Both populations were grown in Beijing (39.54°N). The F$_2$ population was planted in 2009 and the F$_1$ population was planted in the fall of 2010. A total of 7 agronomic traits were investigated. These traits included the plant height (cm) (PH), number of spikes per plant (NSP), number of spikelets per spike (NSS), spike length (cm) (SL), number of grains per spike (NGS), grain yield per plant (GYP) and 1000-grain weight (TGW).

Microsatellite analysis

Total DNA was extracted from the sample leaves using the CTAB method (Murray and Thompson 1980). Each PCR reaction was conducted in a total volume of 15 μl containing 1.2 μl of template DNA (approximately 50 ng), 0.09 μl of Taq DNA polymerase (5 U/μl, Fermentas), 1.5 μl of 10× PCR buffer [200 mM (NH$_4$)$_2$SO$_4$, 750 mM Tris-HCl (pH 8.8, 25°C)], 1.2 μl of 25 mM MgCl$_2$, 0.12 μl of dNTP (25 mM), 1.2 μl of primers (2 μM) and 9.69 μl of H$_2$O. The amplification experiments were subjected to 94°C for 5 min. This step was followed by 35 cycles of 94°C for 45 s, 48–63°C (depending on the microsatellite primer annealing temperature) for 45 s and 72°C for 45 s. The extension step was performed at 72°C for 10 min. Each PCR product was mixed with 3 μl of loading buffer (98% formamide, 0.3% of each bromophenol blue and xylene cyanol and 10 mM of EDTA), denatured at 95°C for 5 min and immediately cooled on ice. Electrophoresis was carried out in a 5% denatured polyacrylamide gels with 1× TBE (90 mM Tris-borate, 2 mM EDTA) for 90 min. A silver-staining experiment was later performed according to the method of Tixier et al. (1997).

Linkage analysis

The F$_2$ generation derived from crossing the yellow-green leaf mutant ygld and T. durum cultivar Langdon was used for mapping. The phenotypes of the F$_2$ individuals were confirmed by investigating the F$_1$ families in the field at different growth stages. A total of 794 F$_2$ individuals were used for genetic mapping. To determine the map position of the ygld genes on the wheat chromosomes, 546 pairs of SSR markers on the whole durum wheat chromosomes were selected from Somers et al. (2004), Paux et al. (2008) and Xue et al. (2008). The polymorphic markers between parents were first picked out. Then BSA (bulked segregant analysis) method was used to screen for the polymorphic markers between the green and yellow-green leaf DNA bulks. Each bulk was composed of 6 individuals from the F$_2$ generation. To exclude the possibility of other reasons which could cause the yellow-green leaf phenotype, all the DNA samples of F$_2$ were phenotypically validated using the F$_1$ families. The MAPMAKER/EXP ver. 3.0 program, which is based on the maximum likelihood method, was used for linkage analysis (Lander et al. 1987). The map distances between markers and genes were derived from the Kosambi function (Kosambi 1944).

Results

Phenotypic characterization of the ygld mutant

Leaf-color mutations are diverse and can occur at different growth stages. The plants with the mutations conferring severe Chl deficiency died early. Some plants with other mutations eventually regained the normal green leaf color (Chen et al. 2009, Dong et al. 2007, Pereira et al. 1997). Compared with the wild-type, the ygld mutant exhibited yellow-green leaves from germination through maturity. Even though the mutant was less vigorous and was delayed in its development, the yellow-green leaf color mutation in the ygld mutant was not lethal (Fig. 1).

The TEM analysis showed that there were no differences in the size or number of the chloroplasts between the mutant and wild type plants. However, the shape and structure of the chloroplasts were different between groups. The wild-type plants had ellipsoidal chloroplasts. In the ygld mutant, the shape of chloroplasts changed from ellipsoidal to circular. The mutant grana stacks were highly variable in size and...
appeared disorganized in the stroma region. In the wild type, the chloroplasts had uniform diameters and had approximately parallel grana stacks and lamellae (Fig. 2).

Pigment content and fluorescence kinetic parameter analysis

The quantity of chlorophyll $a$ (Chl $a$), chlorophyll $b$ (Chl $b$) and carotenoid (Car) as well as the ratio of chlorophyll $a$/chlorophyll $b$ (Chl $a$/Chl $b$) were measured. These measurements were compared between groups at different times during development (Table 1). The content of the pigments changed with developmental stage, but the levels of Chl $a$, Chl $b$ and Car in the mutant were lower throughout development compared with the wild type. Chl accumulation in the mutant was slower than that in wild type and did not reach the wild-type level. Additionally, increasing the Chl $a$/Chl $b$ ratio meant that Chl $b$ was more reduced than Chl $a$ in the mutant. Consequently, the $ygld$ mutant is deficient especially in Chl $b$.

The fluorescence kinetic parameters (Table 2) in Fo and Fm of the yellow-green leaf mutant were decreased significantly compared with the wild type. This reduction corresponded to the reduction in chl. The $\phi$PSII, NPQ and ETR were decreased by 16.7%, 16.6% and 25.5%, respectively. These results indicated that photosynthesis efficiency was decreased in the mutant. The Fv/Fm values of the wild type and the mutant were not significantly different. This result implied that the primary light energy conversion of PSII in the mutant was retained at wild-type levels (Wang et al. 2009).

The effects of the mutations on yield traits

The yield-related traits of the F$_2$ and parts of the F$_3$ populations were investigated in the 2 crop seasons. To comprehensively understand the effects of the $ygld$ phenotype on the yield-related traits, a survey of 7 agronomic traits was carried out (Table 3). We measured the plant height (cm) (PH), number of spikes per plant (NSP), number of spikelets per spike (NSS), spike length (cm) (SL), number of grains of spike (NGS), grain yield per plant (GYP) and 1000-grain weight (TGW). With the exception of SL, all of the agronomic traits in the mutant were significantly reduced compared with wild-type plants. The Chl deficiency throughout development inhibited photosynthesis and consequently affected the accumulation of biomass and the development of the plant.

Genetic analysis of the $ygld$ phenotype

The F$_2$ population, which was derived from crossing the $ygld$ mutant with Langdon, was used to investigate the Mendelian segregation ratio. The F$_2$ population contained 794 individuals in total: 51 of them exhibited yellow-green leaves, whereas the other individuals showed normal green leaves. A $\chi^2$ analysis was applied to test for the deviation of the $ygld$ mutant in the F$_2$ population from the expected segregation ratio of 15:1 ($\chi^2_{15:1} = 0.0056$, p > 0.9). The segregation ratio indicated that the $ygld$ phenotype was controlled by two recessive nuclear genes. Neither gene alone could give rise to the leaf color abnormality. The genes were designated as $ygld1$ (yellow-green leaf durum 1) and $ygld2$ (yellow-green leaf durum 2).

Genetic mapping of the two recessive genes

To map the $ygld1$ and $ygld2$ genes, 546 pairs of markers were screened for polymorphisms. Among these markers, 132 pairs showed polymorphisms between the two parents. Six and 3 SSR markers were linked with the $ygld1$ and $ygld2$ genes, respectively (Table 4 and Fig. 3). The gene $ygld1$ was located on chromosome 5AL and was flanked by the SSR markers cfa2185 and cfa126 at genetic distances of 16.0 cM and 8.5 cM, respectively. Moreover, the SSR marker cfa2185
The ygld2 gene was mapped onto chromosome 5BL with 3 markers. Barc142 and barc308 were both at a distance of 8.0 cm and the SSR marker wmc28 cosegregated with ygld2 (Fig. 3).

**Discussion**

Chloroplasts and Chl are essential for photosynthesis in higher plants. This fundamental process sustains the life on earth (Chen et al. 2005, Inaba and Ito-Inaba 2010, Wang et al. 2009). Chl a is a component of the light-harvesting complexes (LHCs) and photosynthetic reaction centers. Chl b is located in the light-harvesting pigment protein complexes of the PSI and PSII (Masuda et al. 2003, Oster et al. 2000). Previous studies have indicated that mutants with a higher Chl a/Chl b ratio have lower LHC levels. As a result of the
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limited chl content, these mutants have abnormal thylakoid membranes (Falbel and Staehelin 1996, Marco et al. 1989). The ygld mutant with the yellow-green leaf phenotype is a chlorophyll deficient mutant. This mutant has a high chl a/chl b ratio and has decreased chl a, chl b, carotenoid levels. These results indicate that the LHC content may be lower in the mutant than in the wild type. Furthermore, the PSII of the ygld mutant was not significantly affected according to our measurements of the fluorescence parameters. This result indicated that the PSI in the ygld mutant was impaired by the mutation. Thus, the abnormal pigment content and chloroplastic structure in the ygld mutant may have contributed to the partial repression of chl synthesis. Plants with normal leaf colors have higher chlorophyll content and normal chloroplasts. These characteristics allow the plants to absorb more energy and be more efficient at photosynthesis (Lawlor 2009, Luo and Ren 2006, Wang et al. 2003, Williams et al. 1985). Therefore, the wild-type plants grow vigorously. The chloroplasts in many leaf color mutants are irreversibly abnormal from the early stages of leaf development (An et al. 2011). Therefore, the mutations affect the accumulation of both Chl and biomass throughout plant development. The ygld mutant can carry out photosynthesis but grows slowly compared to the wild type. Almost all of the agronomic traits were reduced significantly in ygld, but the influence on SL was limited. Further study on the genes of ygld would contribute to the improvement in breeding. The study could be useful to understand genetic mechanism of chloroplast biogenesis and exploit in the improvement for wheat photosynthesis. Moreover the differentiation in leaf color could be a significant marker. Leaf color mutant has been used successfully in rice breeding to monitor seed purity in seed production as a phenotypic marker (Chen et al. 2007). Many of these yellow-green leaf mutations have been genetically characterized and used as genetic markers. In rice, 11 yellow-green leaf mutants (chl 1 to10 and ygl1) have been identified. All of these phenotypes are controlled by a single recessive gene (www.gramene.com). Moreover, several yellow-green leaf mutants have been identified from tetraploid wheat (Triticum turgidum L.). Further analysis showed that most yellow-green leaf mutants are also controlled by a single recessive gene (Klindworth et al. 1995, Luo and Ren 2006, Williams et al. 1985). However, Smith identified a mutant controlled by a dominant gene (Smith 2007).

Table 3. The relationship between the phenotypes and seven yield-related traits

| Phenotype | Trait | PH  | TGW  | SL  | NSP | NSS  | NGS | GYP |
|-----------|-------|-----|------|-----|-----|------|-----|-----|
| Wild type | 132.9 ± 11 | 34.6 ± 8.1 | 9.5 ± 4.6 | 23.4 ± 2.7 | 46.8 ± 9.1 | 272.6 ± 171.0 | 9.5 ± 6.5 |
| Mutant    | 102.8 ± 14.5** | 20.4 ± 5.8** | 7.4 ± 1.3 | 19.5 ± 3.4** | 31.1 ± 8.5** | 45.7 ± 23.1** | 1.0 ± 0.6** |

plant height (cm) (PH), number of spikes per plant (NSP), number of spikelets per spike (NSS), spike length (cm) (SL), number of grains of spike (NGS), grain yield per plant (GYP), 1000-grain weight (TGW), ** significant at P < 0.01 by T-test.

Table 4. Molecular markers used for mapping the genes of ygld1 and ygld2

| Marker | Tm (°C) | Forward primer (5’→3’) | Reverse primer (5’→3’) |
|--------|---------|------------------------|------------------------|
| cfa2155 | 60      | TTTGTGTTGAAAGAAGAAGAAGAGG | TTTGTGTTGAAAGAAGAAGAAGAGG |
| cfa2141 | 60      | GAATGGAAGGCGGACATAGA | GCCCTCCAACAGCCATAATT |
| cfa2185 | 60      | TTTCTCGAGTTGTTGGG | TTTGTGCTGAAAGAAATCCATCG |
| wmc110  | 61      | GCAGATGAGTTGATGTTGATTGTG | GTACTTGAAACCTGTTGTTG |
| wmc28   | 51      | ATCACGCATGTCTCAGCATGAC | GTTGATGAGTATGCGAGG |
| gwm291  | 60      | CATACCTACCGCAGCTCGG | AAAGATGATGACCATCAGG |
| barc308 | 55      | GCCATCCTGCTGCTGCTGCTG | GCCGTTGAGTCAAGTTGAACAAAT |
| barc142 | 52      | CGGTAGGAGGACTACAA | GGCATGTCATATGACCTGAAG |
| wmc28   | 51      | ATCAAGGCTATGCTGCTGCTG | ATTAAGACCATGAAAGACCTG |
leaf color alterations. Thus, the previously reported genes associated with yellow-green segregating markers wmc110 and wmc28, respectively and to their locations. The analysis of the mapping locations and mapped onto chromosome 5AL and 5BL with the co-

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Literature Cited

Acknowlegements

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An,S.J., D.Pandeya, S.W.Park, J.Li, J.K.Kwon, S.Koeda, M.Hosokawa, N.C.Paek, D.Choi and B.C.Kang (2011) Characterization and genetic analysis of a low-temperature-sensitive mutant, sy-2, in Capsicum chinense. Theor. Appl. Genet. 122: 459–470.

Asakura,Y., S.Kikuchi and M.Nakai (2008) Non-identical contributions of two membrane-bound cp SRP components, cpFtsY and Alb3, to thylakoid biogenesis. Plant J. 56: 1007–1017.

Bellemare,G., S.G.Bartlett and N.H.Chua (1982) Biosynthesis of chlorophyll a/b-binding polypeptides in wild type and the chlorina f2 mutant of barley. J. Biol. Chem. 257: 7762–7767.

Chen,G., Y.R.Bi and N.Li (2005) EGY1 encodes a membrane-associated and ATP-independent metalloprotease that is required for chloroplast development. Plant J. 41: 364–375.

Chen,T., Y.Zhang, L.Zhao, Z.Zhu, J.Lin, S.Zhang and C.Wang (2007) Physiological character and gene mapping in a new green-revertible albino mutant in rice. J. Genet. Genomics 34: 331–338.

Chen,T., Y.Zhang, L.Zhao, Z.Zhu, J.Lin, S.Zhang and C.Wang (2009) Fine mapping and candidate gene analysis of a green-revertible albino gene gtr at in rice. J. Genomics 36: 117–123.

Dong,H.L., Y.Deng, J.Y.Mu, Q.T.Lu, Y.Q.Wang, Y.Y.Xu, C.C.Chu, K.Chong, C.M.Liu and J.R.Zuo (2007) The Arabidopsis Spontaneous Cell Death1 gene, encoding a zeta-carotene desaturase essential for carotenoid biosynthesis, is involved in chloroplast development, photo protection and retrograde signalling. Cell Res. 17: 575–575.

Eckhardt,U., B.Grimm and S.Hortensteiner (2004) Recent advances in chlorophyll biosynthesis and breakdown in higher plants. Plant Mol. Biol. 56: 1–14.

Falbel,T.G., J.B.Meechl and L.A.Staehelin (1996) Severity of mutant phenotype in a series of chlorophyll-deficient wheat mutants depends on light intensity and the severity of the block in chlorophyll synthesis. Plant Physiol. 112: 821–832.

Falbel,T.G and L.A.Staehelin (1996) Partial blocks in the early steps of the chlorophyll synthesis pathway: A common feature of chlorophyll b-deficient mutants. Physiol. Plantarum 97: 311–320.

Flood,P.J., J.Harbinson and M.G.Aarts (2011) Natural genetic variation in plant photosynthesis. Trends Plant Sci. 16: 327–335.

Giardi,M.T., T.Kucera, J.M.Briantais and M.Hodges (1995) Decreased photosystem II core phosphorylation in a yellow-green mutant of wheat showing monophasic fluorescence induction curve. Plant Physiol. 109: 1059–1068.

Hui,Z., F.X.Tian, G.K.Wang, G.P.Wang and W.Wang (2012) The antioxidative defense system is involved in the delayed senescence in a wheat mutant tasg1. Plant Cell Rep. 31: 1073–1084.

Inaba,T. and Y.Yit-Inaba (2010) Versatile roles of plastids in plant growth and development. Plant Cell Physiol. 51: 1847–1853.

Jarvis,P., P.Dormann, C.A.Peto, J.Lutes, C.Benning and J.Chory (2000) Galactolipid deficiency and abnormal chloroplast development in the Arabidopsis MGD synthase 1 mutant. Proc. Natl. Acad. Sci. USA 97: 8175–8179.

Jung,K.H., J.Hur, C.H.Ryu, Y.Choi, Y.Y.Chung, A.Miyao, H.Hirochika and G.An (2003) Characterization of a rice chlorophyll-deficient mutant using the T-DNA gene-trap system. Plant Cell Physiol. 44: 463–472.

Kilworth,D.L., N.D.Williams and M.E.Duysern (1995) Genetic-analysis of chlorina mutants of durum-wheat. Crop Sci. 35: 431–436.

Kosambi,D.D (1944) The estimation of map distance from recombination values. Ann. Eugen. 12: 172–175.

Kosuge,K., N.Watanabe and T.Kubo yama (2011) Comparative genetic mapping of homoeologous genes for the chlorina phenotype in the genus Triticum. Euphytica 179: 257–263.

Lander,E.S., P.Green, J.Abrahamson, A.Barlow, M.J.Daly, S.E. Lincoln and L.A.Newberg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181.

Lawlor,D.W. (2009) Musings about the effects of environment on photosynthesis. Ann. Bot. 103: 543–549.

Lichtenthaler,H.K. (1987) Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce,R. and L.Packer (eds.) Methods Enzymol., 148, Academic Press Inc., New York, pp. 350–382.

Liu,Z., S.W.Hong, M.Escobar, E.Vierling, D.L.Mitchell, D.W.Mount and J.D.Hall (2003) Arabidopsis UVH6, a homolog of human XPD and yeast RAD3 DNA repair genes, functions in DNA repair and is essential for plant growth. Plant Physiol. 132: 1405–1414.

Luo,P.G. and Z.L.Ren (2006) Wheat leaf chlorosis controlled by a single recessive gene. J. Plant Physiol. Mol. Biol. 32: 330–338.

Marco,G.D., N.D’Ambrosio, M.T.Giardi, A.Massacci and D.Tricolli (1989) Photosynthetic properties of leaves of a yellow green mutant of wheat compared to its wild type. Photosynth. Res. 21: 117–122.

Maskhina,E.V. and E.P.Gus’kov (2002) Cyto genetic effect of temperature on the sunflower varieties. Tsiitologiya 44: 1220–1226.

Masuda,T., N.Fusada, N.Oosawa, K.Takamatsu, Y.Y.Yamamoto, M.Ohno, K.Nakamura, K.Goto, D.Shibata, Y.Shirano et al. (2003) Functional analysis of isoforms of NADPH: protochlorophyllide oxidoreductase (POR), PORB and PORC, in Arabidopsis thaliana. Plant Cell Physiol. 44: 963–974.

Mei,M., Y.Qiu, Y.Sun, R.Huang, J.Yao, Q.Zhang, M.Hong and J.Ye (1998) Morphological and molecular changes of maize plants after seeds been flown on recoverable satellite. Adv. Space Res. 22: 1691–1697.

Moon,S., C.Giglione, D.Y.Lee, S.An, D.H.Jeong, T.Mein nel and G.An (2008) Rice peptide deformylase PDF1B is crucial for
development of chloroplasts. Plant Cell Physiol. 49: 1536–1546.

Morita, R., M. Kusaba, S. Iida, H. Yamaguchi, T. Nishio and M. Nishimura (2009) Molecular characterization of mutations induced by gamma irradiation in rice. Genes Genet. Syst. 84: 361–370.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Oster, U., R. Tanaka, A. Tanaka and W. Rudiger (2000) Cloning and functional expression of the gene encoding the key enzyme for chlorophyll b biosynthesis (CAO) from Arabidopsis thaliana. Plant J. 21: 305–310.

Paux, E., P. Sourdille, J. Salse, C. Saintenac, F. Choulet, P. Leroy, A. Korol, M. Michalak, S. Kianian, W. Spielmeyer et al. (2008) A physical map of the 1-gigabase bread wheat chromosome 3B. Science 322: 101–104.

Pereira, T.N., H. Ilarslan and R.G. Palmer (1997) Genetic and cytological analyses of three lethal ovule mutants in soybean (Glycine max; Leguminosae). Genome 40: 273–285.

Preiss, S. and J.P. Thornber (1995) Stability of the apoproteins of light-harvesting complex I and II during biogenesis of thylakoids in the chlorophyll b-less barley mutant chlorina f2. Plant Physiol. 107: 709–717.

Smith, L. (1952) A rare dominant chlorophyll mutant in durum wheat: Induced by atomic bomb irradiation. J. Hered. 43: 125–128.

Somers, D.J., P. Isaac and K. Edwards (2004) A high-density wheat microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 109: 1105–1114.

Tixier, M.H., P. Sourdille, M. Röder, P. Leroy and M. Bernard (1997) Detection of wheat microsatellites using a non radioactive silver-nitrate staining method. J. Genet. Breed. 51: 175–177.

Tomarchio, L., L. Triolo and G. Dimarco (1983) Photosynthesis, ribulose-1,5-bisphosphate carboxylase, electron transport, and ribulose 1,5-bisphosphate of virescent and normal green wheat leaves. Plant Physiol. 73: 192–194.

Varughese, G.S. and M.S. Swaminathan (1968) A comparison of the frequency and spectrum of mutations induced by gamma rays and EMS in wheat. Indian Journal of Genetics and Plant Breeding 28: 158–165.

Wang, B., T. Lan, W.R. Wu and W.M. Li (2003) Mapping of QTLs controlling chlorophyll content in rice. Acta Genetica Sinica 30: 1127–1132.

Wang, L., M. Ouyang, Q. Li, M. Zou, J. Guo, J. Ma, C. Lu and L. Zhang (2010) The Arabidopsis chloroplast ribosome recycling factor is essential for embryogenesis and chloroplast biogenesis. Plant Mol. Biol. 74: 47–59.

Wang, Q.S., X.C. Sang, Y.H. Ling, F.M. Zhao, Z.L. Yang, Y.F. Li and G.H. He (2009) Genetic analysis and molecular mapping of a novel gene for zebra mutation in rice (Oryza sativa L.). J. Genet. Genomics 36: 679–684.

Watanabe, N. and S.E. Koval (2003) Mapping of chlorina mutant genes on the long arm of homoeologous group 7 chromosomes in common wheat with partial deletion lines. Euphytica 129: 259–265.

Williams, N.D., L. Joppa, M.E. Duysen and T.P. Freeman (1985) Inheritance of three chlorophyll-deficient mutants of common wheat. Crop Sci. 25: 1023–1025.

Wu, Z., X. Zhang, B. He, L. Diao, S. Sheng, J. Wang, X. Guo, N. Su, L. Wang, L. Jiang et al. (2007) A chlorophyll-deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. Plant Physiol. 145: 29–40.

Xue, S., Z.Z. Zhang, F. Lin, Z.X. Kong, Y. Cao, C. Li, H.Y. Yi, M.F. Mei, H.L. Zhu, J.Z. Wu et al. (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor. Appl. Genet. 117: 181–189.

Zhang, H., J. Li, J.H. Yoo, S.C. Yoo, S.H. Cho, H.J. Koh, H.S. Seo and N.C. Park (2006) Rice chlorina-1 and chlorina-9 encode ChlD and ChlI subunits of Mg-chelatase, a key enzyme for chlorophyll synthesis and chloroplast development. Plant Mol. Biol. 62: 325–337.