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Short communication

Reduced chlorophyll biosynthesis in heterozygous barley magnesium chelatase mutants

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ABSTRACT

Chlorophyll biosynthesis is initiated by magnesium chelatase, an enzyme composed of three proteins, which catalyzes the insertion of Mg^{2+} into protoporphyrin IX to produce Mg-protoporphyrin IX. In barley (*Hordeum vulgare* L.) the three proteins are encoded by *Xantha-f*, *Xantha-g* and *Xantha-h*. Two of the gene products, XanH and XanG, belong to the structurally conserved family of AAA+ proteins (ATPases associated with various cellular activities) and form a complex involving six subunits of each protein. The complex functions as an ATP-fueled motor of the magnesium chelatase that uses XanF as substrate, which is the catalytic subunit responsible for the insertion of Mg^{2+} into protoporphyrin IX. Previous studies have shown that semi-dominant *Xantha-h* mutations result in non-functional XanH subunits that participate in the formation of inactive AAA complexes. In the present study, we identify severe mutations in the barley mutants *xantha-h.38*, *-h.56* and *-h.57*. A truncated form of the protein is seen in *xantha-h.38*, whereas no XanH is detected in *xantha-h.56* and *-h.57*. Heterozygous mutants show a reduction in chlorophyll content by 14–18% suggesting a slight semi-dominance of *xantha-h.38*, *-h.56* and *-h.57*, which otherwise have been regarded as recessive mutations.

1. Introduction

Plants require chlorophyll to harness light energy for transforming water and carbon dioxide into carbohydrates and oxygen. Magnesium chelatase (EC 6.6.1.1) catalyses the insertion of Mg^{2+} into protoporphyrin IX and thereby is the first committed enzyme of the chlorophyll biosynthetic pathway. Mutations in barley magnesium chelatase were induced between 1953 and 1981 by different chemical and physical treatments such as triethylenemelamine, sodium azide and ethyl methane-sulfonate, and irradiation by neutrons, gamma-rays and X-rays (Simpson et al., 1985; Henningsen et al., 1993). Mutant seedlings are yellow due to inhibition of the chlorophyll biosynthesis but an intact synthesis of carotenoids. The seedlings die after 2–3 weeks when the carbohydrate from the kernel has been consumed (Fig. 1). The lethal mutant alleles have to be kept as heterozygous stocks. Crosses between different magnesium chelatase mutants revealed three complementation groups – *xantha-f*, *xantha-g* and *xantha-h* (von Wettstein et al., 1974). This observation suggested that magnesium chelatase is a protein complex built up by three different gene products, which has been confirmed by later studies. In barley, the *Xantha-f*, *Xantha-g* and *Xantha-h* genes encode proteins of 153, 83, and 45 kDa, respectively (Jensen et al., 1996; Hansson et al., 1999; Olsson et al., 2004; Axelsson et al., 2006). XanH and XanG belong to the structurally conserved family of AAA+ proteins (ATPases associated with various cellular activities) (Fodje et al., 2001). They form a two-ring structure with six XanH subunits in one layer and six XanG subunits in the other layer (Lundqvist et al., 2010). Members of the AAA+ family are known to act as molecular machines that remodel their various target molecules in an ATP-dependent manner (Iyer et al., 2004). The XanF subunit is the substrate of the XanH-XanG motor unit and the subunit where the insertion of Mg^{2+} into protoporphyrin IX is supposed to occur (Hansson et al., 2013). Previous studies have explored recessive barley mutants in *Xantha-g* and semi-dominant mutants in *Xantha-h* to give insights into the function of the AAA+ protein complex (Hansson et al., 2002; Axelsson et al., 2006; Lundqvist et al., 2013). The semi-dominant mutations *Xantha-h.clo 125*, *Xantha-h.clo 157* and *Xantha-h.clo 161* lead to changes of conserved amino-acid residues in the XanH subunit due to point mutations in *Xantha-h* (Hansson et al., 1999, 2002). Four recessive mutations in the

Abbreviations: AAA, ATPases associated with various cellular activities.

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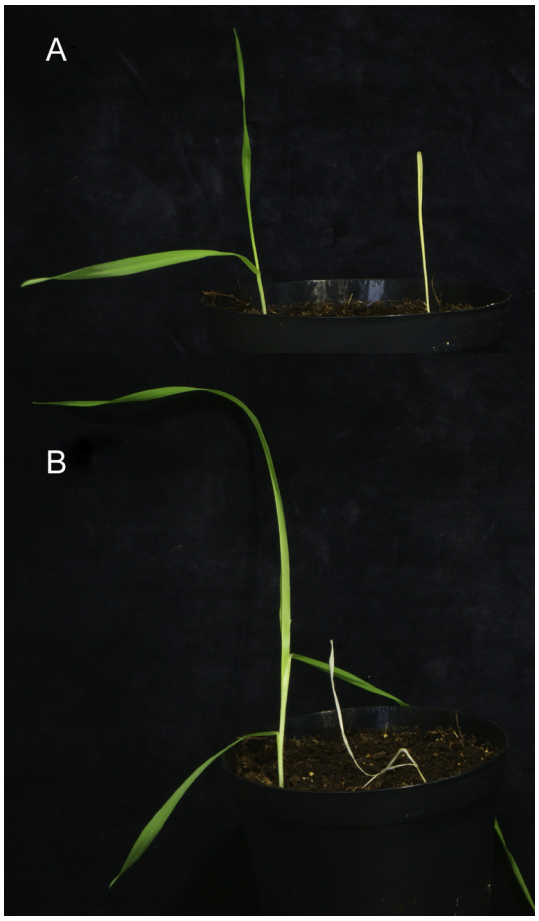

Fig. 1. Barley cultivar Bonus (left) and mutant *xantha-h.38* (right) grown in greenhouse. Photo taken after 8 days (A) and 20 days (B), respectively. (A) The yellow homozygous mutant synthesizes carotenoids but not chlorophyll. (B) The mutant dies after 2–3 weeks.

Xantha-h gene (*xantha-h.30*, *-h.38*, *-h.56* and *-h.57*) are also available (Henningsen et al., 1993). Previous studies showed that the recessive mutants lack the protein encoded by the *Xantha-h* gene (Jensen et al., 1996; Lake et al., 2004), but until today the mutations have never been described at DNA level.

The semi-dominant mutants segregate into a 1:2:1 ratio with dark-green wild-type plants, light-green heterozygous mutants and yellow homozygous mutants (Hansson et al., 1999). The light-green heterozygous plants have a reduced amount of chlorophyll due to a limited number of functional XanH-XanG AAA+ complexes. It has been demonstrated that the gene products from wild-type and mutant *Xantha-h* form mixed complexes and that most, if not all, combinations of mixed complexes are inactive (Lundqvist et al., 2013). It is very likely that mixed inactive complexes are also formed in plastids of heterozygous plants and thereby reduce the catalytic capacity of the magnesium chelatase, which results in light green plants. The recessive mutants segregate into a 3:1 ratio with dark green plants and yellow homozygous mutants. Visual inspection cannot differentiate green wild-type plants from green heterozygous *xantha-h.30*, *-h.38*, *-h.56* and *-h.57* mutants (Henningsen et al., 1993). In the present study, we characterize the recessive *xantha-h* mutations at the DNA level and test whether the heterozygous plants show any tendencies of reduced magnesium chelatase activity monitored as level of chlorophyll content.

2. Results and discussion

The barley genome has not yet been sequenced but a gene space assembly embedded in a physical map enriched with sequenced BAC clones and whole genome shotgun contigs is available (International Barley Sequencing Consortium, 2012). The gene model representing the *Xantha-h* gene is MLOC_11877.2, which is anchored to chromosome 7HL at 109.73 cM (Ariyadasa et al., 2014). The genomic sequence of MLOC_11877.2 could be found in BAC clone HVVMRXALLhA0265N11. Primers were designed according to the genomic sequence of the *Xantha-h* gene and they were used to amplify *Xantha-h* from the four recessive barley mutants *xantha-h.30*, *-h.38*, *-h.56* and *-h.57*. The barley *Xantha-h* gene is 1462 bp and contains two introns of 104 and 95 bp, respectively (Fig. 2). The deduced polypeptide is 420 amino-acid residues of which the first 73 residues are predicted to constitute a chloroplast transit peptide sequence (ChloroP; (Emanuelsson et al., 1999)).

Mutant *xantha-h.38* has an in-frame deletion of 255 bp removing amino-acid residues 165 to 249 of the unprocessed protein (Fig. 2, Table 1). A partial deletion of the *Xantha-h* gene in mutant *xantha-h.38* was suggested earlier due to a smaller hybridizing DNA fragment in a Southern blot analysis and a shorter mRNA in a Northern blot experiment (Jensen et al., 1996). The observed deletion results in a truncated XanH protein of 335 amino-acid residues. The deletion spans the Walker B motif of the XanH protein (Fodje et al., 2001), which can explain the severe effect of the *xantha-h.38* mutation. A truncated version of XanH, with an approximate molecular weight of 30 kDa, could be detected by Western blot analysis using total protein extract of homozygous mutant *xantha-h.38* (Fig. 3). XanH of wild-type barley migrates as a 39 kDa protein. Both protein sizes correspond to those of the processed proteins, i.e. also the truncated XanH protein must be imported into the chloroplast. The truncated XanH appear to be less abundant in protein extract of mutant *xantha-h.38* (Fig. 3). This is probably due to degradation of miss-folded XanH. However, the

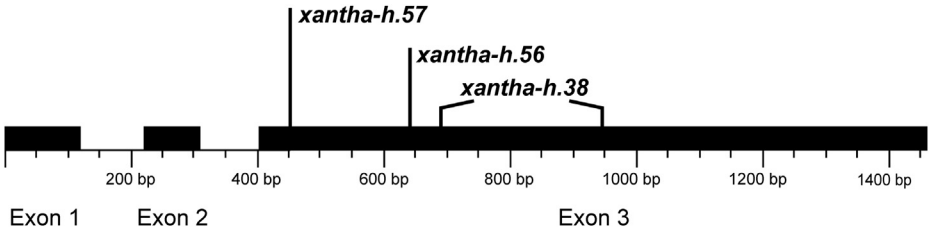

Fig. 2. Gene structure of barley *Xantha-h* (MLOC_11877.2) and the location of the three identified *xantha-h.38*, *-h.56* and *-h.57* mutations. The gene contains three exons (black bars) and two introns (lines). The genomic sequence of *Xantha-h* between start and stop codon was sequenced in the barley cultivar Bonus and in the four available recessive *xantha-h* mutants. The three exons comprise a 1263 bp coding sequence encoding a protein of 420 amino-acid residues. The first 73 residues are predicted to constitute a chloroplast transit peptide (ChloroP; (Emanuelsson et al., 1999)). Single nucleotides are affected in *xantha-h.56* and *-h.57*, while the mutation of *xantha-h.38* is an in-frame deletion of 255 nucleotides (Details in Table 1). The *Xantha-h* DNA sequence of cultivar Bonus has been deposited at the GenBank database under accession number KF974532.