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Multiple genes of mevalonate and non-mevalonate pathways contribute to high aconites content in an endangered medicinal herb, *Aconitum heterophyllum* Wall

Nikhil Malhotra, Varun Kumar, Hemant Sood, Tiratha Raj Singh, Rajinder Singh Chauhan *

Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat 173234, Himachal Pradesh, India

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ABSTRACT

Aconitum heterophyllum Wall, popularly known as Atis or Patis, is an important medicinal herb of North-Western and Eastern Himalayas. No information exists on molecular aspects of aconites biosynthesis, including atisine- the major chemical constituent of *A. heterophyllum*. Atisine content ranged from 0.14% to 0.37% and total alkaloids (aconites) from 0.20% to 2.49% among 14 accessions of *A. heterophyllum*. Two accessions contained the highest atisine content with 0.30% and 0.37% as well as the highest alkaloids content with 2.22% and 2.49%, respectively. No atisine was detected in leaves and shoots of *A. heterophyllum*, thereby, suggesting that the biosynthesis and accumulation of aconite alkaloids occur mainly in roots. Quantitative expression analysis of 15 genes of MVA/MEP pathways in roots versus shoots, differing for atisine content (0–2.2 folds) showed 11–100 folds increase in transcript amounts of 4 genes of MVA pathway; HMGS, HMGR, PMK, IPPI, and 4 genes of MEP pathway; DXPS, ISPD, HDS, GDPS, respectively. The overall expression of 8 genes decreased to 5–12 folds after comparative expression analysis between roots of high (0.37%) versus low (0.14%) atisine content accessions, but their relative transcript amounts remained higher in high content accessions, thereby implying their role in atisine biosynthesis and accumulation. PCA analysis revealed a positive correlation between MVA/MEP pathways genes and alkaloids content. The current study provides first report wherein partial sequences of 15 genes of MVA/MEP pathways have been cloned and studied for their possible role in aconites biosynthesis. The outcome of study has potential applications in the genetic improvement of *A. heterophyllum*.

1. Introduction

Aconitum heterophyllum Wall, locally called as Atis, is an important medicinal herb in the North-Western and Eastern Himalayas of India. It is a biennial herb found between 2400 and 3600 m altitude above mean sea level. It has been listed as a 'critically endangered species' by the International Union for Conservation of Nature and Natural Resources (CAMP, 2003; Nautiyal et al., 2002; IUCN, 1993). The aqueous extract of root is used for the treatment of chronic fever and cold. The plant is used as an active ingredient in the herbal formulation Diarex Vet with other important medicinal plants for the treatment of indigestion, flatulence and diarrhea (Mitra et al., 2001). The paste of *A. heterohyllum* dried tubers mixed with water and sugar is taken orally to treat body ache. It is also used as an aphrodisiac and tonic (Semwal et al., 2009). Kutajghan Vati is a classical Ayurvedic anti-dysentery lozenge prepared from *A. heterophyllum* and other medicinal plants (Lather et al., 2010). Recent studies have shown that its roots are used for curing arthritis (Subramoniam et al., 2013) as well as in the preparation of Caspa Drops – a polyherbal formulation for improving digestion and preventing abdominal distension (Sojitra et al., 2013).

Tuberous roots of genus *Aconitum* contain alkaloids like atisine, aconitine, hetidine, and heterophyllinine which are known to possess medicinal properties like analgesic, aphrodisiac, dyspepsia, etc. (Jabeen et al., 2006; Zhaohong et al., 2006). The plant possess anti-inflammatory, antibacterial and enzyme inhibition activity (Verma et al., 2010; Nisar et al., 2009) due to the presence of non-toxic, amorphous alkaloids like atisine, hetisine and

Abbreviations: HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; PMK, phosphomevalonate kinase; IPPI, isopentenyl pyrophosphate isomerase; DXPS, 1-deoxy-D-xylulose 5-phosphate synthase; ISPD, 2-C-methylerythritol 4-phosphate cytidyltransferase; HDS, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase; GDPS, gerenyldiphosphate synthase.

* Corresponding author. Tel.: +91 1792 239231.
E-mail address: rajinder.chauhan@juit.ac.in (R.S. Chauhan).

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heteratisine (Chauhan, 2006). Atisine in particular is known to act as antiperiodic and aphrodisiac when given in the form of a tonic stimulating CNS and respiratory system (Rastogi and Mehrotra, 1991).

The aconites, including atisine represent major constituents as well as marker compounds of *A. heterophyllum* (Srivastava et al., 2011; Giri et al., 1997). Aconites biosynthesis follows the common MVA/MEP route for isoprenoid production (Fig. 1) but no molecular data is available as of today on the relative importance of MVA/MEP pathways in the biosynthesis of these medicinally important moieties. The biosynthesis and accumulation of isoprenoids is controlled by various structural and regulatory genes (Olofsson et al., 2011). Isopentenyl pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP) – the building blocks of isoprenoids, condense to form geranyl diphosphate (GPP) (Suzuki et al., 2009). Plants utilize both MVA and MEP pathways for isoprenoid biosynthesis, even though they are localized in different compartments (Rodríguez-Concepción and Boronat, 2002; Lange et al., 2000). The MEP pathway starts with the formation of 1-deoxy-D-xylulose 5-phosphate (DXP) from D-glyceraldehyde 3-phosphate and pyruvate by the catalytic action of a 1-deoxy-D-xylulose 5-phosphate synthase (Sprenger et al., 1997) and then DXP is converted into IPP by a series of enzymes. A total of 15 genes are involved, 8 coding for MEP pathway enzymes, and 6 for MVA pathway enzymes, and the final gene coding for IPPI that interconverts DMAPP and IPP in isoprenoid biosynthesis (Wise and Croteau, 1998). To understand the relative role of MVA/MEP pathways gene(s) in aconites biosynthesis, the cloning and expression analysis of all 15 genes in relation to aconites content was studied for the first time in *A. heterophyllum*.

2. Results and discussion

2.1. Quantification of atisine and total alkaloids content

Atisine content in roots of 14 accessions of *A. heterophyllum* ranged from 0.14% to 0.37% and total alkaloids (aconites) from 0.20% to 2.49% (Fig. 2). Two accessions, namely AHCR and AHSR showed the highest atisine content of 0.30% and 0.37% as well as the highest total alkaloids content of 2.22% and 2.49%, respectively. The differences in atisine/aconites content among different accessions of *A. heterophyllum* are most likely due to variation in their genetic potential, not the environmental factors because all accessions were planted under controlled conditions in the green house so as to provide uniformity in growing conditions. Most of the studies on characterization of variation in chemical constituents in different plant species have been done in accessions collected from different geographical locations that differ for altitude and environmental components, thereby, limiting the determination of genetic variations. The effect of climate, altitude and geographical components have been found to significantly influence the production of metabolites in plants such as hyperforin and hypercin content in *Hypericum perforatum* (Nikolic and Zlatkovic, 2010), picrosides content in *Picrorhiza kurroa* (Katoch et al., 2011), camptothecin content in *Nothapodytes nimmoniana* and reserpine content in *Rauwolfia serpentina* (Kumar et al., 2010). The atisine content was estimated in roots of plants of different age groups.

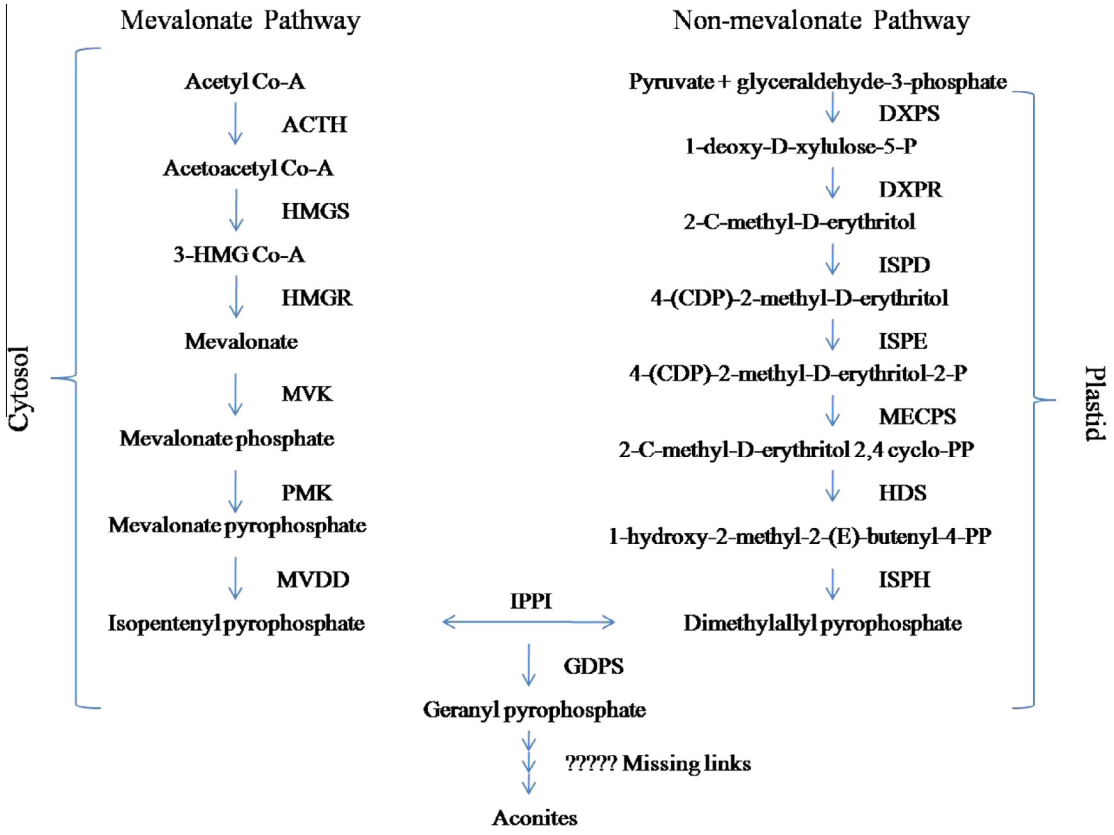

Fig. 1. Common isoprenoid pathway for aconites biosynthesis (adapted from Rodríguez-Concepción and Boronat, 2002). MVA pathway: ACTH acetoacetyl-CoA thiolase, HMGS 3-hydroxy-3-methylglutaryl-CoA synthase, HMGR3-hydroxy-3-methylglutaryl-CoA reductase, MVK mevalonate kinase, PMK phosphomevalonate kinase, MVDD mevalonatediphosphate decarboxylase, IPPI isopentenyl pyrophosphate isomerase, GDPS gerenyldiphosphate synthase. MEP pathway: DXPS 1-deoxy-D-xylulose 5-phosphate synthase, DXPR 1-deoxy-D-xylulose 5-phosphate reductoisomerase, ISPD 2-C-methylerythritol 4-phosphate cytidyltransferase, ISPE 4-(cytidine-50-diphospho)-2-Cmethylerythritol kinase, MECPS 2-C-methylerythritol-2,4-cyclophosphate synthase, HDS 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase, ISPH 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase.

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