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Identification of Amaryllidaceae alkaloids in bulbs and in vitro cultures in various species of Narcissus using GC-MS and LC-MS

Ameneh Tarakemeh1, Majid Azizit, Vahid Rowshan2, Hassan Salehi3, Rosella Spina4, François Dupire4,5, Hossein Arouie6, Dominique Laurain-Mattard4

a) Department of Horticultural Science, Ferdowsi University of Mashhad, Mashhad, Iran; b) Fars agricultural and Natural Resources Education and Research Center, Shiraz, Iran; c) Department of Horticultural Science, Shiraz University, Shiraz, Iran; d) Université de Lorraine, CNRS, L2CM, F-54000 Nancy, France; e) Université de Lorraine, MassLor – Mass Spectrometry Platform, F-54000 Nancy, France

Correspondence: francois.dupire@univ-lorraine.fr

INTRODUCTION
Various species of Narcissus, bulbous flowering plants belonging to the Amaryllidaceae family, are known to synthesize Amaryllidaceae alkaloids (galanthamine, lycorine and tazettine) from the same precursor: 4'-O-methyl-norbelladine. There is a growing interest for these plants due to the medicinal use of one of the major alkaloids in this genus. Narcissus bulbs are considered one of the most important natural sources of galanthamine (selective, reversible and competitive acetylcholinesterase inhibitor) other than synthetic substances. Amaryllidaceae alkaloids possess some interesting biological activities such as anti-malarial, antiviral, antitumor, antifungal and insect antifeedant activity [1].

The aim of work is the evaluation of Amaryllidaceae alkaloids diversity in various varieties of N.tazetta (N. tazetta var. Shastpar (G1), N. tazetta var. Meskin (G2), N. tazetta var. Panjehgorbel (G3) and N. tazetta var. Shahla (G4)) and Narcissus papyraceus (G5), growing wild populations in Iran using GCMS ans LCMS and in vitro culture.

MATERIALS AND METHODS

Plant materials: the in vitro bulbs of the four varieties of Narcissus tazetta and Narcissus papyraceus were harvested 2 months after full flowering stage from Behbahan, Ahvaz province in Iran in April 2016. The in vitro cultures are initiated using thin slices of leaves (about 3–5 mm) isolated from N. tazetta var. Meskin (G2) bulbs. Initial explants were cultivated on the Murashige and Skoog medium supplemented with various auxins, 2,4-dichlorophenoxyacetic acid (2,4-D), picloram or naphthalene acetic acid (NAA) at different concentrations (25 µM and 50 µM) and the cytokinin 6-benzylaminopurine (BAP, 0.5 µM) for induction of calllogenesis and organogenesis. Calli and bulblets of N. tazetta var. Meskin (G2) were subcultured in medium containing precursor 4’-O-methyl-norbelladine at various concentrations (0.05 and 0.15 g/L) and incubated for two periods of time (10 and 20 days) [1].

Preparation of sample (Figure 1): lyophilized start material was extracted, overnight, with acidified methanol (HCOOH) with addition of internal standard hyoscyamine. 

GCMS method: Column DB5MS (30’0.25 mm), mobile phase: He at 1mL/min, injector : 280°C

LCMS method: Column Mixed mode HILIC (150’2,1 mm), mobile phase A : acetonitrile, mobile phase B : water 10mM HCOONH4, Flow : 200 µL/min

RESULTS:

For the in vivo bulbs: Thirty (30) alkaloids were identified among them nine alkaloids were observed by both the methods of analysis. The variety Meskin of N. tazetta (G2), showed the highest diversity of alkaloids and the highest content in galanthamine (red square in tables 1 and 2). By GCMS, six compounds were found in all Narcissus varieties bulblets and showed MS fragmentation patterns characteristic of the Amaryllidaceae alkaloids.

CONCLUSION

➢ It is the first time that these various varieties of Narcissus tazetta were analysed by GC-MS and LC-MS for their alkaloid diversity
➢ N. tazetta var. Meskin (G2) is a potential source of Amaryllidaceae alkaloids of interest.

REFERENCES: