Industrial Crops & Products 109 (2017) 897–904

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research paper

Phytochemical evaluation of major bioactive compounds in different cytotypes of five species of *Rumex* L.

Syed Mudassir Jeelani[a,*], Umer Farooq[b], Ajai Prakash Gupta[c], Surrinder K. Lattoo[a,*]

[a] Plant Biotechnology Division, CSIR- Indian Institute of Integrative Medicine,Canal Road, Jammu Tawi, 180001, India
[b] Department of Botany, Punjabi University, Patiala, Punjab 147002, India
[c] Quality Control and Quality Assurance Division, CSIR- Indian Institute of Integrative Medicine,Canal Road, Jammu Tawi, 180001, India

ARTICLE INFO

Keywords:
Rumex
Cytotypes
LC–MS
Meiosis
Polyploidy
Phytoconstituents

ABSTRACT

The species of genus *Rumex* represent a reservoir of important phytoconstituents reputed for their pharmacological activities. In the present study, identification and quantification of major bioactive compounds in five species of *Rumex* based on tissue- and location-specific comparison among extant cytotypes has been carried out for the first time. Detailed cytological investigations of their populations from NW Himalayas revealed occurrence of different chromosomal races in *R. crispus* (n = 30, 40), *R. dentatus* (n = 20, 60) and *R. nepalensis* (n = 30, 40, 50, 60). Out of ten identified cytotypes, two were identified as novel polyploid types with chromosome counts of 80 and 120 (n = 40 in *R. crispus* and n = 60 in *R. dentatus*) respectively. The tissue-specific chemo-profiling revealed relative dominance of different phytoconstituents in root followed by leaf and stem respectively. Concentration of phytoconstituents (emodin, physcion, piceatannol, resveratrol and rutin) as determined by LC–MS showed positive correlation with the increasing ploidy status and altitudinal gradients. These results indicate that the species/cytotypes thriving at higher altitudes tend to accumulate copious amounts of secondary metabolites. Chromosomal plasticity in terms of polyploidy seems to enhance the adaptability range of a species particularly at higher altitudes. Further, the genus *Rumex* appears to be rich repository of desirable bioactive metabolites with robust cytotypic and chemical diversity for its exploitation to develop cultivars of commerce for trade and industry.

1. Introduction

The species of genus *Rumex* L. (Polygonaceae) are widely distributed at 1600–4000 m (asl) in the Kashmir Himalayas. The roots and aerial parts of *Rumex* species are used in traditional systems of medicine since antiquity. Different species of *Rumex* are used for the treatment of specific and broader set of ailments and diseases. For instance, the root of *R. nepalensis* is purgative and is used as a substitute for *Rheum* species (Manandhar, 2002), while its leaves bear antiseptic properties and are utilized for the treatment of syphilic and colic ulcers (Kirtikar and Basu, 1987). The root and leaf extracts of *R. dentatus* are used for curing constipation (Farooq et al., 2014), whereas, leaves and roots of *R. hastatus* treat jaundice (Abbasi et al., 2015). The plants of Polygonaceae are known to produce large number of biologically important secondary metabolites, such as anthraquinones, naphthalenes, stilbenoids, steroids, flavonoids, glycosides, leucoanthocyanidins and phenolic acids (Jang et al., 2005; Wegiera et al., 2007; Liang et al., 2010; El-Hawary et al., 2011; Gescher et al., 2011). Several bioactive compounds have been reported and isolated from different species of the genus *Rumex*. For example, emodin, chrysophanol and physcion have been isolated from roots of *R. dentatus* (Liu et al., 1997), and from roots and fruits of *R. crispus* (Fairbairn and El Muhtadi, 1972); emodin, emodin glycoside, chrysophanol and chrysophanol glycoside from leaves of *R. nepalensis* (Farooq et al., 2013); from roots of *R. nepalensis* and *R. hastatus* (Zhang et al., 2009; Liang et al., 2010); and rutin from the roots of *R. hastatus* (Zhang et al., 2009). Stilbenes and rumexoids are also produced in the roots of *R. bucephalophorous* (Kerem et al., 2006). The activity of these compounds have been reported against tumour, inflammation, constipation (Zhang et al., 2008), cancer, cardiovascular and neurodegenerative (Fremont, 2000; Ignatowicz and Baer-Dubowska, 2001; Latruffe et al., 2002) as well as fungal disorders (Jayatilake et al., 1993; Gonzalez et al., 2003).

The present investigation was aimed at to investigate chemical diversity in different cytotypes of five species of *Rumex* where the ploidy level range from diploidy to dodecaploidy ($2n = 18 = 2x$ to $2n = 120 = 12x$) from Kashmir Himalayas. These cytological investigations were corroborated with comparative chemo-profiling to have an insight into the chemical diversity in relation to genome

* Corresponding authors.
E-mail addresses: sandrabi555@gmail.com (S.M. Jeelani), sklattoo@iiim.ac.in (S.K. Lattoo).

http://dx.doi.org/10.1016/j.indcrop.2017.09.015
Received 28 May 2017; Received in revised form 1 August 2017; Accepted 9 September 2017

Table 1
Information on chromosome count, ploidy level and locality recorded in different populations of five species of genus *Rumex* from Kashmir Himalayas.

S. no.	Name of species	Observed chromosome number ('*n*')	Ploidy level	Locality & voucher number
P1	*R. crispus* L.	30	Hexaploid (2*n* = 6*x* = 60)	Gurinullah (34°34′ N, 75°44′E;2500 m)/RRL23227
P2		30	Hexaploid (2*n* = 6*x* = 60)	Kanzalwan (34°36′N, 74°42′E;2500 m)/RRL23226
P3		40	Octaploid (2*n* = 8*x* = 80)	Dawar (34°38′N,74°47′E; 2600 m)/RRL23228
P4	*R. dentatus* L.	20	Tetraploid (2*n* = 4*x* = 40)	Izmarg (31°20′ N,78° 20′E;2300 m)/RRL23235
P5		20	Tetraploid (2*n* = 4*x* = 40)	Gurinullah (34°34′ N, 75°44′E;2400 m)/RRL23236
P6		20	Tetraploid (2*n* = 4*x* = 40)	Aharbal (33°38′ N, 74°47′E;2300 m)/PUN57702
P7		20	Tetraploid (2*n* = 4*x* = 40)	Doodhganga (34°01 'N, 74°48′E;2400 m)/PUN57839
P8		60	Dodecaploid (2*n* = 12*x* = 120)	Kanzalwan (34°36 ′N, 74°42′E;2700 m)/RRL23237
P9		60	Dodecaploid (2*n* = 12*x* = 120)	Yosmarg (33°47 ′N, 74°39′E;3000 m)/PUN57711
P10	*R. hastatus* D.Don	9	Diploid (2*n* = 2*x* = 18)	Kanzalwan (34°36 ′N, 74°42′E;2300 m)/RRL23238
P11		9	Diploid (2*n* = 2*x* = 18)	Gurinullah (34°34′ N, 75°44′ E; 2400 m)
P12		9	Diploid (2*n* = 2*x* = 18)	Ferozpur nalla (34°10 ′N, 74°25′E;2100 m)/PUN57640
P13	*R. nepalensis* Spreng	30	Hexaploid (2*n* = 6*x* = 60)	Dawar (34°38′ N, 74°47′E;2400 m)/RRL23233
P14		30	Hexaploid (2*n* = 6*x* = 60)	Kanzalwan (34°36 ′N, 74°42′E;2500 m)/RRL23230
P15		40	Octaploid (2*n* = 8*x* = 80)	Patalwan (34°35′E; 2700 m)/RRL23234
P16		40	Octaploid (2*n* = 8*x* = 80)	Tulial (34°37′N, 74°59′E;2600 m)/RRL23231
P17		50	Decaploid (2*n* = 10*x* = 100)	Aharbal (33°38 ′N, 74°47′E;2800 m)/PUN57679
P18		60	Dodecaploid (2*n* = 12*x* = 120)	Izmarg (31°20′ N,78° 20′E;2700 m)/RRL23232
P19		60	Dodecaploid (2*n* = 12*x* = 120)	Mahadev (34°10′N, 75°00′E;2900 m)/PUN57844
P20	*R. orientalis* Bernh. ex Schult.f.	30	Hexaploid (2*n* = 6*x* = 60)	Gurinullah (34°34′ N, 75°44′E;2500 m)/RRL23229
P21		30	Hexaploid (2*n* = 6*x* = 60)	Izmarg (31°20′ N, 78°20′E;2600 m)
P22		30	Hexaploid (2*n* = 6*x* = 60)	Dras (34°27′ N,75°46′E; 2900 m)/PUN57880
P23		30	Hexaploid (2*n* = 6*x* = 60)	Zawoora (34°44′N, 74°48′E;2300 m)/PUN57877

organisation of different cytotypes/species of *Rumex*. Standard LC–MS method was employed for phytochemical analysis based on seven major compound viz. rutin, piceatannol, resveratrol, naringenin, kaempferol, emodin and physcion. There were significant differences in the accumulation pattern of bioactive metabolites in five different species of *Rumex* and also appreciable variability was observed interms of tissue-specificity and ploidy status of the species. Further, the study entails the identification of elite cytotypes for optimum production of desired metabolites for commercial and conservation purposes.

2. Materials and methods

2.1. Plant materials

The wild plants of *R. crispus*, *R. dentatus*, *R. hastatus*, *R. nepalensis* and *R. orientalis* were collected from different localities of the Kashmir Himalayas (Table 1). The plant collections were identified at Herbaria Botanical Survey of India (BSI), Northern circle, Dehradun (India); Janaki Ammal Herbarium, CSIR-Indian Institute of Integrative Medicine (Acronym, HRRL) Jammu, India and Punjabi University, Patiala, India (PUN). Voucher specimens of these plants were deposited in the HRRL and PUN with accession numbers as given in Table 1.

2.2. Meiotic analysis

The young floral buds, 3–4 days prior to anthesis were fixed in freshly prepared Carnoy's fixative (6 alcohol: 3 chloroform: 1 acetic acid v/v/v) for 24 h and then preserved in 70% alcohol at 4 °C in the refrigerator. The cytological preparations were made using the squash technique in 2% acetocarmine (Kumar et al., 2013). To confirm the chromosome number, around 50 pollen mother cells (PMCs) were observed at different stages of meiosis. Photomicrographs of chromosome counts were taken from freshly prepared slides using Nikon 80i Eclipse microscope/Nikon YS 100 ProCam. The previous chromosome reports were compiled from various catalogues to plant chromosome numbers (Darlington and Wylie, 1995; Moore, 1967–1974; Kumar and Subramanian, 1986; Khatoon and Ali, 1993) besides, IPCN database (http://www. tropicos.org).

2.3. Chemicals

MS-grade acetonitrile, water and formic acid, used in the study, were purchased from Merck, Germany. Investigated compounds (rutin, piceatannol, resveratrol, naringenin, kaempferol, emodin and physcion) were procured from Sigma–Aldrich, St. Louis, USA (Fig. 1a–g). Rest of the chemicals and solvents used were of analytical grade.

2.4. Extraction procedure

The extraction was carried out following the protocol provided by National Cancer Institute (NCI, US) (McCloud, 2010). The root, stem and leaf samples of different cytotypes of *R. crispus* (hexaploid and octaploid), *R. dentatus* (tetraploid and dodecaploid), *R. hastatus* (diploid), *R. nepalensis* (hexaploid, octaploid, decaploid and dodecaploid) and *R. orientalis* (hexaploid) were dried under a gentle air stream (temperature of 25 °C ± 2 °C and relative humidity of 65% ± 5%) and pulverized separately to fine powder using a mortar and pestle. The 50 g powder of each sample was serially extracted (33 × 100 mL) with DCM: methanol in the ratio of 1:1 (v/v). The extractions were carried out for a period of 72 h (24 × 3) at room temperature, and every time fresh solvents were used for the left out marc. The filtrates were pooled and filtered through Whatman No. 1 paper, and solvents were removed at 45 °C under reduced pressure using a rotary evaporator (Buchi) to yield the extract.

2.5. Sample preparation of standards and tissue extracts for LC–MS

The stock solutions of standards were prepared in volumetric flasks separately in methanol- water (1:1; v/v). Standard working solutions were then obtained by mixing and making appropriate dilutions of stock solutions using methanol. The concentration utilized for the preparation of eight point calibration curve ranged between 0.39–100 ng/mL for rutin, piceatannol, resveratrol, naringenin, kaempferol, emodin and physcion. The crude extracts of all the root, stem and leaf of collected plant samples were also dissolved in the methanol- water (1:1; v/v) to get 20 mg/mL sample concentration. The standard solutions and crude extracts were filtered through a 0.25 µm disposal membrane filter (Millipore). The stock and working solutions were stored at +4 °C.