EVOLUTIONARY PATTERN AND ALKALOID BIO- SYNTHESIS IN *THALICTRUM*[1]

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Thalictrum L. is one of the large genera in *Ranunculaceae*. More than 120 species are known to be widespread, mostly in the climatically moderate zones of the northern hemisphere (10,66). All species are perennials, usually with creeping rhizomes. In many species large clones may exist along with the heterozygous populations. The plants have alternate and 2–4 pinnate or ternate leaves. Their flowers are usually small, in terminal bracteate panicules or racemes, which are considered to be more specialized and derived from leafy inflorescences like these in *Isopyrum* L. (67). Most of the species are wind pollinated, but this is believed to be not a primary but a secondary reversal from insect pollination (68,69). Another evolutionary trend in the flower biology of *Thalictrum* is the transfer from bisexual to unisexual (monoecious or diecious) flowers (67). Polyploidy is the other major trend in the evolution of the genus. Being perennials, the species of *Thalictrum* easily overcome the setbacks of hybridization and, thus, realize different levels of alopolyploidy. In the higher levels of polyploidy this is most probably combined often with autopolyploidy.

At present some 70 species, more than a half of the species in the genus, have been cytologically studied (70,71,72). For 13 species only a diploid chromosome number is known. In three of the species, *Thalictrum aquilegifolium*, *T. alpinum*, *T. minus* complex, diploid and polyploid cytotypes are known (see table 1). The remaining 54 species are polyploid, and chromosome numbers are recorded ranging from 4x to 22x (70,71,72). Two groups of polyploids can be distinguished. In the first group most of the species have only one cytotype known to date, but in several of the species two cytotypes are known. In the second group, on the contrary, all species have several polyploid cytotypes (70,71,72).

In the variety of polyploid cytotypes a clear difference between the polyploids of the first and the second group can be descerned. In the first group, the hybridization and polyploidization have produced a single cytotype proven to be successful and to propagate freely in different ecological niches. In the second group, however, it seems that the evolutionary changes are still continuing. Thus in different parts of the range of the species and in different ecological niches, different alopolyploids have been established.

This type of evolutionary pattern (differentiation between two groups of species) is further supported by phytochemical data concerning the isoquinoline alkaloids in the species of *Thalictrum*. At present, only seventeen of the species with known chromosome numbers have been studied for their alkaloid content (table 1). For nine species for which alkaloids have been recorded the chromosome number is not yet known (73). Certainly not all species which have been cytologically studied have been thoroughly investigated in a like manner for their alkaloid content (e.g. species such as *Thalictrum aquilegifolium*, *T. tubiferum*, and *T. actaefolium* in table 1). In some of the species only a few of the alkaloids have been recorded, mostly monomers and all in very small quantities. In other diploids, *Thalictrum foetidum*, *T. foliolosum* and *T. alpinum*, the biosynthesis is predominantly monomeric producing mainly protoberberines and only small quantities of dimers. Only in *Thalictrum alpinum* with a known diploid, an

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TABLE 1. Chromosome numbers and alkaloid content in 17 species of *Thalictrum* L.

Taxa	Chromosome number ploidy level 2n=nx (Reference no.)	Alkaloid content[b]									
		Monomers						Dimers			
		simple isoquinolines	benzyltetrahydroisoquinolines	aporphines	protoberberines	other monomers	total monomers	bisbenzylisoquinolines	aporphine-benzylisoquinolines	total dimers	grand total
1	2	3	4	5	6	7	8	9	10	11	12
Sect. Macrogynes Lec.											
Subject. Anomalocarpes Lec.											
1. *T. fendleri* Engelm.	28–4x (1,2,3,4,5, 47) 42–6x (5) 56–8x (5) 70–10x (5)		1	5	4	1	11	2	1	3	14
Subsect. Homalocarpes Lec.											
2. *T. polygamum* Muhl.	84–12x (8,9) 154–22x (3,11)	2	2	4	5	8	21		7	7	28
3. *T. dioicum* L.	28–4x (12) 42–6x (7,12,13, 49)	1	1	2	4	2	10		5	5	15
4. *T. dasycarpum* F.M.	100–14x (7,49) 451–22x (3)	1	1	1	1	3	7	1	2	3	10
Sect. Thalictrum											
Subsect. Thalictrum											
5. *T. aquilegifolium* L.	14–2x (1,2,3,4,7, 14,15,16,17,18, 19,21,48,49,50, 51) 28–4x (20)			1			1				1
6. *T. tubiferum* Maxim.	14–2x (3,80)				1		1				1
7. *T. actaefolium* S.Z.	14–2x (21)				1		1				1
8. *T. foetidum* L.	14–2x (1,2,3,37, 38,57)			1	1		2	2	2	4	6
9. *T. foliolosum* DC.	14–2x (39)	1		1	4		6	4		4	10
10. *T. alpinum* L.	14–2x (1,3,21, 22,23,24,25,26, 27,28,29,30,31, 32,43,44,45,46, 47,79) 16–2x+2(33) 21–3x (43)	2		3	6		11	7	1	8	19
11. *T. isopyroides* C.A.M.	42–6x (2)			1	1	1	3	3		3	6
12. *T. lucidum* L.	28–4x (1,2,3,18, 19,56)			1	6	1	8	7		7	15
T. bulgaricum	28–4x (2)										
13. *T. minus* complex	14–2x (56) 40–6x−2 (24) 42–6x (1,7,18, 19,34,35,49,52, 53,54,60,65,78) 48–7x−1 (40) 70–10x (18,19, 29,57) 72–10x+2 (18, 19,29) 84–12x (41,42)	4	3	6	5	6	24	22	10	32	56
14. *T. rugosum* Poir.	28–4x (2)	3		2	7	6	18	14		14	32

TABLE 1. Continued.

Taxa	Chromosome number ploidy level 2n=nx (Reference no.)	Alkaloid content[b]									
		Monomers						Dimers			
		simple isoquinolines	benzyltetrahydroisoquinolines	aporphines	protoberberines	other monomers	total monomers	bisbenzylisoquinolines	aporphine-benzylisoquinolines	total dimers	grand total
1	2	3	4	5	6	7	8	9	10	11	12
15. *T. simplex* L.	28–4x (63) 56–8x (1,2,18,19,34,46,63) 70–10x (2,3)			3	1	2	6	6		6	12
16. *T. flavum* L.	28–4x (11) 84–12x (1,2,3,18,19,34,35,36,55)	1		1	1	2	5		1	1	6
17. *T. sacchaliense* Lec.	70–10x (64)			3	1		4	1		1	5

[a]Indicated is the number of alkaloids, but not their quantity.
[b]For reference see literature cited 73.

aneuploid and a triploid cytotype, has the isolation of eleven different monomers and eight dimers (seven bisbenzylisoquinolines and a single aporphine-benzylisoquinoline) been recorded. Detailed study of this species is needed in order to investigate the possible presence of a polyploid correlated alkaloids. Several tetraploids like *Thalictrum fendleri*, *T. lucidum*, and *T. simplex* are characterized mainly by monomeric and by dimeric alkaloid biosynthesis which does not differentiate them clearly from the diploids. In *Thalictrum sacchalinense*, a decaploid, only four monomers have been reported to date. At present, it is not known whether protoberberines are precursors of other more complex structures. This may well show that some species are at a more primitive stage in the development of *Thalictrum*. On the other hand they may well form a separate branch adapted to the biosynthesis of the isoquinoline alkaloids in the genus.

In species like *Thalictrum flavum* and *T. dioicum* protoberberines also prevail, but dimeric aporphine-benzylisoquinolines are present even more abundantly in *T. flavum*. *Thalictrum isopyroides*, a hexaploid, has only three monomers in small quantities, while the three bisbenzylisoquinolines are predominant. *Thalictrum polygamum* and *T. dasycarpum* are highly polyploid, reaching up to 2n=22x, and their alkaloid content is rich in aporphine-benzylisoquinoline dimers.

In the complex *Thalictrum minus* the alkaloid pattern is rather complicated (73). A clear correlation between the polyploidy level and the type of the alkaloid produced is known. In hexaploids, the dimeric alkaloids are bisbenzylisoquinolines; in the decaploids only aporphine-benzylisoquinolines are found. Protoberberine alkaloids, mainly berberine, are characteristic for both cytotypes. In some parts of the geographical range, i.e. in the USA, the plants of *Thalictrum minus* complex with unknown chromosome numbers, have been recorded to produce mostly dimeric alkaloids (74), while the protoberberines occur only in very small quantities. Monomers characterize plants of the complex in other parts of its area (USSR) (73), and the dimers that are present are mostly bisbenzylisoquinolines.

Bearing in mind that the phytochemical and biosystematic investigations on *Thalictrum* species are not yet equally advanced, a preliminary picture of the alkaloid biosynthesis may be postulated. In the variety of isoquinoline alkaloids found in different *Thalictrum* species, some characteristic features are observed. In all species studied, quaternary salts of the protoberberines, mainly berberine and/or aporphines, mainly magnoflorine, were isolated. In the greater part of the species dimeric alkaloids have been found (73). In accordance with the scheme suggested by Barton (74), the benzylisoquinoline alkaloids with hydroxyl groups are the precursors of the bisbenzylisoquinoline alkaloids. An intramolecular phenolic coupling leads to the proaporphine-benzylisoquinoline dimers which rearrange into aporphine-benzylisoquinoline alkaloids (75). In 1973, Markekov and Mollov (76), and Shamma in 1977 (77) suggested reticuline to be a precursor of the aprophine portion of the thalicarpine molecule forming the entire structure of the dimer by coupling with the second reticuline molecule. This idea was corroborated by the work of Marekov and Sidjimov (62), who studied the incorporation of labeled benzylisoquinoline precursors and the different biogenetic route of the formation of aporphine-benzylisoquinoline alkaloids in *Thalictrum minus* complex. As to the other scheme of Shamma (71), considering proaporphine-isoquinoline dimers as possible intermediates of aporphine-benzylisoquinolines can be valid for *Berberidaceae* but not for *Thalictrum* plants (59).

In *Thalictrum* species, benzylisoquinoline alkaloids rarely occur. Till now, eight individual alkaloids of this group have been found, five only having a free hydroxyl group (73). According to the present biogenetic theory, only the latter are able to dimerize. All of them have been found in plants in minute or trace quantities. Evidently in these plants, the processes of biosynthesis including the dimerization, is prevailing. The presence of traces of non-dimerized benzylisoquinolines can be explained by their full methylation or conversion into protoberberines, after which they are prevented from dimerization. The benzylisoquinoline alkaloids with the free hydroxyl group are very rare and occur only in traces; this phenomenon corresponds to the concept that their dimerization or synthesis of protoberberine alkaloids occurs more rapidly than their biosynthesis.

Aporphines in *Thalictrum* are also few; there are some eighteen alkaloids with only seven of them having a free hydroxyl group (73). Four of these alkaloids contain this group in position 1. Evidently, at this position, it is inappropriate for them to take part in the dimerization process. In fact, both ortho-positions are occupied and unable then to link in an oxygen bridge. The linkage of such a bridge in position 1 through the available free hydroxyl group has not been reported to date. Therefore, in *Thalictrum* plants, those aporphine alkaloids are principally present which could not be converted into dimeric alkaloids.

Isoquinolones found in some *Thalictrum* species are the result mainly of the oxidation of benzylisoquinolines or dimers. All other monomers are found in all species in very small quantities.

Generally, when comparing the diploid species with different polyploid cytotypes of *Thalictrum* species, the number of monomeric and dimeric alkaloids increases in the second group. In the first group, the biosynthesis affords mostly the production of monomers (mainly protoberberines). Dimers are not found in some diploids and, when present, are few and mostly of the benzylisoquinoline type. Polyploids, ranging from 4x to 22x, are generally richer in alkaloids, monomers and dimers; in the dimers both benzylisoquinolines and aporphine-benzylisoquinolines are present. One can think that the genetic changes in the polyploids have opened the way to higher phases of biosynthesis and for a greater number of alkaloids with a more complex structure. There are even cases when only aporphine-benzylisoquinoline alkaloids of the dimers are known to be biosynthesized in high polyploids such as *Thalictrum dasycarpum*, *T. polygamum* and some cytotypes of *T. minus* complex.

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