

Table S1. 5S and 26S rDNA loci numbers and chromosomal positions in 42 *Trifolium* species belonging to subgenus *Chronosemium* and subgenus *Trifolium* (7 sections).

| Subgenus/Section | Trifolium species | 2n | Loci number per 2n | | Position | |
|---|-------------------------------|--|--------------------|--------|----------|---------|
| | | | 5S | 26S | | |
| Chronosemium | | | | | | |
| | T. aureum ^a ▪ | 2x=16 | 4 | 2 | AS OCP | |
| | | 2x=14 | 4 | 2 | AS OCP | |
| | T. badium ^a ▪ | 2x=14 | 2 | 4 | S | |
| | | | | 2 | 2 | S |
| | T. campestre ^a ▪ | 2x=14 | 2 | 2 | S | |
| | T. micranthum ^a ▪ | 2x=16 | 2 | 2 | S | |
| | T. dubium ^a ▪ | 4x=30 | 4 | 4 | S | |
| | | | | | | |
| Trifolium | | | | | | |
| Trifolium | T. alpestre ^a ▪ | 2x=16 | 10 | 2 | CBS1 AS | |
| | | | 11 | 2 | CBS1 AS | |
| | T. arvense ^a ▪ | 2x=14 | 2 | 2 | S | |
| | T. bocconeii ^a ▪ | 2x=12 | 2 | 2 | S | |
| | T. cherleri ^a ▪ | 2x=10 | 4 | 10 | CBS1 AS | |
| | T. diffusum ^a ▪ | 2x=16 | 2 | 2 | S | |
| | T. hirtum ^a ▪ | 2x=10 | 6 | 2 | AS OCP | |
| | | | | | | |
| | T. ligusticum ^a ▪ | 2x=12 | 2 | 2 | S | |
| | | 2x=14 | 2 | 2 | S | |
| | T. pallidum ^a ▪ | 2x=16 | 4 | 2 | CBS1 AS | |
| | T. purpureum ^a ▪ | 2x=14 | 2 | 2 | S | |
| | T. rubens ^a ▪ | 2x=16 | 4 | 2 | S | |
| | T. squamosum ^a ▪ | 2x=16 | 4 | 2 | S | |
| | T. stellatum ^a ▪ | 2x=12 | 4 | 2 | S | |
| | | 2x=14* | 4 (2w) | 2 | S | |
| | T. pannonicum ^a ▪ | 16x=128 | 16 | 16 | S | |
| | | T. pratense ^b ▪ | 4x=28 | 8 | 8 | CBS2 AS |
| | | T. medium ^b ▪ | 8x=64 | 12 | 8 | S |
| | Trichocephalum | T. subterraneum subsp. subterraneum ^a ▪ | 2x=16 | 2 | 4 (2w) | S |
| T. subterraneum subsp. subterraneum ^c ▪ | | 2x=16 | 2 | 2 | S | |
| T. subterraneum subsp. brachycalycinum ^c | | 2x=16 | 2 | 4 (2w) | S | |
| T. israeliticum ^c ▪ | | 2x=12 | 10 | 4 | CBS2 AS | |
| | | | | | | |
| Vesicastrum | T. fragiferum ^a ▪ | 2x=16 | 2 | 2 | AS OCP | |
| | | 2x=16 | 2 | 2 | AS OCP | |
| | T. resupinatum ^a ▪ | 2x=14 | 2 | 2 | AS OCP | |
| | | | 2 | 2 | S | |
| | T. spumosum ^a ▪ | 2x=16 | 4 | 2 | CBS1 AS | |
| Trifoliastrum | T. glomeratum ^a ▪ | 2x=16 | 2 | 2 | S | |
| | T. montanum ^a ▪ | 2x=16 | 2 | 2 | S | |

| | | | | | |
|---------------|---|--------|--------|----|---------|
| | <i>T. occidentale</i> ^d ▪ | 2x=16 | 4 | 2 | CBS1 AS |
| | <i>T. pallescens</i> ▪ | 2x=16 | 2 | 2 | AS OCP |
| | <i>T. thalii</i> ▪ | 2x=16 | 2 | 2 | S |
| | <i>T. repens</i> ^d ▪ | 4x=32 | 4 | 2 | CBS1 AS |
| | <i>T. uniflorum</i> ^d ▪ | 4x=32 | 4 | 4 | CBS2 |
| | <i>T. nigrescens</i> subsp. <i>nigrescens</i> ^d | 2x=16 | 2 | 2 | CBS1 |
| | <i>T. nigrescens</i> subsp. <i>petrisavii</i> ^d ▪ | 2x=16 | 2 | 2 | S |
| | <i>T. ambiguum</i> ^d ▪ | 2x=16 | 2 | 2 | S |
| | <i>T. hybridum</i> ^d ▪ | 2x=16 | 2 | 2 | CBS1 |
| | <i>T. isthmocarpum</i> ^d ▪ | 2x=16 | 2 | 6 | CBS1 AS |
| Involucrarium | <i>T. chilense</i> ▪ | 2x=16 | 4 | 2 | CBS1 AS |
| | <i>T. microdon</i> ▪ | 2x=16 | 2 | 2 | CBS1 |
| | <i>T. microcephalum</i> ▪ | 2x=16 | 16 | 16 | CBS8 |
| | | | 16 | 2 | CBS1 AS |
| Paramesus | | | 4 | 2 | CBS1 AS |
| | <i>T. glanduliferum</i> ▪ | 2x=16 | 5 (1w) | 2 | CBS1 AS |
| | | | 4 | 2 | S |
| | <i>T. strictum</i> ▪ | 2x=14 | 2 | 2 | S |
| Lupinaster | <i>T. lupinaster</i> ▪* | 4x=28* | 8 | 4 | CBS2 AS |
| | | 4x=32 | 8 | 4 | CBS2 AS |

* Newly described chromosome number. Published in ^aAnsari et al. [33], ^bDluhošová et al. [37], ^cFalisticco et al. [13], ^dAnsari et al. [36]. w – weaker signals than the others; S – signals on separate chromosomes; AS OCP – all signals on one chromosome pair; CBSn AS – co-localization of both signals on n-chromosome pairs, another on separate chromosomes; CBSn – co-localization of both signals on n-chromosome pairs. ▪ indicates sequences used in the tree (Figure S7), with *A. thaliana* used as an outgroup.