An improved method for high-temperature induced embryo sac chromosome doubling in *Populus simonii* Carr. × *P. nigra* var. *italica* (Moench.) Kochne, an interspecific hybrid of *Tacamahaca* and *Aigeiros* poplars

Wenting Xu¹, ²#, Liqin Guo¹, ³#, Yan Zhang¹, Jian Zhao¹, Zunzheng Wei⁴*, Jinfeng Zhang¹*

¹Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, National Engineering Laboratory for Tree Breeding, Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants of Ministry of Education, Key Laboratory of Forest Trees and Ornamental Plants biological engineering of State Forestry Administration, College of Biological Sciences and Technology, Beijing Forestry University, Beijing, 100083, P. R. China

²State Key Laboratory of Subtropical Silviculture, School of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou, 311300, P. R. China

³Beijing Academy of Forestry and Pomology Sciences, Beijing, 100093, P. R. China

⁴Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Ministry of Agriculture, Key Laboratory of Urban Agriculture (North), Ministry of Agriculture, Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100091, P. R. China

*Corresponding Authors

Jinfeng Zhang: zjf@bjfu.edu.cn

Zunzheng Wei: weizunzheng@163.com

#These authors contributed equally to this work.
### Supplementary table S1 Developmental process of megasporocytes after pollination in *P. simonii × P. nigra var. italica*

| Time after pollination (h) | Meiosis I | Meiosis II | Uni-nucleate embryo sac | Two-nucleate embryo sac | Four-nucleate embryo sac | Eight-nucleate embryo sac and post | Total numbers |
|----------------------------|------------|------------|--------------------------|--------------------------|--------------------------|----------------------------------|---------------|
| 0                          | 8          | 18         | 23                       | 9                        | 5                        | 63                               |               |
| 12                         | 3          | 20         | 21                       | 11                       | 4                        | 1                                | 60            |
| 24                         | 2          | 22         | 24                       | 13                       | 6                        | 1                                | 68            |
| 36                         | 2          | 12         | 23                       | 16                       | 7                        | 3                                | 63            |
| 48                         | 10         |            | 20                       | 21                       | 9                        | 4                                | 66            |
| 60                         | 18         |            | 23                       | 15                       | 7                        | 4                                | 67            |
| 72                         | 11         |            | 22                       | 12                       | 13                       | 9                                | 67            |
| 96                         | 3          |            | 12                       | 13                       | 17                       | 14                               | 59            |
| 120                        | 11         |            | 27                       | 23                       | 9                        | 67                               | 61            |
| 144                        |            |            | 21                       | 32                       | 13                       | 17                               | 53            |

### Supplementary table S2 Survival capsules in high temperature preliminary experiment

| Treatment duration (h) | Temperature (°C) | CK       |
|------------------------|------------------|----------|
|                        | 36               | 41       | 46       |               |
| 1                      | 99/113           | 108/119  | 4/131    | 104/118       |
| 3                      | 122/135          | 61/127   | 0/116    |               |
| 5                      | 88/102           | 0/133    | 0/124    |               |