In vitro propagation and genetic stability assessment of *Plectranthus amboinicus* and *Thymus* × *citriodorus*

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Material and Method

- *Plectranthus amboinicus* (Lour.) Spreng and *Thymus* × *citriodorus* (Pers.) Schreb.

In vitro culture

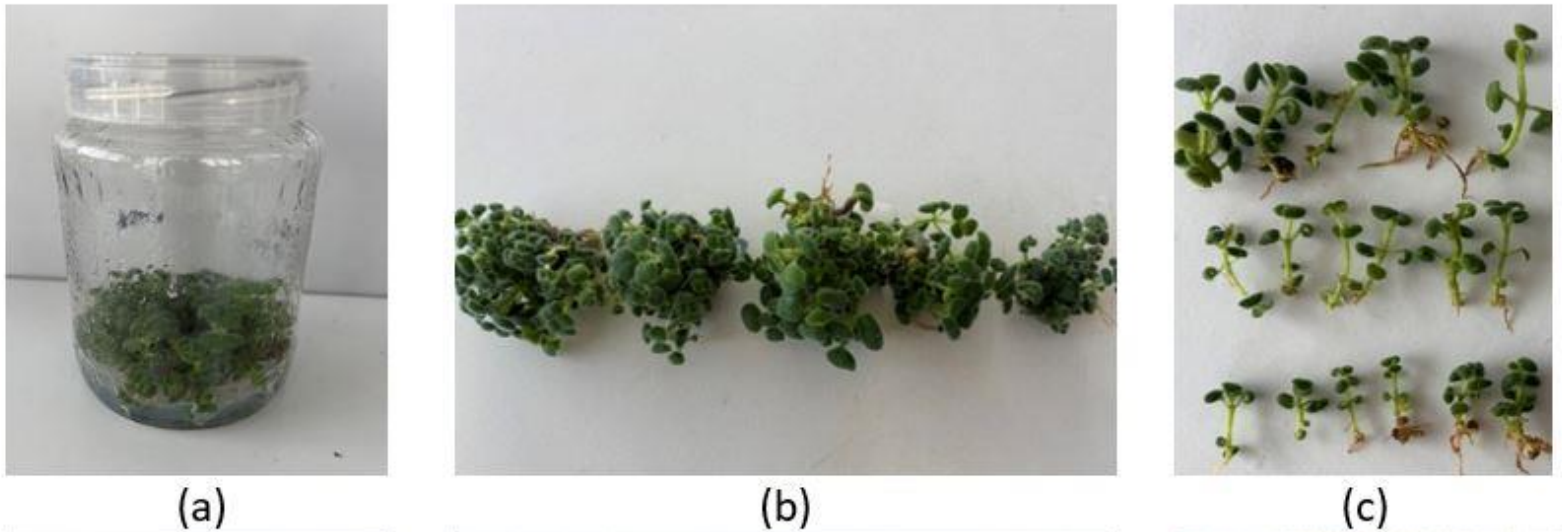
- culture medium for shoot proliferation was Driver and Kuniyaki Walnut (DKW) + 0.5 mg/L 6-benzyladenine (BA) + 30 g/L sugar +0.5 % (w/v) Plant agar. pH = 5.8 before adding the agar, autoclaved at 120 °C and 1 atm for 20 minutes;
- five explants were inoculated in each jar;
- in vitro rooting - DKW medium without PGRs -20 seedlings/jar;
- after inoculation, the culture dishes were incubated in the growth chamber in a controlled environment (22 ± 1 °C, 32.4 mmol·m^{-2}·s^{-1}, 16 h photoperiod).

Genetic Fidelity

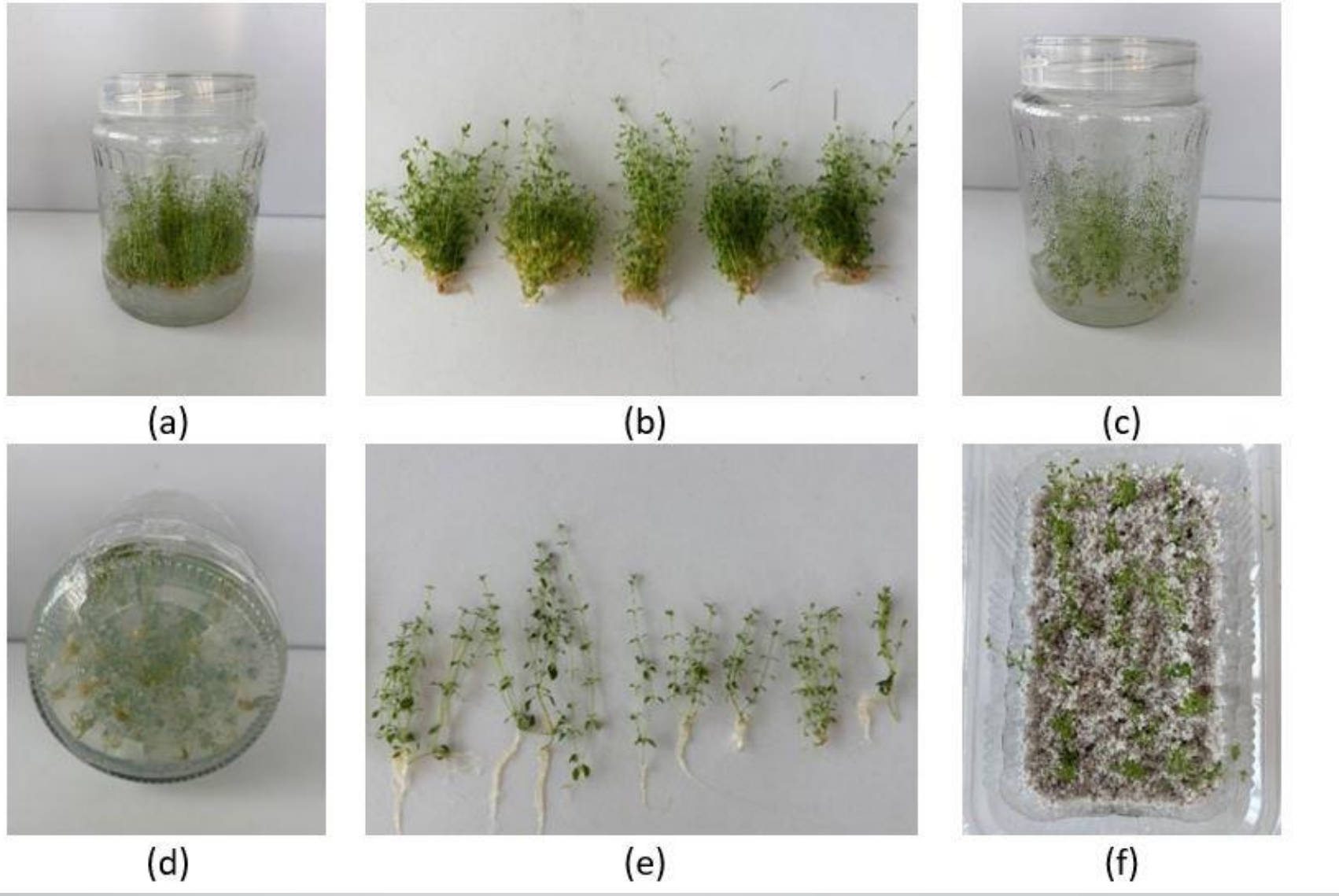
Primer name	Primer sequence 5'-3'
SCoT 1	CAACAATGGCTACCACCA
SCoT 3	CAACAATGGCTACCACCG
SCoT 4	CAACAATGGCTACCACCT
SCoT 10	CAACAATGGCTACCAGCC
SCoT 11	AAGCAATGGCTACCACCA
SCoT 25	ACCATGGCTACCACCGGG

To assess genetic uniformity between in vitro raised plants and the mother plants, SCoT gel images were analyzed using TotalLab software. The number and size range (base pairs) of amplified bands were recorded, noting that band intensity was not considered in scoring.

Results and Discussion

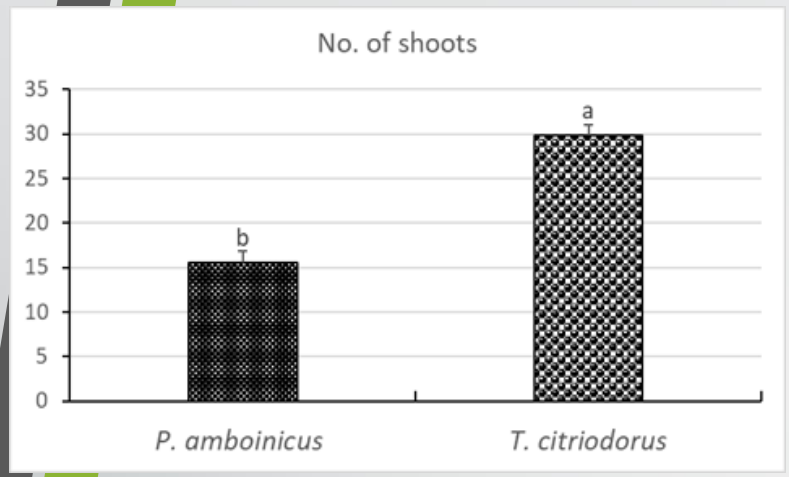


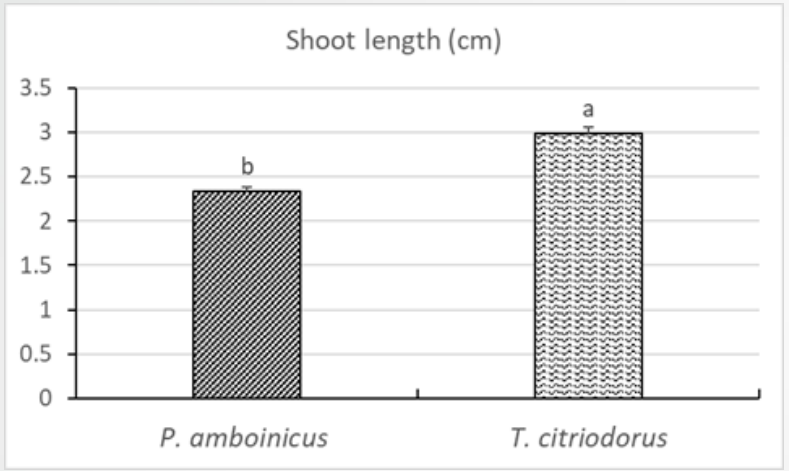
In vitro propagation of *P. amboinicus*: (a–c) Multiplication stage on the medium DKW + 0.5 mg/L BA; (d-e) in vitro rooted plantlets on hormone-free DKW; (f) Acclimatization stage.

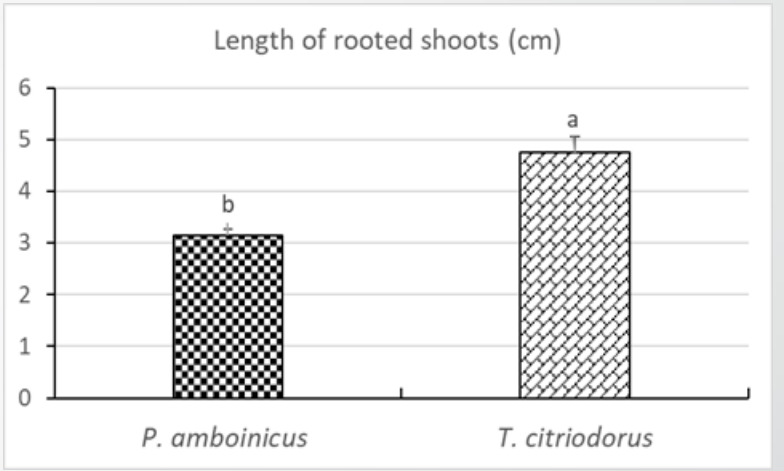


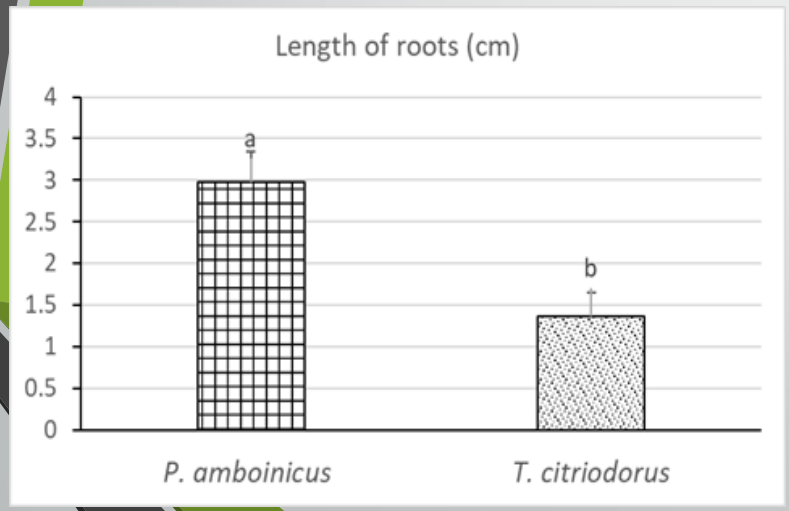
In vitro propagation of *T. citriodorus*: (a–b) Multiplication stage on the medium DKW+0.5 mg/L BA; (c-e) in vitro rooted plantlets on hormone-free DKW; (f) Acclimatization stage.

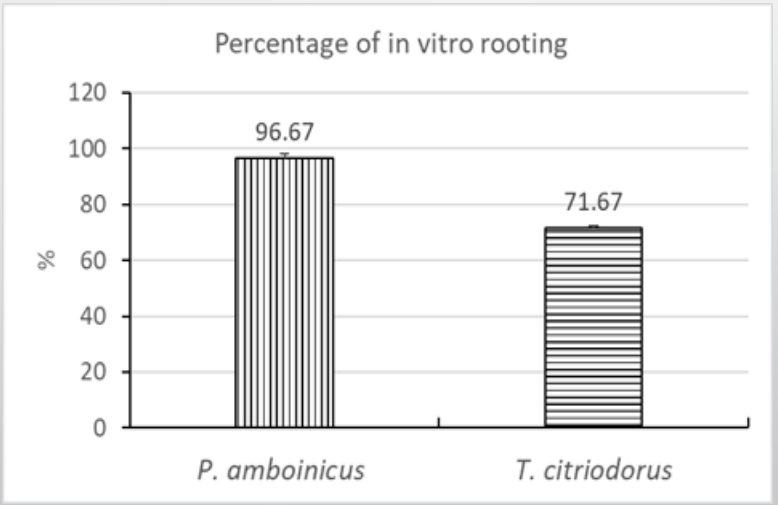
In vitro propagation and acclimatization of *P. amboinicus and T. citriodorus*

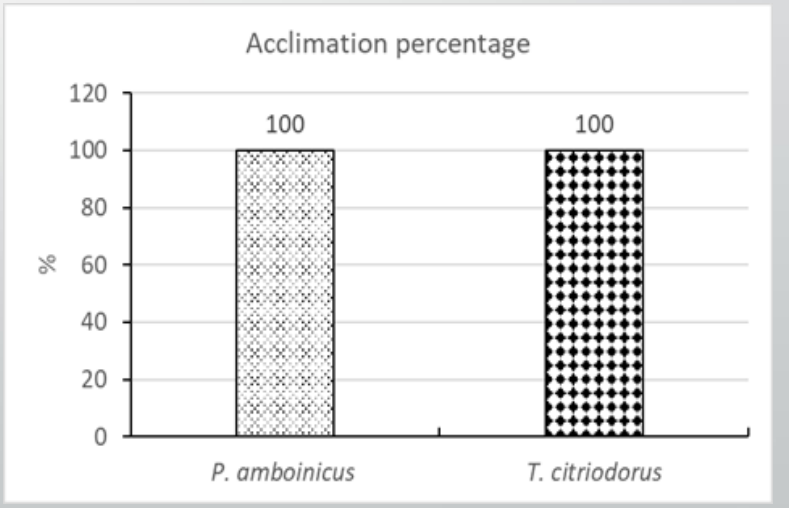






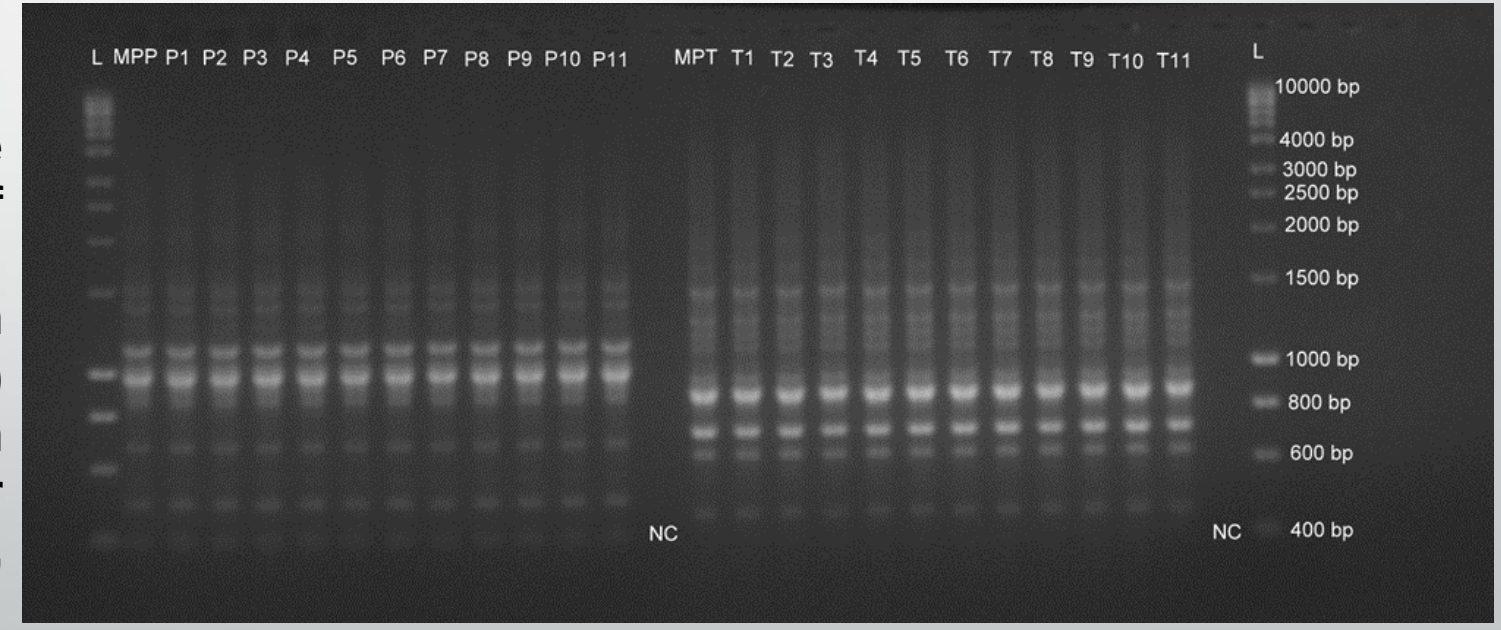






Primer name	Primer sequence 5′-3′	No. of monomorphic bands		Size range of bands (bp)	
		Plectranthus amboinicus	Thymus × citriodorus	Plectranthus amboinicus	Thymus × citriodorus
SCoT 1	CAACAATGGCTACCACCA	7	6	1000-3500	900-3000
SCoT 3	CAACAATGGCTACCACCG	10	9	400-2500	450-2000
SCoT 4	CAACAATGGCTACCACCT	8	7	500-2000	400-1500
SCoT 10	CAACAATGGCTACCAGCC	7	6	500-2000	450-2000
SCoT 11	AAGCAATGGCTACCACCA	9	8	400-2500	400-2000
SCoT 25	ACCATGGCTACCACCGGG	8	7	400-2500	300-3000
Total		49	43		

Number and size range of monomorphic amplified SCoT bands detected in the medicinal plant species *Plectranthus amboinicus* and *Thymus × citriodorus*.



The genetic profile generated with the SCoT 3 primer for the mother plants of *Plectranthus amboinicus* (MPP) and *Thymus × citriodorus* (MPT) and their in vitro raised plants (P1-P11; T1-T11) illustrates the genetic identity from each medicinal plant species L – molecular marker, 1 kb Ladder (Fermentas, Leon-Rot, Germany); NC-negative control.

Conclusions:

- DKW culture medium can be successfully used in both the multiplication and in vitro rooting phases for the two aromatic plant species, *P. amboinicus* and *T. citriodorus*.
- BA at a concentration of 0.5 mg/L effectively induces the proliferation of a high number of shoots in both species.
- The SCoT markers system is a valuable tool for assessing genetic fidelity and can be used to test the in vitro micropropagated plants of *P. amboinicus* and *T. citriodorus.*

Acknowledgements:

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