Application of In vitro Techniques and X-ray Irradiation to Enhance the Variability of Morphological Traits of *Turnera ulmifolia* L. a Potential New Ornamental

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Objective

Turnera ulmifolia is with its striking yellow flowers a potential new ornamental. The production of extra floral nectar makes it to an insect-friendly flower plant in gardens.

For propagation and improvement of morphological traits such as providing *T. ulmifolia* with a more compact growth or a longer flowering an in vitro protocol was elaborated. As explants were used nodal segments as well as leaf pieces.

Key issue

- Elaboration of an in vitro protocol.
- Are there somaclonal variations?
- Use of X-rays to increase the variations.

Results

In vitro culture of nodal explants

Induction medium: **DA-4**:
MS, 2.2 µM BAP, 0.9 µM TDZ, 3% sucrose

6 weeks

Shoot growth medium **DA-1-Fe**:
MS, 11.0 µM BAP, 100 µM FeNaEDTA, 3% sucrose (2 subcultures)

6 weeks

Rooting media:

- MS, 100 µM FeNaEDTA, 3% sucrose
- MS, 2.5 µM IBA, 100 µM FeNaEDTA, 3% sucrose (1 subculture each)

8 – 12 weeks

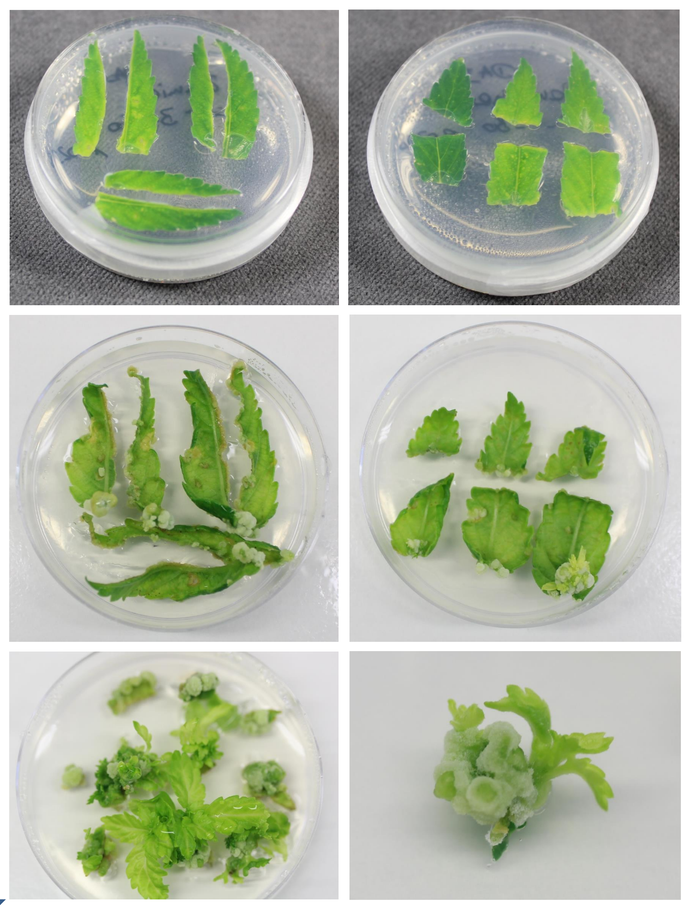
Acclimation to soil and transfer to greenhouse

The protocol enables a rapid plant multiplication from nodal explants. The addition of FeNaEDTA was essential to grow green plants. A short callus stage took place. The rooting frequency amounted 87.2%. 99.0% of rooted plants (69 plants) were transferred to the greenhouse. Compared to the control plants (plants without tissue culture), in vitro derived plants showed often a more compact growth after transfer into soil. Variations in flower colour or flower structure were not detected. However, out of 67 plants 10 had a broad crenate stem. Somaclonal variation or the hormone treatment during the tissue culture might be the reasons. The DNA content was estimated by flow cytometry. It was the same for all plants at 2.88 ± 0.02 pg.

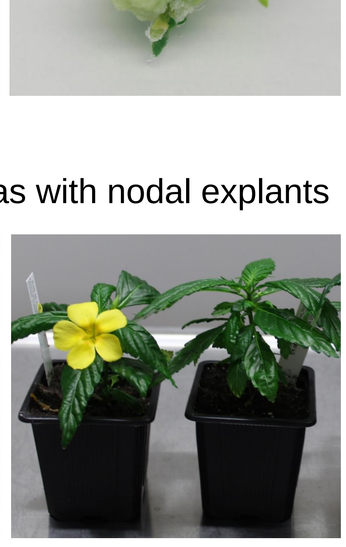
In vitro culture of leaf segments

Induction medium: **DA-1-Fe**:
MS, 11.0 µM BAP, 100 µM FeNaEDTA, 3% sucrose (3 subcultures)

12 weeks



Rooting and acclimation as with nodal explants



Leaf explants are suitable for both for propagation and irradiation. For a high efficiency the abaxial leaf surface should be orientated to the medium. Callus and subsequently new shoots evolved at the fresh cutting sites. Of 54 explants, 33 developed calli (61.1%). At the calli numerous shoots emerged. 64 shoots were transferred to rooting media as the plants from nodal explants. Rooting frequency amounted 76.6%. All 46 rooted plants were successfully acclimated to greenhouse. Seven plants derived from leaf explants showed crenate stems.

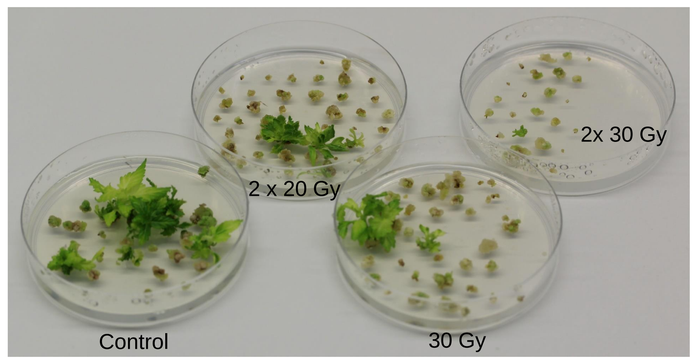
X-ray irradiation of leaf segments

For conditioning leaf explants were cultured on induction medium DA-1-Fe for 4 days. Then, the X-ray irradiation was performed. After two subcultures shoots were transferred to rooting medium MS, 2.5 µM IBA, 100 µM FeNaEDTA.

Table 1. Survey about X-ray irradiations

X-ray treatment	№ of explants	№ of calli	№ of shoots	№ of rooted plants
30 Gy 3 Gy min^{-1}	36	71	18	-
2 x 30 Gy 3 Gy min^{-1}*	36	24	2	-
2 x 20 Gy 3 Gy min^{-1}*	36	80	20	-
10 Gy 3 Gy min^{-1}	72	81	19	4
10 Gy 0.9 Gy min^{-1}	36	15	5	2
2 x 10 Gy 3 Gy min^{-1}*	36	19	2	-
5 Gy 0.9 Gy min^{-1}	36	23	28	7
2x 5 Gy 0.9 Gy min^{-1}*	36	27	20	5
Control	80	132	34	3

*Interval irradiation with a pause of 4 hrs between the treatments



- After all eight X-ray irradiations from 5 – 40 Gy calli have grown on leaf explants (Table 1).
- A surprisingly high number of calli was observed at 30 Gy and 2 x 20 Gy, respectively.
- The shoot development was inhibited. The shoots continued to grow only delayed and died.
- Currently, the few rooted irradiated plants are transferred to the greenhouse.

Plants in greenhouse:
Regenerated plants from nodal explants

Plant with crenate stem

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