In vitro cultures of *Perovskia atriplicifolia*

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***Perovskia atriplicifolia* 'Hybrida'**
photo Stan Shebs

Roots of Perovskia

Introduction

Perovskia, the genus closely related to Salvia and a sister taxon to Rosmarinus, comprises seven species, widespread throughout Central Asia (mainly Afghanistan, Iran and Usbekistan). Several varieties of the species *P. atriplicifolia* are planted worldwide as ornamentals, known under a name Russian Sage. The main active compounds of Perovskia roots are quinoid diterpenes called tanshinones. However, they have been reported so far only from *P. abrotanoides*. As in other related members of Lamiaceae, a presence of abundant fraction of hydroxycinnamic derivatives, was also confirmed.

Our aim was to investigate the possibility of obtaining true to type plants of *P. atriplicifolia* by in vitro regeneration. We have studied the ability of explants from shoot apical buds to develop multiple axillary shoots upon hormonal treatment..

Material and Methods

The seeds were collected from the four years old plants of *P. atriplicifolia cv 'Hybrida'* and wild-type *P. atriplicifolia* cultivated in the **Botanical Garden of Medicinal Plants** in our department. Seeds were disinfected by washing in ethanolic solution of chlorhexidine for 2 min followed by sodium hypochlorite for 15 min. The seed, after washing 4 times in sterile distilled water were placed on ½ MS basal solid medium in Petri dishes and incubated in the dark for germination. The aseptically grown seedlings, were used for excision of apical buds. The apices were incubated on regeneration medium selected during preliminary experiment (modified MS medium solidified with 6% agar). The cultures were maintained under fluorescent illumination with photoperiod of 16 h , at a constant temperature 25 °C. After 30 days of culture,, following parameters were recorded: percentage of responding explants, number of axillary shoots, length of regenerating shoots after 30 days of culture.

The well developed, regenerated axillary shoots were excised and rooted in ½ MS agar medium containing. 0.1 mg/L IAA.

The rooted plantlets were potted and acclimatized to the greenhouse conditions for hardening, before transfering to the garden.

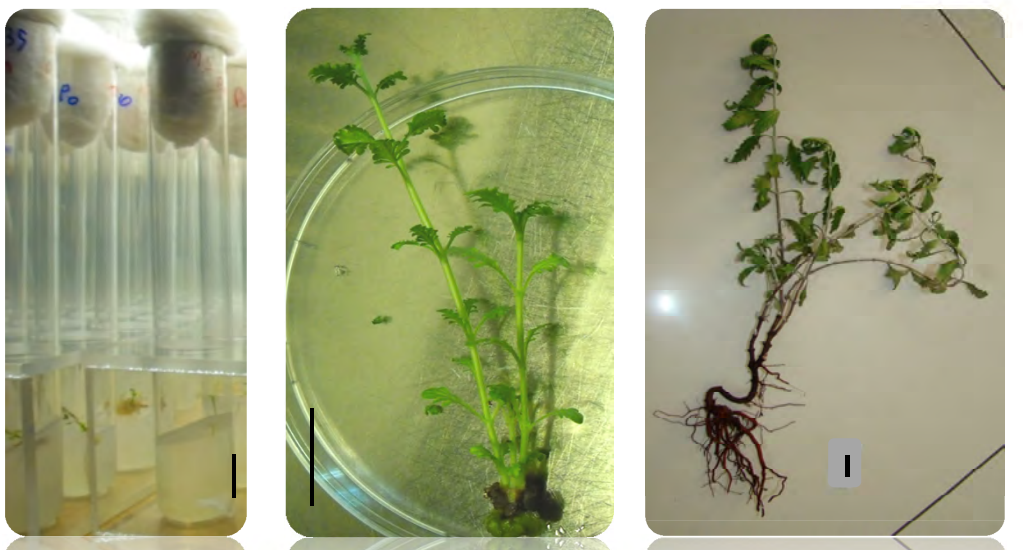
Results

Axillary bud break

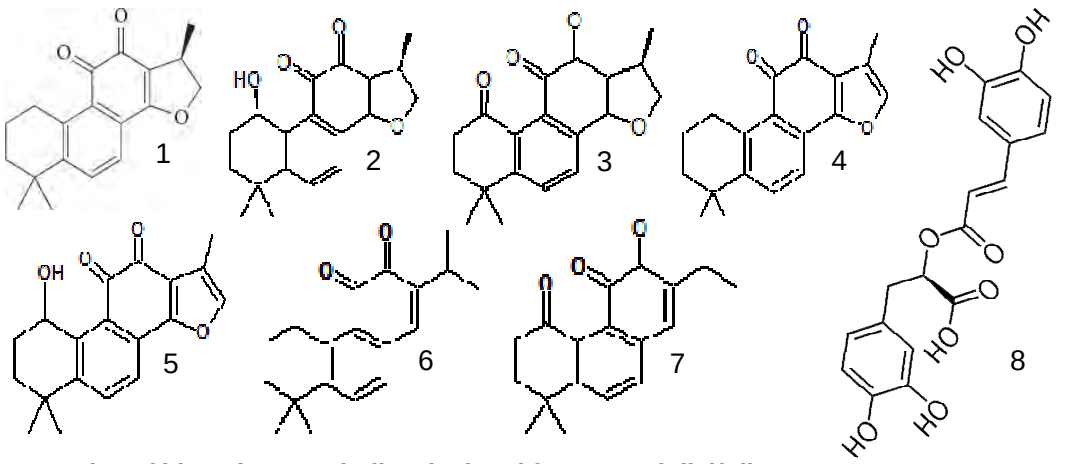
The optimal medium for this stage of culture was MS medium enriched with 30 g/l sucrose, MS vitamins and plant growth reuglators: auxin IAA – 0.1 mg/L and cytokinin BAP – 1 mg/mL. On the control medium without PGRs, only the single main shoot elongation was observed, with rarely occurring one lateral shoot (in no more than 25% of all explants). Two varietes responded differently in terms of axillary shoot regeneration percentage, but other differences were not significant.

Rooting and hardening of regenerants

For rooting, the selected medium was 100% efficient. Also, the hardening of the plantlets and transfer into the garden was without losses. The plants obtaiined from in vitro culture were morphologically identical to the parent plant, and the roots that are important source of bioactive compounds were thick and well developed.



Regeneration of apical explants:
A: primary explants in test tubes;
B: Regenerated shoots;
C: Whole plant obtained from rooted shoots after one season in garden conditions – red-colored roots indicate high tanshinone content. Bar = 2 cm



Examples of bioactive metabolites isolated from *P. atriplicifolia* roots.
1 – cryptotanshinone; **2** – 1-β-hydroxycryptotanshinone; **3** – 1-oxocryptotanshinone; **4** – tanshinone IIA; **5** – 1-β-hydroxytanshinone IIa; **6** – miltirone; **7** - 1-oxomiltirone; **8** – rosmarinic acid

Regeneration efficiency of ornamental variety 'Hybrida' and wild type of *Perovskia atriplicifolia* on control medium without hormones and on medium enriched in exogenous IAA and BAP

	P.a. 'Hybrida' MS – control	P.a. 'Hybrida' IAA/BAP	P. atriplicifolia MS control	P. atriplicifolia IAA/BAP
% explants with axillary shoots	11.1 (n=45)	72.5 (n=80)	25.0 (n=28)	59.3 (n=81)
mean number of shoots	1	1.81	1	1.64
mean shoot length [cm]	6.7	6.7	5.6	4.4

Conclusion

✓The results of *in vitro* regeneration experiment are preliminary and the efficiency of the multiplication of plantlets is still not satisfactory (less than two axillary shoots per explant on average).

✓ However, the regenerated plants are of good quality and the *in vitro* technique can be a useful way for obtaining a high number of cloned, superior material. For this purpose, the culture conditions have to be optimized fo improved mulitplication rate with preserving the uniformity of regenerants. The respective experiments have been initiated, as well as the evaluation of phytochemical quality of the *in vitro* derived plants with respect to tanshinone and rosmarinic acid content.