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BRIEF COMMUNICATION

# Plant micropropagation and callus induction of some annual *Salsola* species

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## Abstract

Micropropagated plants of two annual haloxerophytic Asiatic *Salsola* species (*S. pestifer* and *S. lanata*) were obtained from zygotic embryos cultured on Murashige and Skoog (MS) agar medium supplemented with 0.5 μM benzylaminopurine (BAP) and 0.3 μM indole-3-acetic acid (IAA) or with 0.5 μM 6 γ,γ-dimethylallylaminopurine and 0.3 μM IAA. The callus induction from shoot and leaf explants derived from plants propagated *in vitro* were obtained on MS agar medium with various concentration of auxins and cytokinins. The best medium for growth and proliferation of calluses of both studied species was MS medium containing 9.0 μM 2,4-dichlorophenoxyacetic acid. It was also determined that beginning of plant regeneration from callus of *S. lanata* was induced by 8.8 μM BAP.

*Additional key words*: *Chenopodiaceae*, *in vitro* culture, *Salsola lanata*, *Salsola pestifer*.

The annual *Salsola* species such as *S. pestifer* and *S. lanata* are widespread haloxerophytes growing in open communities of inland salt marshes and/or marshy steppe communities under harsh desert environments of Southern Kyzylkum. *S. lanata* forming a distinct and almost monospecific community in salt/affected arid lands have a high competitive vigour to such environments. *S. pestifer* with slender leaves occurs on dry and sandy saline soils (Li 2000). These annual *Salsola* species are a good desert fodder plant in spring (*S. pestifer)* and in autumn-winter *(S. lanata)* period for sheep, goats and camels. Our previous data have shown that due to their salt-tolerance these species can be considered as excellent source for selection of more salt-tolerant group of plants for improvement of arid/semiarid lands using *in vitro* cell and tissue culture (data not published). However, there are no information about tissue culture techniques within the genera *Salsola.* So, the aim of our study was to elaborate the procedure for plant micropropagation and to obtain actively growing calluses of *S. pestifer* and *S. lanata.*

For experiments performed in the spring-summer season of 2001, the following species were used: *Salsola lanata* Pall. (= *Climacoptera lanata*, Pyankov *et al.* 2001) and S. *pestifer* A. Nelson. Mature seeds of *S. lanata* were collected from plants growing in Southern Kyzylkum desert (Central Asia). The seeds of *S. pestifer* were obtained from two populations growing under different ecological conditions: Bukhara population (from Southern Kyzylkum desert) and Samarkand population (from Zerafshan valley). Before excision of the embryos the seeds were surface-sterilized in 70 % ethanol for 1 min, then transferred to 50 % *Chlorox* for 10 min and subsequently washed 3 times with sterile distilled water. Embryos isolated under the stereo microscope were placed on two types of media: MS medium solidified with 0.8 % agar containing 2.3 μM kinetin and 2 % sucrose and

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*Abbreviations*: BAP - 6-benzylaminopurine, 2,4-D - 2,4-dichlorophenoxyacetic acid, 2iP - 6 γ,γ-dimethylallylaminopurine, IAA - indole-3-acetic acid, IBA - indole-3-butyric acid, KIN - kinetin, MS - Murashige and Skoog (1962) medium.

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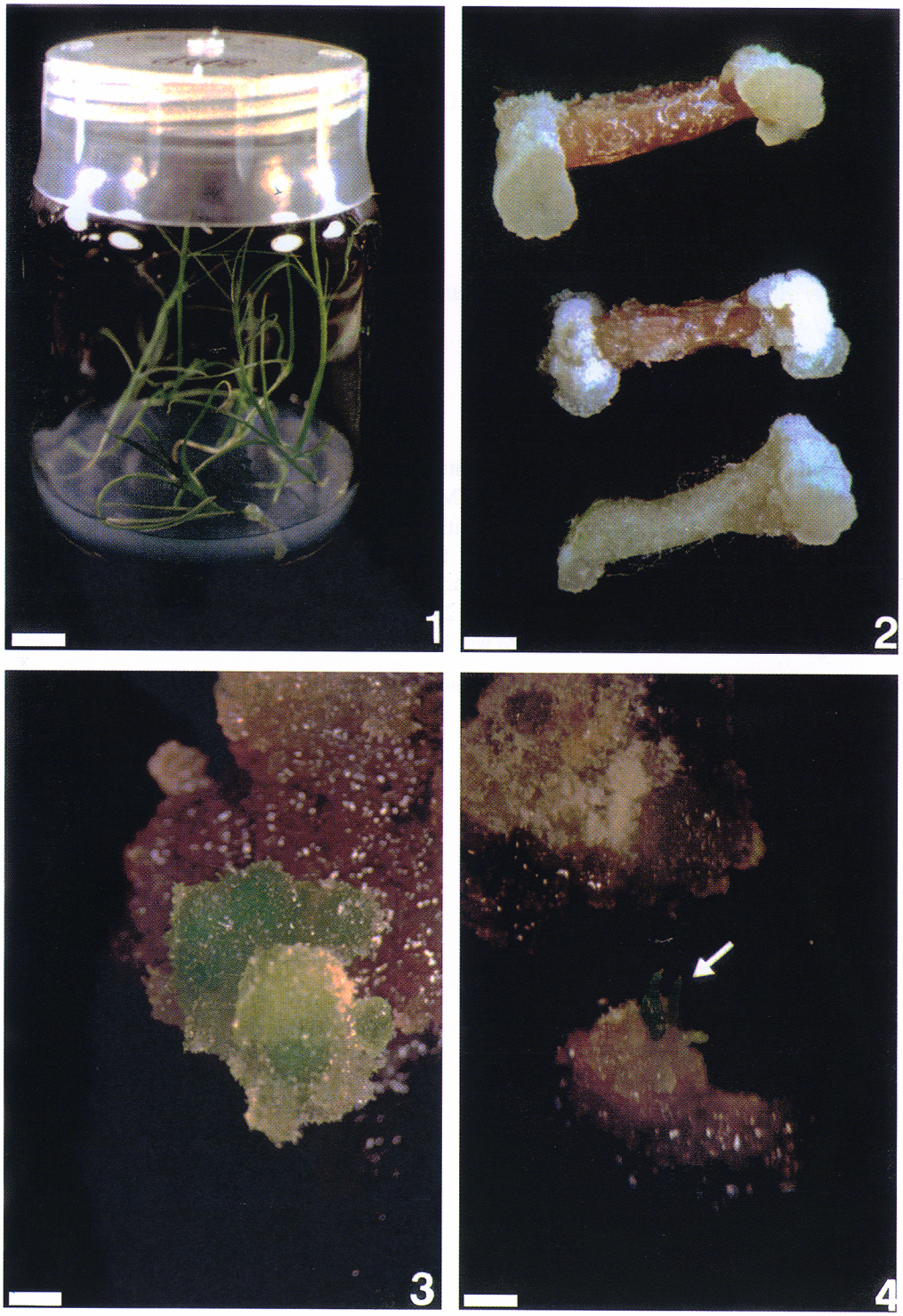


Fig. 1. Green and branching young plants of *S. pestifer* (desert population) after 6 weeks on micropropagation medium (MS + 0.5 μM BAP + 0.3 μM IAA) (*bar* = 1.1 cm).
Fig. 2. Cream-yellow callus covered both cut ends of shoot explants of *S. lanata* (2nd week on MS medium with 9.0 μM 2,4-D) (*bar* = 1.0 mm).
Fig. 3. Green centers on the surface of *S. lanata* callus after one week on regeneration medium (MS + 8.8 μM BAP) (*bar* = 0.5 mm).
Fig. 4. Adventitious bud (*arrow*) developed from *S. lanata* callus after 4 weeks on regeneration medium (MS + 8.8 μM BAP) (*bar* = 0.5 mm).

Table 1. Percentage of *Salsola* explants producing callus on media with various growth regulators (GR).

| GR | [μM] | *S. pestifer* (valley population) | | | | *S. pestifer* (desert population) | | | | *S. lanata* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | shoot | | leaf | | shoot | | leaf | | shoot | | leaf | |
| | | total number | with callus [%] | total number | with callus [%] | total number | with callus [%] | total number | with callus [%] | total number | with callus [%] | total number | with callus [%] |
| BAP | 8.8 | 46 | 100.0 | 77 | 81.8 | 48 | 91.7 | 90 | 68.9 | 58 | 5.2 | 90 | 38.1 |
| BAP | 22.0 | 7 | 42.9 | 46 | 15.2 | 2 | - | 49 | - | 11 | - | 49 | 4.8 |
| KIN | 9.3 | 20 | 40.0 | 20 | 65.0 | 19 | 94.7 | 16 | 100.0 | 18 | - | 16 | 64.7 |
| 2iP | 4.9 | 19 | - | 20 | - | 18 | 100.0 | 14 | 28.6 | 23 | - | 14 | 80.0 |
| IBA | 4.9 | 20 | 95.0 | 20 | 35.0 | 25 | 100.0 | 21 | 100.0 | 19 | 31.6 | 21 | 31.6 |
| 2,4-D | 9.0 | 13 | - | 13 | - | 18 | 94.4 | 20 | 100.0 | 18 | 100.0 | 20 | 93.3 |

also on pure water with 0.8 % agar. The pH of both media was adjusted to 5.5 before autoclaving. Embryos were cultured in Petri dishes in darkness at 24 °C.

For rapid multiplication, and to obtain mature plants, seedlings (reaching a size of 5 cm) were transferred onto the basic MS medium in two combinations. One of them was supplemented with 0.5 μM BAP, the other with 0.5 μM 2iP. Both media contained also 0.3 μM IAA, 0.05 % charcoal, 2 % sucrose, 0.8 % agar and their pH was adjusted to 5.5. Seedlings and plants were cultured in glass jars at 23 °C, and light was provided by white fluorescent tubes for 16 h daily (irradiance of 30 μmol $m^{-2}$ $s^{-1}$). Cultures were maintained by regular subculturing onto the same fresh media every four weeks.

Explants for callus induction were obtained from 1-month-old plants of both tested species clonally propagated on MS medium with 0.5 μM BAP and 0.3 μM IAA. The leaf blades and internode segments of the shoot were cut into 0.5 cm long fragments and placed horizontally on basic MS callus induction medium (pH 5.5) containing 2 % sucrose, 0.8 % agar and 6 different concentrations of various plant growth regulators (Table 1). Cultures were kept in Petri dishes and incubated in darkness at 25 °C. Calluses were maintained by regular subculturing every four weeks.

For regeneration purposes, these calluses were transferred onto MS medium containing 8.8 μM BAP, 2 % sucrose and 0.8 % agar (pH 5.5). Cultures were grown in Petri dishes at 23 °C with light provided by white fluorescent tubes (30 μmol $m^{-2}$ $s^{-1}$) for 16 h a day.

Isolated embryos of both tested species started to germinate shortly after the transfer to the media. Embryo germination was better on pure agar medium than MS medium with kinetin, especially it was characterised for S. *pestifer* from desert conditions (Bukhara population). Swelling of the embryos was noticed already on the second day of the culture and at the end of the first week small, green seedlings with roots and leaves were observed. The seedlings grew further on these same media for next 2 weeks. When seedlings were transferred on micropropagation media, they started to axillary buds develop. Within next 6 weeks on these media plantlets developed many axillary shoots, but considerable variation between individual plantlets was observed. Some of them produced no branches and others produced up to 10 or more. Shoot proliferation was noticed on MS medium supplemented with BAP and IAA as well as on MS medium with 2iP and IAA. On these both media green, well-branching and rooting young plants of all tested species were obtained (Fig. 1). The earliest sign of callus induction of both tested species was visible within one week of culture. Shoot and leaf explants became swollen and later on began to form callus tissue on cut surfaces. Both populations of *S. pestifer* formed white, soft or yellow, compact calluses. Explants of *S. lanata* produced only white and compact callus (Table 1).

The most suitable medium for callus induction for both shoot and leaf explants of desert population of *S. pestifer* was medium with BAP, but the best medium to induce callus of valley population of the same species was medium with IBA (Table 1). The best response of callus induction from shoot and leaf explants of *S. lanata* was demonstrated on the medium with 2,4-D (Table 1).

Obtained callus grew intensively and, on the 6$^{th}$ week of culture, the quantity of callus exceeded the surface area of the explants. For further growth and proliferation callus separated from the explants was transferred on medium with 2,4-D (Table 1). On this medium callus proliferated relatively fast, doubling its volume at about four week intervals (Fig. 2).

One week after transfer to the regeneration medium and exposure to photoperiod, green centers appeared only within the callus of *S. lanata* (Fig. 3). From these centers adventitious buds differentiated during the next 3 weeks of culture (Fig. 4).

Several scientific works were carried out using some species of genera *Atriplex*, *Kochia* and *Suaeda* by *in vitro* tissue culture (Kenny and Caligari 1996, Thiyagarajah *et al.* 1996). However, since now there are no experimental data about tissue culture techniques within

the *Chenopodiaceae* family. This is the first report on plant micropropagation and callus induction of some annual *Salsola* species.

In our work we used for plant micropropagation zygotic embryos derived from mature seeds. According to George (1993) seeds have several advantages such as they are produced in large number, easily distributed as well as plants grown from seed are without most of the pest and diseases which may have afflicted their parents. In this study, we obtained green, well-branching and rooting plants of both tested species in the presence of cytokinins (BAP or 2iP) and auxin (IAA). In numerous experiments different parts of plant such as annual and perennial shoots, leaves, flowers, ovary, roots, *etc.* have been used as explants to obtain callus tissue (Bulgakov and Juravlev 1989, Upadhyay *et al.* 1992, Castellar and Iborra 1997, Luo and Jia 1998, Elhaak 1999). In our study we used fragments of shoots and leaves derived from plant stock collection propagated *in vitro* to induce callus. It was determined that the growth regulators have a different influence on callus induction even inside the same species. So, the best response of callus induction of *S. pestifer* (desert population) was in the presence of BAP, but IBA was the most effective growth substance to induce callus tissue of valley population of the same species. According to some authors, 2,4-D is the most frequently employed auxin to initiate callus tissues (George 1993, Luo and Jia 1998, Zheng and Konzak 1999). In our work, 2,4-D was the most suitable growth regulator to induce callus tissue only for *S. lanata,* however, we found that 2,4-D was the best for proliferation and maintenance of calluses of both tested species. The process of callus induction also depended on type of explant. We noticed, that callus of both populations of *S. pestifer* developed better from shoots explants than from leaves. In contrast, the leaf explants of *S. lanata* formed callus more often than shoot explants. It is important to note that callus formation started very fast, within one week in culture.

The present work is a first step in determining the effects of NaCl at the cellular level. With these results we can start to isolate more salt-tolerant cell lines of *Salsola* species.

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