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Biotechnological aspects of obtaining salt-tolerant plants

Evgeny Aleksandrovich GLADKOV[1], Yuliya Ivanovna DOLGIKH[1],
and Olga Victorovna GLADKOVA[2]

[1] K.A. Timiryazev Institute of Plant Physiology Russian Academy of Sciences, IPP RAS, 35 Botanicheskaya St.,Moscow, 127276 Russia, *corresponding author*: gladkovu@mail.ru
[2] Independent scientist, Moscow, *corresponding author* : olgav.gladkova@mail.ru

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Abstract

Soil salinization is an important ecological problem in urban ecosystems and agro-ecosystems. Salinization has direct adverse effects on plants. Sodium chloride showed greater toxicity than bischofite to *Festuca rubra*. We used cell selection to increase the salt tolerance of the plants. We used sodium chloride in all stages of cultivation.
We have obtained salt-tolerant *Festuca rubra* plants. Most *Festuca rubra* regenerants showed increased salt tolerance. Thus, biotechnological methods can be used to obtain lawn grasses that are resistant to salinity.

Keywords: Soil salinization, *Festuca rubra,* biotechnological methods

1. Introduction

Soil salinization is an important ecological problem in urban ecosystems and agro-ecosystems. Globally, this environmental factor is one of the main land-degrading threats influencing soil fertility (Hassani *et al*., 2020). Soil salinity and sodicity, called collectively salt-affected soils, affect approximately 932 million ha of land globally (Wong *et al*., 2010). 20% of cultivated land in the world, and 33% of irrigated land, are salt-affected and degraded (Machado *et al*., 2017).

The use of deicing reagents causes salinisation of urban ecosystems (Gladkov, Gladkova, 2022). Salinization has an adverse effect on plants. Indeed , salinity stress significantly reduced the plant growth, leaf water potential and relative water content in five Coleus species (Kotagiri *et al*., 2017). The salinity affected both growth and physiological attributes of the maize species (Zahra *et al*., 2020).

Crop loss due to soil salinization is an increasing threat to agriculture worldwide (Van Zelm *et al*., 2020). This environmental factor endangers the development of ecological agriculture (Chen *et al*., 2022).

Alternative uses for saline land are proposed. High salinity land may provide an alternative resource for the cultivation of dedicated biomass crops (Stavridou *et al*. 2017).

Improving plant resistance to salinity is an important task for many researchers. The use of biotechnological methods to improve salt tolerance is a promising development. These methods are used to increase resistance to salinization and other stresses (Gladkov *et al*., 2021; Gladkov & Gladkova, 2022). The salt tolerance of some crop plants and cell lines has been increased. Cell lines in *Solanum tuberosum* resistant to sodium chloride have been obtained (Queiros *et al*., 2007).

The regenerants obtained from the salt-treated showed improved growth characteristics(Al-Khateeb *et al*., 2020).

An efficient regeneration protocol was applied to regenerate shoots on salt stress-tolerant calli lines of aubergine (*Solanum melongena*) (Hannachi *et al*., 2021).
Biotechnological methods can be used in urban greening (Gladkov, Gladkova, 2021).Cell selection has been used to obtain salt-tolerant *Agrostis stolonifera* plants. (Gladkov *et al*.,2014; Gladkov, Gladkova, 2022).

The aim of the present work was to develop the biotechnological method of increasing the salt tolerance of the lawn grass *Festuca rubra*.

2. Materials and Methods

The phytotoxicity of sodium chloride and magnesium chloride (deicing reagent bischofite, magnesium chloride hexahydrate ($MgCl_2.6H_2O$)) was assessed in water solutions of the toxicants. 10 seeds each were placed in Petri dishes on filter paper moistened with toxicant solutions. Water was used as a control.

Calli of *Festuca rubra* was obtained from seeds on Murashige and Skoog modified medium with 3 mg/l 2,4- dichlorophenoxyacetic acid (Gladkov *et al*., 2014).

Determination of the sensitivity of callus cultures to sodium chloride was carried out by morphogenesis ability. We counted the percentage of morphogenic calli in relation to all calli. Only surviving calli were evaluated. Calli with green buds were considered morphogenic (Gladkov *et al*., 2014).

To select tolerant clones calli of *Festuca rubra* cultivated on Murashige and Skoog modified medium with 1 mg/l 2,4-diclorophenoxiacetic acid and 1% sodium chloride. The cultures were maintained in a growth room at 26 °C, exposed to a 16-h photoperiod in the light and humidity 70% (Gladkov *et al*., 2014).

The tolerance of the obtained regenerants was evaluated in soil. We assessed the increase in plant growth.

3. Results and discussion

Various deicing reagents are used in Russia. Deicing reagents are among the priority pollutants of the Moscow soils. All reagents used that contain chlorine are hazardous to plants. Sodium chloride is widely used as deicing reagent. It showed greater toxicity than magnesium chloride (bischofite, magnesium chloride hexahydrate) to *Festuca rubra* (Figure 1). Therefore, obtaining plants resistant to sodium chloride is the most appropriate.

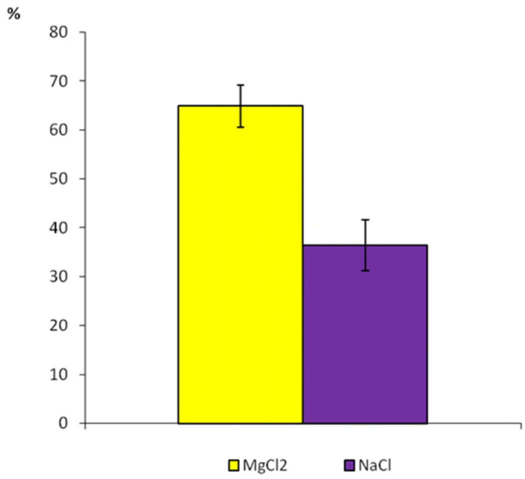

Figure 1. Effect of 1% deicing reagent (sodium chloride; bischofite) on *Festuca rubra* plants

We used cell selection to increase the salt tolerance of the plants.

When developing a cell selection scheme, the sensitivity of calli to toxicant (Gladkov, Gladkova, 2020), the choice of selective concentration and the duration of cultivation are of prime importance. An inhibitory effect on the callus was observed at 1% sodium chloride. 56% of the surviving calli retained the ability for morphogenesis. The concentration of 1% NaCl was chosen as selective because some surviving callus retained their morphogenic ability. Our research has shown that the use of higher concentrations of sodium chloride significantly reduces the possibility of obtaining regenerants. The scheme for cell selection to obtain salt-tolerant plants can vary depending on the duration of cultivation (Figure 2).

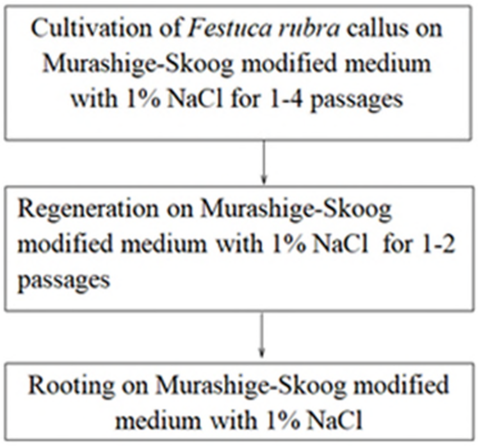

Figure 2. Cell selection scheme for obtaining *Festuca rubra* plants tolerant to sodium chloride

The duration of cultivation is an important biotechnological aspect (Table 1). 300 calli were studied. Prolonged cultivation resulted in the death of most callus. The regenerants obtained after prolonged cultivation were very impaired and slow root formation was observed in the surviving regenerants which were planted again on modified Murashige and Skoog medium for rooting.

However, most of the regenerants did not take root in the soil after prolonged cultivation. Consequently, the duration of cultivation had an influence on the regeneration capacity of the callus and the obtaining of salt-tolerant plants.

Table 1. Selection of NaCl-tolerant plants

Cultivation duration, number of passages	Number of calli	Regenerants rooted in the soil
2	100	6
3	100	4
4	100	2

We used two passages to cultivate calli in further studies. In further studies, we also used one passage for the cultivation of calli. The use of one passage is less effective, due to the lower tolerance of many plants to salinity.

We used sodium chloride in all stages of cultivation.

We have obtained salt-tolerant plants. Most of the regenerants showed increased salt tolerance. Initial studies showed increased salt tolerance of the regenerants at sodium chloride concentrations of 0.5- 2%. Some regenerants were able to survive at 2% sodium chloride. The original plants did not survive at this concentration. Further studies were for concentrations of 0.5-1% sodium chloride. There was no growth retardation in most of the regenerants studied at 0.5 % sodium chloride. Only one regenerant showed growth like the original plants. The growth of most of the regenerants was more than twice that of the original plants at 1% salinity. One of the most salt-tolerant regenerants showed no growth retardation.

In perspective, it is of interest to compare the development of the descendants of the regenerants with the original plants.

The concept developed for obtaining salt-tolerant plants can be used for other plant species. Earlier, salt-tolerant *Agrostis stolonifera* plants were obtained (Gladkov, Gladkova, 2022). Thus, biotechnological methods can be used to obtain lawn grasses that are resistant to salinity. Lawn grasses will be able to grow at higher levels of salinity in the soil and retain their ornamental qualities.

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