SCREENING OF BULGARIAN RASPBERRY CULTIVARS AND ELITES FOR OSMOTIC TOLERANCE IN VITRO

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ABSTRACT

Abiotic stresses such as drought, high or low temperatures limit in great extend plant distribution and productivity. This is particularly true for perennial crops, including small fruits. Classical breeding for drought, heat and freezing tolerance is a very complex and time-consuming process. Biotechnology approaches could significantly contribute in speeding up the procedures and to make them less dependent on the environment conditions.

Using PEG as selective agent under in vitro conditions we were able to distinguish Bulgarski rubin and Elite 1 as osmotic tolerant. Shopska alena, Samodiva, Lyulin and Elite 3 were with reduced growth and membrane integrity of the tissues. Further studies are in progress to compare the reaction to osmotic stress in vitro with the response to drought under field conditions. This will help us to improve and speed up the raspberry breeding program.

Introduction

It is generally accepted that the unfavorable environmental conditions in great extend limit plant distribution and yield. This is particularly true for the perennial crops. The reaction to abiotic stress is a very complex trait. Most often environmental stresses affect plant water status, reducing the water potential and thus, impairing many functions (2). The contemporary raspberry cultivars should respond to increased requirements. Among the most important traits are high yielding, suitability for mashie harvesting, uniformity in fruit ripening, increased tolerance to abiotic stress – high and low temperatures, drought. Classical breeding for tolerance is difficult and time-consuming procedure. On the other hand, information for application of in vitro methods in this area is still limited. The biotechnological approaches, including in vitro selection for stress tolerance will continue to have a significant place in the strategy of establishing plant systems with optimal stress reaction and productivity. Polyethylene-glycols (PEG) of high molecular weights have long been used to simulate drought stress in plants as a non-penetrating osmotic agent lowering the water potential similarly to soil drying (9).

Our aim was to develop procedure for in vitro screening for osmotic tolerance of valuable raspberry genotypes.

Materials and Methods

Plant material

The wide-spread Bulgarian cultivars Bulgarski rubin, Shopska alena, Samodiva and Ly-

Abbreviations: MS – Murashige & Skoog plant tissue culture medium, PEG – polyethylene glycol, RWC – Relative Water Content.

ulin (1), as well as. Elite 1 and Elite 3 were tested for osmotic stress tolerance. In vitro plants were obtained as previously described (7). In short: initial explants were taken form 1-year old shoots of 2-years old mother plants grown under greenhouse conditions. Standard procedure for sterilization was applied. Cultures were initiated on MS (11) (Duchefa) with 0.1 mg/l IBA, 0.3 mg/l BAP and 0.1 mg/l GA3 and transferred to MS basal medium.

Osmotic stress

In vitro cloned plantlets of the six genotypes were grown on MS (11) basal medium. Two weeks later they were transferred in tubes, containing liquid MS medium with 20 or 25% PEG 6000 (Duchefa) where plantlets were placed on filter paper bridges. The stress treatment continued for 40 days. The fresh weight of the plants was evaluated before and after the exposure to osmotic stress. Plants maintained on MS basal medium were used as controls.

Electrolyte leakage

The degree of membrane integrity was assessed by the leakage of electrolytes from the upper fully expanded leaf of plants (3). One leaf per plant from every group of treatment was weighted and then immersed in exact volume of bidistilled and deionized water for 20 h in the dark with continuously shaking. The amount of electrolyte leakage was measured conductometrically (Mettler Toledo MC 226) and expressed as µS per gram fresh weight (FW).

Relative Water Content (RWC)

RWC was used as additional marker for membrane integrity. To evaluate RWC the upper fully developed leaf per plantlet was detached after 40 days osmotic stress and manipulated. We used the formula:

RWC. = (FW-DW) / (TW-DW) x 100

Where FW is Fresh Weight, DW – Dry Weight, obtained after 48 h at +80^{0}Ñ, TW – Turgor Weight, obtained after 24 h immersing in distilled water in dark.

Results and Discussion

Commercial growing of raspberry under the conditions of Southern Hemisphere, North America and Southern Europe requires the development of cultivars, tolerant to abiotic stress (13). In many cases, the breeding programs are based on the involvement of wild species as donors of tolerance (10)**.** On the other hand, screening under natural conditions is long-lasting process fully dependent on the climatic conditions of the specific region for several years in a row. The development of a screening procedure under controlled conditions is a very attractive goal. Using PEG as a selective agent, it was possible to distinguish osmotic tolerant and sensitive alfalfa cultivars (12) or to select somaclonaal variants with improved drought and salt tolerance (4, 5, 6). We were able to show also that freezing-tolerant transgenic tobacco lines were more osmotic tolerant than their wild type genotype (8).

Based on our previous experience we developed procedure for screening in vitro for PEG tolerance in raspberry.

In preliminary studies (data not shown) we found that it is very difficult to establish any differences between the reactions to osmotic stress of the raspberry genotypes on PEG concentrations lower than 20%. Similar observations have been reported for mulberry (14). High PEG doses (30 and 40%) were very damaging. Thus, we used 20 and 25% PEG as selective concentrations.

On MS basal medium (no stress) the raspberry plants increase their weight in genotype-specific manner (**Fig. 1**). The plants of Lyulin and Elite 1 grew very intensively and increased their weight twice, while Bulgarski rubin and Samodiva showed only about 30-40% increase. Shopska alena and Elite 3 were with moderate growth.

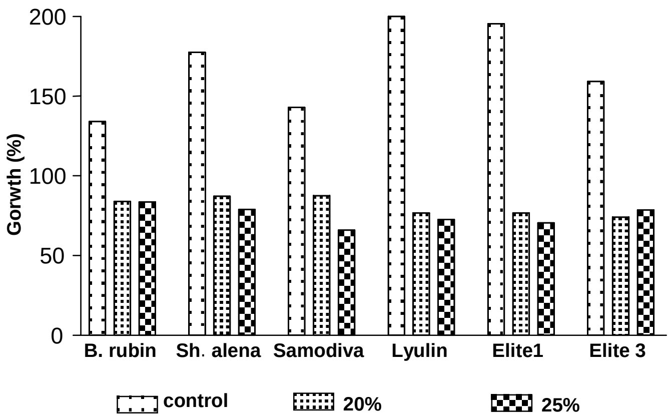

Fig. 1. Plants of various raspberry genotypes were grown for 40 days under normal conditions and osmotic stress (20 and 25% PEG 6000). Data presented are the average of at least four replicates, obtained from two independent experiments and statistically processed with Prism Plot.

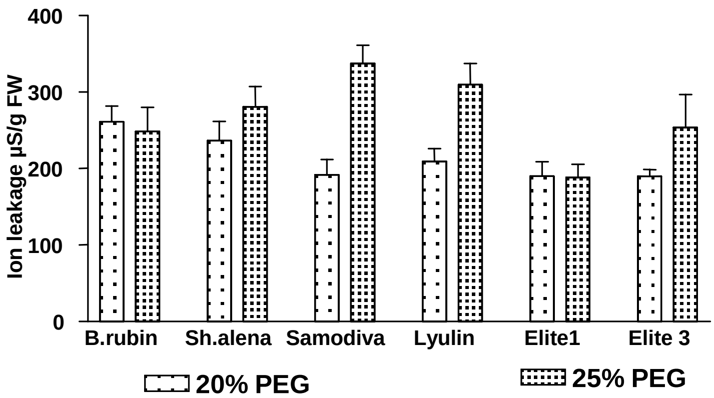

Fig. 2. Ion leakage from plants of various raspberry genotypes under osmotic stress conditions in vitro. Data presented are the average of at least four replicates, obtained from two independent experiments and statistically processed with Prism Plot.

The response to osmotic stress was again genotype-specific (Fig. 1). While Bulgarski rubin, Elite 3 and Lyulin showed practically no differences in the reduction of their growthunder both PEG concentrations, Samodiva and in lesser extent Elite 1 and Shopska alena reduced their growth under higher PEG.

The membrane integrity of the raspberry plantlets was reduced by the applied osmotic stress. The procedure allowed to distinguish putative tolerant from sensitive forms (**Fig. 2**). The lowest levels of leakage on 20% PEG were for Elies 1 and 3 and Samodiva while Bulgarski rubin and Shopska alena were with highest. Applying higher osmotic stress (25% PEG) we were able to establish the differences in greater details. There was

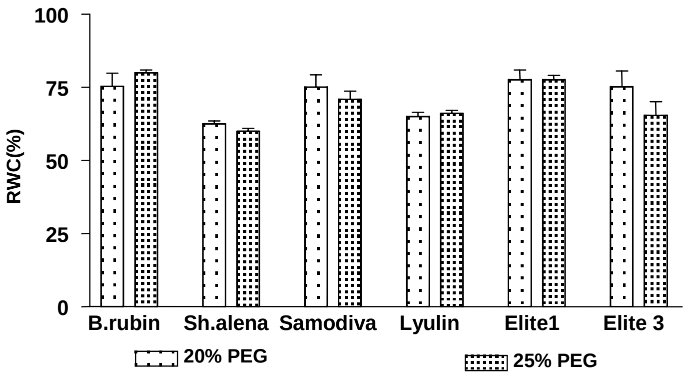

Fig. 3. Relative Water Content of plants from various raspberry genotypes under osmotic stress conditions in vitro. Data presented are the average of at least four replicates, obtained from two independent experiments and statistically processed with Prism Plot.

no increase ion leakage in Elite 1 and Bulgarski rubin plants. Elite 3 and Shopska alena showed moderate sensitivity, while Lyulin and especially Samodiva lost drastically their membrane integrity.

The changes in Relative Water Content appeared to be less informative under the conditions of our experiment. Relative reduction of RWC was found in Samodiva and Elite 3 when stronger osmotic stress was applied (**Fig. 3**).

Using PEG as selective agent we were able to show that under in vitro conditions plants of Bulgarski rubin and Elite 1 appear to be more osmotic tolerant than the rest of the tested genotypes. Further studies are in progress to compare the reaction to osmotic stress in vitro with the response to drought under field conditions. This will help us to improve and speed up the raspberry breeding program.

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