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# Effect of NaCl Stress and Sucrose on Potato Microtuberization

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## ABSTRACT

*In vitro* microtuberization of potato (*Solanum tuberosum* L.) cvs. 'Marfona' and 'Agria' was studied by the use of individual excised single-node cuttings cultured on semi-solid MS medium supplemented with 5 mg $L^{-1}$ BAP and 80 g/L sucrose in the presence of 0, 25, 50, 75, 100 and 150 mM NaCl or 30, 40, 60, 80, 100 and 120 g/L sucrose. Microtuberization decreased significantly with increasing salinity; the highest NaCl level (150 mM) completely inhibited microtuber development in both cultivars. Microtuber production was significantly increased by increasing sucrose concentration up to 80 g/L then reduced at higher concentrations.

**Keywords:** BAP, microtuber, salinity, single-nod cutting, *Solanum tuberosum* L.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is a vegetable crop of major economical importance and widely distributed in the world (Mahmood 2002). It is grown in about 140 countries, more than hundred of which are located in the topical and subtropical zones (Gandonou *et al*. 2005). Salinity is one of the major physiochemical stresses reducing the yield of a wide variety of crops all over the world (Sha Valli Khan *et al*. 2007). In potato, soil salinity reduces plant growth and tuber yield (Silva *et al*. 2001).

Tissue culture techniques have widely been used for crop breeding purposes, especially in selection for stress tolerance (Tam 2003) Propagation of potato by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange and conservation. Potato tuber formation is regulated through a photoperiodic developmental process. This technique provides a uniform and convenient experimental model for (micro) tuberization studies (Silva *et al.* 2001). Microtubers are now massively produced for several purposes throughout the world. They can be used to produce minitubers or for genotype screening for all important tuber characteristics such as color, shape, yield and average weight (Mohammad Javad *et al*. 2005). Gopal *et al*. (2004) reported that *in vitro*-cultured potato plants produced tubers in short days. They also demonstrated that the sucrose level in the culture medium determines microtuberization. Some other exogenous factors, including temperature and plant growth regulators (PGRs), also affect tuberization. The involvement of PGRs, for example cytokinins, abscisic acid and gibberellins in the regulation of *in vitro* potato (micro)tuber formation has been reported. Among these, gibberellins are suggested to be the inhibitors of tuberization. However, the roles of many endogenous factors as well as other plant hormones have yet to be clarified (Gao *et al*. 2003). Although salinity affects a range of developmental processes in potato, there is still little information on the effects of salt stress on *in vitro* tuberization. In the present study, microtuber formation capacity of two important potato cultivars, 'Marfona' and 'Agria', which are being cultivated in most regions of Iran, was evaluated under different levels of NaCl salinity stress or various sucrose concentrations.

## MATERIALS AND METHODS

### Micropropagation

Disease-free tubers of both cultivars were provided from the Hamedan Agricultural Research Station, washed under tap water, surface sterilized in 3% sodium hypochlorite solution for 15 min followed by 3 washes with sterile distilled water, put in disposable plastic containers under aseptic conditions and incubated at 25 ± 2°C and a 16-h photoperiod with 30 μmol/$m^2$/s illumination provided by cool white fluorescent lamps. Shoots emerged from the tubers within 4 weeks, were excised when 2 cm in length, and were cultured in jars (250 ml capacity) containing 50 ml semi-solid MS (Murashige and Skoog 1962) medium (pH 5.7 ± 0.1) supplemented with 15 g/L sucrose. Sufficiently actively growing rooted cuttings were prepared through successive *in vitro* cutting of the samples.

### Salt and sucrose treatments

In the first part of the study, individual excised single-node cuttings (1.0-1.5 cm in length) were transferred into the same jars containing 50 ml semi-solid MS culture medium supplemented with 5 mg/L BAP and 80 g/L sucrose plus 0 (control), 25, 50, 75, 100 and 150 mM NaCl. In the second part, single-node cuttings were cultured in media of the same composition containing a series of sucrose concentrations (30, 40, 60, 80, 100 and 120 g/L). For any level of salt stress or sucrose concentration, 5 nodal cuttings from each cultivar were placed in each jar and four replications were considered for any treatment. All cultures were incubated in a growth room providing the same conditions as for micropropagation experiments. The number of microtubers per jar was recorded 6 weeks after culture (Omokolo *et al*. 2003). The data were analyzed using MSTATC statistical software and the means were compared by Duncan's multiple range test ($P < 0.05$).

## RESULTS AND DISCUSSION

### Salt stress

Initiation of microtubers occurred in the cultures within 2-3 weeks (**Fig. 1**) and it took about 6 weeks before microtubers could be harvested from the culture vessels. However, well-developed microtubers (5-6 mm in diameter) were obtained from the controls only in the absence of salinity (**Fig. 2**). A

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Short Communication

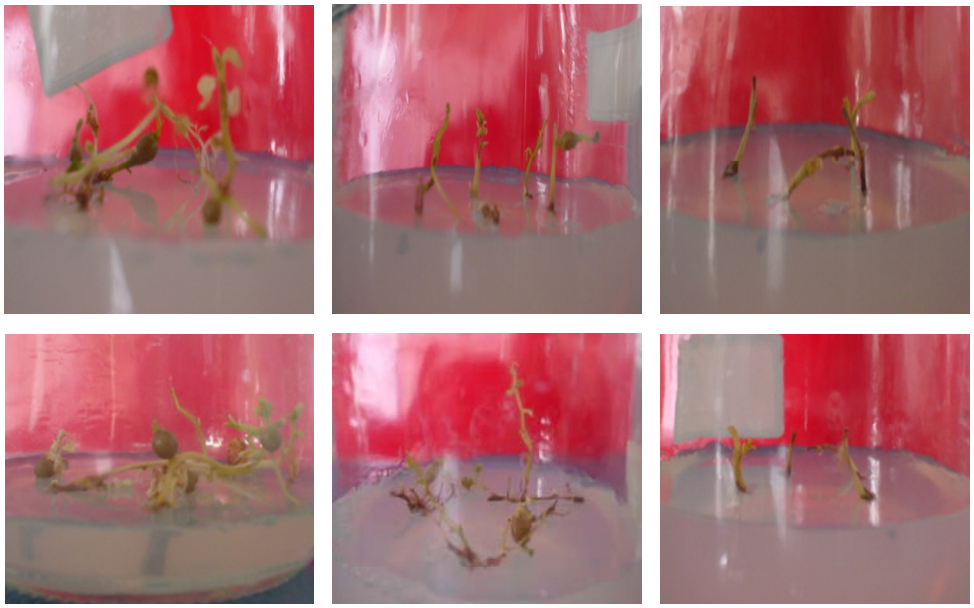

**Fig. 1 The view of 'Agria' (top row) and 'Marfona' (bottom row) potato microtuberization under NaCl stress condition 4 weeks after culture.** From left to right: control (no salt), 100 and 150 mM NaCl, respectively.

linear and significant reduction in growth was observed in microtuber production as salt concentration increased in the culture media for both cultivars.

Similar results have also been reported by Ochatt *et al*. (1999) and Zhang *et al*. (2006). The suppressive effect of salinity on microtuberization of potato was probably a consequence of the osmotic potential reduction in the cells of both stolon and microtuber tissues due to increasing salt levels. Salinization usually causes a reduction in water content and nutrient uptake of plant tissues and this may similarly occur in microtubers. Retardation in microtubers' growth observed in this study could also be due to the accumulation of salt in the plant organs reducing water absorption and deteriorating some metabolic pathways in cells.

There was also significant difference between both cultivars in terms of microtuber production, not only in the control and the absence of salinity, but also at some levels of salt stress. Cultivar played an important role in microtuber production; 'Marfona' always produced more microtubers than 'Agria' (**Fig. 2**). A similar cultivar-dependent result was also reported by Gopal *et al*. (2004).

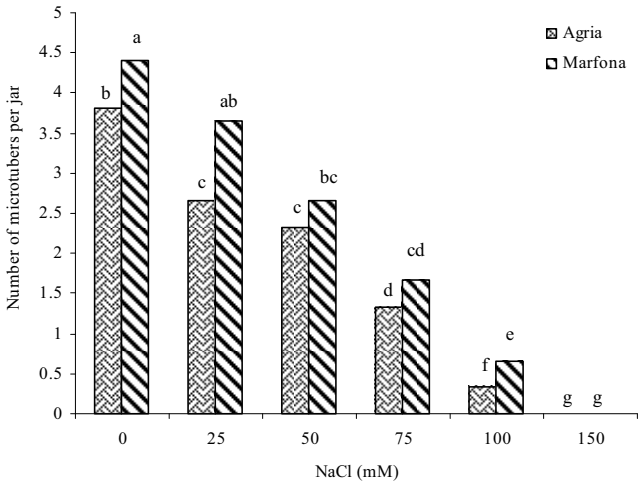


**Fig. 2 Effect of salt (NaCl) stress on microtuber production in two potato cultivars 6 weeks after culture.**

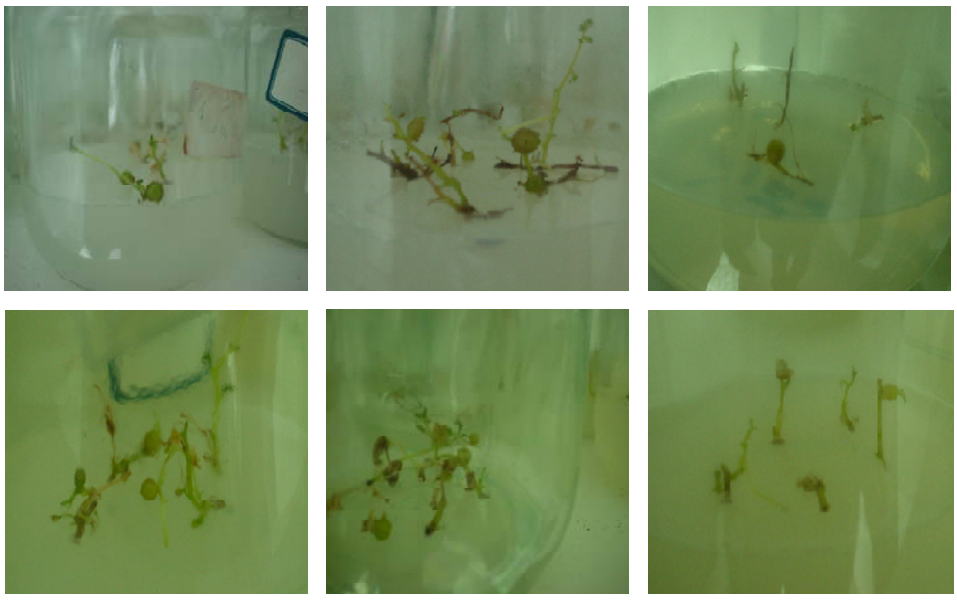

**Fig. 3 The view of 'Agria' (top row) and 'Marfona' (bottom row) potato microtuberization at the presence of sucrose 4 weeks after culture.** From left to right: 30, 80 and 120 g/L sucrose, respectively.

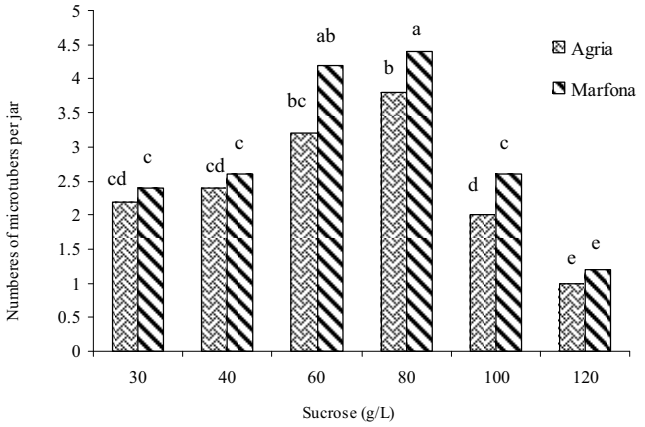


**Fig. 4 Effect of different sucrose concentrations on microtuber production in two potato cultivars 6 weeks after culture.**

## Sucrose concentrations

Microtuberization was significantly induced in the presence of sucrose (**Fig. 3**). The highest number of microtubers was obtained from cultures containing 80 g/L sucrose; a higher sucrose concentration than this level suppressed tuberization in both cultivars (**Fig. 4**). These results are in agreement with the findings of Karam and Al-Majathoub (2000). As in our results, Omokolo *et al*. (2003) could improve microtuberization in potato by increasing the sucrose level from 3 to 8%. It seems that high sucrose levels are needed for the initiation of tuberization. Besides, sugars constitute the main component of tubers and arrowroots. Both cultivars showed significant differences in microtuber production at some levels of sugar and 'Marfona' always produced more microtubers than 'Agria' (**Fig. 4**).

The use of a higher concentration of sucrose is recommended as it promotes microtuberization and produces more larger-sized microtubers. Although high sucrose levels are necessary for optimal microtuber production, this rapidly hydrolyzes into glucose and fructose, making the long-term maintenance of desirable sucrose levels difficult (Levy *et al*. 1993). Therefore, successful strategies to reduce sucrose hydrolysis without inhibiting microtuber growth will improve the efficiency of sucrose utilization in potato microtuber production.

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