Response of garlic callus to salt level

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ABSTRACT

There is a lack of information on the yield and quality response of garlic grown under increasing levels of salinity. This experiment was conducted in vitro to assess effects of salt level on callus induction, survival, and regeneration of the marketable garlic (*Allium sativum* L) Jordan and California. Garlic shoot tips were exposed to vary concentrations of NaCl salt of 0, 50, and 100 mM and cultured in MS media supplemented with different concentrations of hormones (MS-1, MS-2, and MS-3). There were negative, significant, effects for embryogenesis capacity of callus induction and regeneration due to salt level. The best growth and response of callus induction and proliferation were for MS-1 medium, a combination of 1 mL thiamine, 1 mL kinetin, 1 mL BAP, 5 mL 2,4-D and 30 g sucrose for both cultivars regardless of salt concentration. Maximum callus induction was in cv. Jordan (81%) and (72%) in cv. California. The relative fresh weight growth rate for cv. Jordan decreased to 10%, and for cv. California was reduced to 14% when exposed to 100 mM of NaCl compared to the control. Many calli that failed to induce on saline media recovered and grew when transferred to NaCl salt free media. Direct exposure of callus to salinity provides an opportunity to study development of garlic to salt tolerance in laboratory.

Key words: *Allium sativum*, MS medium, callus recovery, in vitro, salinity.

INTRODUCTION

Salinity is a major constraint to crop production (Flowers, 2004), especially in arid and semi-arid regions (Munns and Tester, 2008). It decreases plant growth through osmotic and toxic effects. The osmotic effect can reduce the ability of plants to take up water and the toxic effects can cause premature aging and reduce photosynthetic leaf area of plants to a level that cannot sustain growth (Munns and Tester, 2008; Nemati et al., 2011; Horie et al., 2012). The plant's ability to grow under saline conditions depends on salt concentration, adaptability to low water potential, and presence of high concentrations of Na and Cl ions.

Under laboratory conditions, differences in water potential of the medium may affect growth, because cumulative effects of water stress on callus becomes more extreme in long term cultures.

Plant response to salinity differs between crop species and ranges from tolerant to sensitive with garlic (*Allium sativum* L) classified as being sensitive (FAO, 1994). Extensive research has been carried out on the health promoting and medicinal properties of garlic, also garlic almost never produces fertile seed, and thus it must be propagated vegetatively.

Plant callus and somatic embryos can be used to study selection pressure in vitro, including tolerance to high and low temperature, water stress, salt stress, herbicides and resistance to fungal diseases (Barlass, 1985; Dami and Hughes, 1997). Use of in vitro methods to obtain NaCl tolerant strains can examine physiological aspects of tolerance. The effect of NaCl on growth of garlic callus depends on NaCl concentration, the medium used and variety. Al-Safadi and Faoury (2004) evaluated salt tolerance in garlic cultivars using in vitro techniques, and 34 mM NaCl concentration was found to produce a reduction in shoot length. Francois (1994) reported that...
the salinity threshold of garlic was 3.9 dS/m and at 7.4 dS/m yield was reduced by 50%. Similarly, maximum callus growth at 50 mM and reduction in growth at 150 mM NaCl has been reported by Zheng et al. (2004). Tissue culture studies on A. sativum (Ayabe and Sumi, 2001) regarding callus induction and development using explants have been conducted, but there are no reports related to the effect of salts on callus recovery. This study was conducted to evaluate effects of exposure to salt on callus induction, regeneration and recovery of garlic in vitro.

MATERIAL AND METHODS

This research was conducted in the Laboratory of Tissue Culture in the Vegetables Genetics Laboratory at the University of Wisconsin, Madison, WI.

Garlic bulbs were separated into cloves and peeled from their protective leaves. The cloves were washed with tap water, and immersed in 70% ethanol for 5 min, followed by disinfection in 25% sodium hypochlorite, with 2 drops of Tween 20/100 mL solution for 20 min under constant stirring and washed in autoclaved distilled water. Each explants was excised to expose its 1 cm apical meristem region and each was introduced into a Petri dish (100×100 mm) with a 20 mL of sterilized by autoclaved, basic Murashige and Skooge (MS; Murashige and Skooge 1961) medium. The pH of the medium was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving.

Garlic shoot tips were exposed to various concentrations of NaCl salt of 0, 50, and 100 mM and cultured in MS solid medium (Murashige and Skoog, 1961). Two MS media were used for callus induction and regeneration; each was supplemented with different concentrations of hormones and was categorized into MS-1, MS-2, and MS-3. For callus induction: MS-1 medium was supplemented with 1 mL thiamine, 1 mL kinetin, 5 mL 2,4-D. MS-2 medium was supplemented with 2 mL thiamine, 2 mL kinetin, 6 mL 2,4-D. MS-3 medium was supplemented with 3 mL thiamine, 3 mL kinetin, 7 mL 2,4-D. In addition 30 g sucrose and 2 g/L of phytagel were also used for each medium. For callus regeneration medium: MS-1 medium was supplemented with 1 mL/L thiamine and mL/L BAP, MS-2 medium was supplemented with 2 mL/L thiamine and 2 mL/L BAP, MS-3 medium was supplemented with 3 mL/L thiamine and 3 mL/L BAP. In addition 30 g sucrose and 2 g/L of phytagel were used for each medium.

Isolated shoot pieces (1.0 cm) were cut and transferred to the callus induction medium. Each Petri dish with 25 mL medium contained 5 shoot segments and each cultivar was represented by 9 plates. Cultures were kept in the dark at 27±2°C for 2 min. Subcultures were transferred to fresh media every 4 weeks. For comparison, calli from shoot segments were transferred to new Petri dishes with 3 regeneration media. Calli were incubated at 27±2°C during a 16- h photoperiod and transferred to a fresh medium every 4 weeks. The calli were evaluated for shoot regeneration rate, and the 2 cultivars were compared. To evaluate the ability for callus to recover from exposure to salt, all un-germinated calli exposed to 100 mM of NaCl for 10, 20, 30, 40, and 50 days were transferred to salt free medium for up to 60 days.

The experiment was arranged in a completely randomized design with 3 replicates. Each replicate was represented by one(1) Petri dish and data were evaluated with ANOVA using SAS (ver. 9.4, SAS Institute Inc., Cary, NC). If interactions were significant they were used to explain the data. For interactions that were not significant means were separated, using Tukey’s test.

RESULTS

Callus initiation started from 52 to 74 days and from 58 to 81 days of incubation in ‘Jordan’ and ‘California’, respectively. In all cases MS-1 medium was suitable for callus induction and regeneration. Number of days for callus induction was affected by salt concentration and cultivar (Figure 1). Callus induction frequency and fresh weight decreased due to treatment with 100 mM NaCl in

![Figure 1](image-url)
Figure 2. Callus fresh weight and callus induction percentage of 'Jordan' (JO) and 'California' (CA) garlic due to exposure to levels of NaCl. MS: Murashige and Skoog medium.

Figure 3. Callus induction percent of 'Jordan' (JO) and 'California' (CA) garlic recovery after up to 60 days exposure to salt at 100 mM followed by transfer to Murashige and Skoog medium (MS-1) salt free medium.

Increased NaCl concentration in medium decreased percent callus regeneration in both cultivars compared with normal callus regeneration in the salt free medium. There was a reduction in days to shooting and rooting in both cultivars (Figure 4). Shoot and root formation were highest at 0 and 50 mM compared with 100 mM of NaCl in all types of media (Figure 5). A higher growth rate for both

all media, and callus induction percent was higher in 'Jordan' than 'California' cultivar (Figure 2). Mean callus induction percent decreased with increase in NaCl level to 100 mM in the medium. There were no differences at 50 mM in all media for both cultivars. Callus recovery from saline to free salt medium was reduced after exposure to higher salt for both cultivars (Figure 3).
Figure 4. Similar trends of the days required for shooting and rooting of ‘Jordan’ (JO) and ‘California’ (CA) garlic due to exposure to levels of NaCl. MS: Murashige and Skoog medium.

Figure 5. Effect on number of shoot and root of ‘Jordan’ (JO) and ‘California’ (CA) garlic cultivars due to exposure to levels of NaCl. MS: Murashige and Skoog medium.
cultivars occurred in MS-1 media, this was decreased due to exposure to 100 mM compared with 0 and 50 mM (Figure 6). The MS-1 was best for callus regeneration.

DISCUSSION

Salinity results in dehydration of the cells and reduces availability of nutrients which can lead to growth inhibition (Campos et al., 2013). Both cultivars had better callus growth in control regeneration medium nutrient, which decreased as salt concentration increased. Reduction in callus growth is a common phenomenon in cultured cells grown on medium nutrient to which NaCl is added (Venkataiah et al., 2004; Roa and Patil, 2012) and is interpreted that a certain amount of total energy available for tissue metabolism is channeled to resist stress. In medium with >100 mM of NaCl (data not presented) sensitive callus became soft, necrotic, and stopped growing. Callus obtained with control, and lower concentrations of NaCl (50 mM), were green and compact and showed less injury. Decline of callus growth due to NaCl concentration was observed in many plant tissues (Rains, 1989; Sheldon et al., 2004). Takashi et al. (2002) found that garlic (A. sativum) calli in vitro are affected over a range of salt concentrations in culture medium. Under salt stress, difference in water potential of the medium may affect growth, because cumulative effects of water stress on callus become more extreme in long term cultures. Thus, the importance of water status in tissue culture medium should be considered when optimizing garlic in vitro propagation.

Percent increase in calli was highest in standard Murashige and Skoog medium and reduced when NaCl was added to MS medium. Leland et al. (1994) observed that yield and quality response of garlic exposed to salt reduced all yield components when irrigation water was more than 9.9 dS/m. Due to lack of information pertaining to garlic growth under saline conditions, it was important to determine the yield and quality response of garlic grown under increasing levels of salt stress. The results of this experiment with those of other authors on other crop (Zahra et al., 2015; Yunital et al., 2014; Benderradji et al., 2012) when calli were exposed to NaCl indicate that the response is likely to be universal. Incorporation of NaCl in the medium during callus induction and regeneration will pave the way for studying the effects of salt stress on different stages of development. Screening in vitro to obtain NaCl tolerant strains can be used to explain physiological aspects of tolerance. Use of NaCl to study tolerance in vitro may indicate how plants perform in the field when exposed to similar amount of salt.

Although garlic tends to be slightly more salt tolerant than most vegetables, the need to maintain low soil salinity levels is essential for maximum yield. Once salinity exceeds the 100 mM threshold, yield and quality processing attributes are reduced. It is important to devise an efficient protocol of callus proliferation to begin in vitro selection for salt stress tolerance, and extend opportunities for genetic manipulation of garlic through tissue culture. In vitro techniques could be important to improving crop tolerance and yield through genetic transformation. It is important to devise an efficient protocol of callus proliferation for in vitro selection for salt stress tolerance, so that experiments can be designed to study salt tolerance of garlic under field conditions.

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REFERENCES


