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Research Article

Effect of different concentrations of NaCl on micropropagation of *Mentha viridis*

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Abstract: Wild type species of Mentha was assayed *in vitro* for salinity (NaCl) tolerance. Micro propagated sub cultured stem of Mentha was used to assay shoot length, multi shooting, root length, root number, and the emergence of leaves. Different concentration of NaCl (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml and 0.4 mg/ml) supplemented in MS media was used for different treatments. Data was recorded after various periods (7 days, 14 days, 21 days, and 28 days). The study reveal that shoot length increases favourably at low concentration of NaCl (0.1 mg/ml and 0.2 mg/ml) but at higher concentration (0.3mg/ml and 0.4 mg/ml) there is a reduction in length. Number of emerged shoots increases during every treatment. The number, however, gets inhibited at 0.4mg/ml of NaCl. NaCl's concentration (except 0.4 mg/ml, which completely inhibits emergence) seems to favor the emergence of roots. The number seems to be the greatest at 0.3 mg/ml of NaCl. Every concentration of NaCl except for 0.4 mg/ml (which completely inhibits the growth) inhibits the root length. Every concentration of NaCl has been found to favour the emergence of leaf in the propagated plantlet. A concentration of 0.3 mg/ml has been found to initiate the emergence of leaves the fastest. The emergence of leaves could also be noticed at 0.3 mg/ml and 0.4 mg/ml even after 21 days of incubation. The finding mentioned above can be useful in future research on micropropagation. The details of each finding is described.

Keywords: *Mentha viridis*; salinity; *in vitro;* micropropagation

Introduction

Soil salinity is one of the most critical global problems that negatively affect crop productivity. Salinity impairs plant growth and development via water stress, cytotoxicity due to excessive uptake of ions such as sodium (Na+) and chloride (Cl−), and nutritional imbalance. Additionally, salinity is typically accompanied by oxidative stress due to the generation of reactive oxygen species (ROS) (Tsugane *et al.* 1999; Hernandez *et al.* 2001; Isayenkov, 2012). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy, and lipid metabolism are affected (Parida & Das, 2005). During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. The osmotic effects of salinity stress can be observed immediately after salt application and are believed to continue for the duration of exposure, resulting in inhibited cell expansion and cell division, as well as stomatal closure (Flowers, 1977; Flowers, 2004; Munns, 2002). During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth (Cramer & Nowak, 1992). Excess sodium and, more importantly, chloride can affect plant enzymes and cause cell swelling, resulting in reduced energy

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production and other physiological changes (Larcher 1980; Greenway & Munns, 1980). Ionic stress results in premature senescence of older leaves and toxicity symptoms (Chlorosis, Necrosis) in mature leaves due to high Na+, which affects plants by disrupting protein synthesis and interfering with enzyme activity (Bohnert, 2000; Munns, 2002; Munns & Termaat, 1986). The *Mentha sp.*, is an essential member of the family Lamiaceae that contain several species, different regarding their ploidy levels (Bhat *et al.* 2013). The plant is widely used in the cosmetic and pharmaceutical industries. Tissue culture techniques offer an easy and important tool in developing stress-tolerant variants (Gayathri *et al.* 2015; Salem, 2016; Larit & Saiba, 2018). In the present study, an attempt has been made to evaluate the effect of various salt concentrations on *in vitro* cultivation of subcultured wild *Mentha* plant.

Materials and Methods

Source of explants

Field grown plants of *Mentha viridis* were used as explants for *in vitro* cultivation. Various parts of plants, such as segments of the stem, nodes of the stem, have been used as explants source.

Sterilization

The plant material was first washed in continuous running water for 2 hours and then in sterilized distilled water mixed with Tween 20. Washing in Tween 20 was performed with shakings to remove surface adhered contaminants. (Jimenez *et al.* 1996; Gayathri *et al.* 2015; Salem, 2016).

The plant sample was again washed with sterilized distilled water mixed with Bavistin (01%). The plant sample was then washed three times in sterilized distilled water before transferring to the laminar flow chamber to transfer to culture media. The plant sample was further disinfected with 0.1% (w/v) Mercuric Chloride ($HgCl_2$) for five minutes, followed by thorough rinsing in autoclaved distilled water at least seven to eight times. To avoid bacterial contamination, the plant samples were treated with Mox solution (500 mg Mox dissolved in 100 ml of sterilized distilled water). The surface-sterilized explants contained nodal segments having a length of 0.5 to 01 cm. containing a single node.

Culture medium and condition:

MS medium was for *in vitro* culture explants (Murashige & Skoog, 1962). This media was added 03% sucrose and 0.8% agar (used to solidify the medium). The *p*H of the medium was adjusted to 05. by adding 01N NaOH / 01N HCl and then autoclaved to 121 ^{0}C for 20 minutes. The culture was maintained at 25 $^{-+}$ under16-hour photoperiod provided by white fluorescent tubes. Measurement was done using the centimeter scale.

Results and Discussion

Effect of different concentration of NaCl on micropropagation of cultured sub stem in MS media supplemented with N_1K_1 (1mg of Naphthalene acetic acid and 1 mg of Kinetin) different concentrations of NaCl was added. MS media without NaCl served as control. Ex plants propagated from the stem tip was transferred to each tube and incubated at the desired temperature. The result obtained for each parameter has been described under a separate heading (Plates 1-6).

Effect of different concentration of NaCl on shoot length of subcultured explant of *Mentha viridae s*ubcultured stem was transferred to MS medium containing different concentration of NaCl (0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml and 0.4 mg/ml) media without NaCl served as control. Result obtained has been described in Figure 1.

Plate 1. Growth of *Mentha* subcultured stem on MS media supplemented with $N_1 K_1$

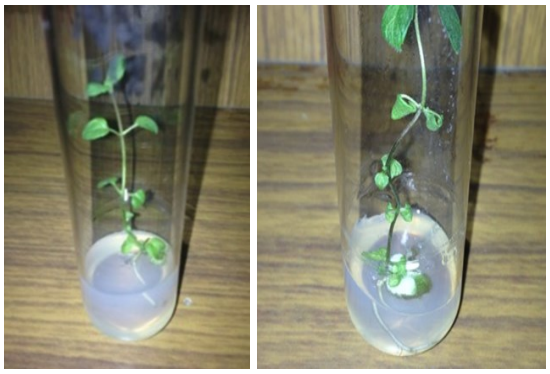

Plate 2, Plate 3. Growth of *Mentha* subcultured stem on MS media supplemented with N_1K_1 and 0.1 mg/ml of NaCl

Plate 4. Growth of *Mentha* subcultured stem on MS media supplemented with N_1K_1 and 0.2 mg/ml of NaCl (Showing stem length and internode length)

Plate 5, Plate 6, Plate 7. Growth of *Mentha* subcultured stem on MS media supplemented with N_1 K_1 and 0.2 mg/ml of NaCl (Showing multi shooting and emergence of the leaf)

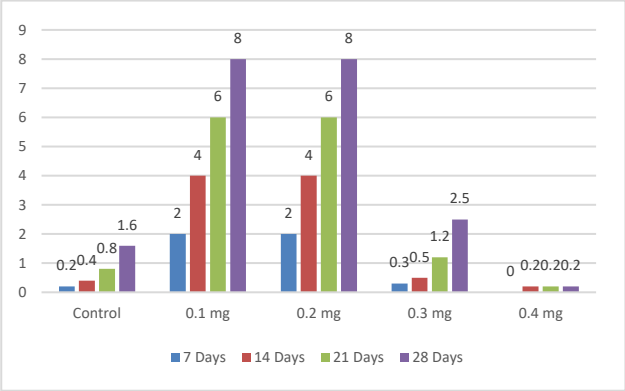

Figure 1. Effect of different concentration of NaCl on shoot length (cm) in *Mentha viridis*

The result described in Fig 1 reveals that shoot length increases slowly in the absence of NaCl, but in the presence of 0.1 mg/ml and 0.2 mg/ml it increases rapidly. The trend of growth has been identical in both treatments. At a concentration of 0.3 mg/ml of NaCl the growth is greatly retarded. This retardation continues during treatment with 0.4 mg/ml of NaCl. Thus, it appears that 0.1 mg/ml and 0.2 mg/ml favors growth at a concentration higher than this value inhibits the growth. Salinity reduces shoot growth by suppressing leaf initiation and expansion, as well as internode growth, and by accelerating leaf abscission in maize (Akram *et al.* 2010a, 2010 b; Qui *et al.* 2012). Salt stress rapidly reduces leaf growth rate due to a reduction in the number of elongating cells and / or cell elongation rate in maize (Szalai & Janda, 2009). A low concentration of salt (0.1 mg/ml and 0.2 mg/ml) has a positive effect on shoot length, and this finding is consistent with the finding of Bouraoui *et al.* (1998).

In a separate set of the experiment, the cultural condition was the same as described above. The number of shoots that emerged during different conditions has been described. Effect of different concentrations of NaCl on number of shoots of the subcultured stem of *Mentha*. Similarly, as described above, freshly subcultured stem of *Mentha* was transferred to MS Medium containing different NaCl concentrations, Media without NaCl served as control. Result has been described in Fig 2.

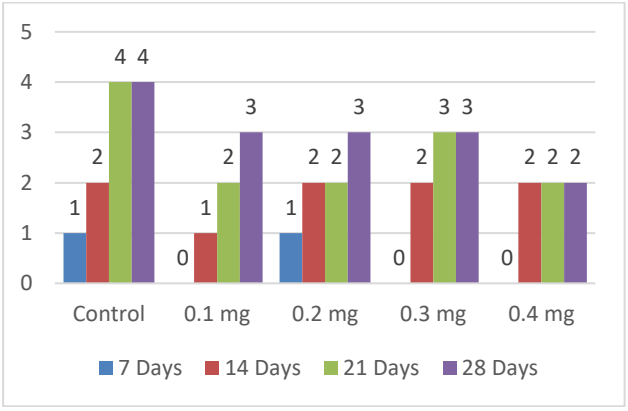

Figure 2. Effect of different concentration of NaCl on number of shoots in *Mentha viridis*

On scanning the data provided in Figure 2 it appears that the number of shoots emerging from the subcultured stem has been almost identical except for control, where the number is marginally more significant and in 0.4 mg/ml of NaCl where it is marginally less. In order to evaluate the role of NaCl on the emergence of root and its length during treatment with NaCl, the experiment was designed.

Effect of different concentrations of NaCl on a number of emerging roots and its length in *Mentha viridis* In order to evaluate the role of NaCl on the number of emerging roots and also root length. After various periods of treatment has been described in Figure 03 and Figure 04.

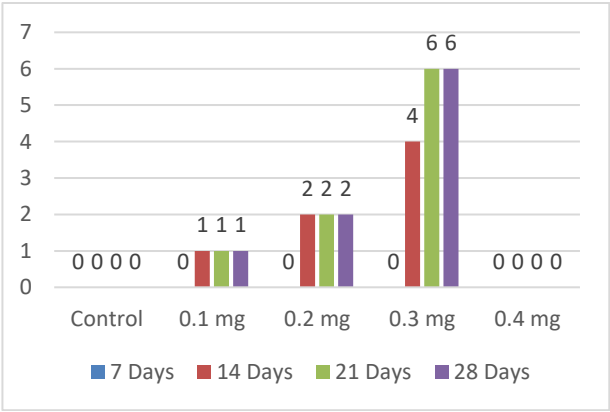

Figure 3. Effect of different concentration of NaCl on the number of roots in *Mentha viridis*

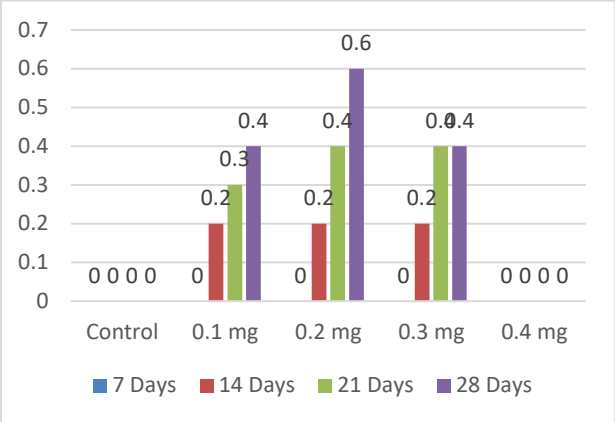

Figure 4. Effect of different concentration of NaCl on root length (cm) in *Mentha viridis*

From the data described in figure 3 it appears that supplementation of NaCl in MS media induces root development in *Mentha* during micropropagation. The trend of emergence continues even at 0.3 mg/ml of NaCl. At a concentration above 0.3 mg/ml of NaCl there is complete inhibition in root number. The trend in rise in root number has been 0.1 mg/ml<0.2 mg/ml<0.3 mg/ml.

On review of data described in Fig-04, it appears that an increase in root length has been similar to an increase in root number. Rise in length of the root begins with 0.1 mg/ml of NaCl, increases further in 0.2 mg/ml of NaCl and then declines at 0.3 mg/ml of NaCl. At a concentration of 0.4 mg/ml the growth is completely inhibited. The root system has been found to decrease in Barley at high concentration of NaCl (Suhayda *et al.*,1992). Guerrier, (1996) reported a slowdown in root growth and leaf growth in glycophytes due to the presence of salinity. NaCl's addition to the culture media decreased the osmotic potential of the media inducing salinity stress that adversely affected the plants' growth of potato cultivars. Several authors reported NaCl's use for *in vitro* salinity screening in potatoes and different plants (Zhang *et al.* 1993; Pour *et al.* 2009). Plants growing in the presence of increasing NaCl concentrations decreased their shoot and root length in all potato cultivars (Zhang *et al.* 1993; Pour *et al.* 2009). Sasikala & Prasad (1994) and Zhang *et al.* (1993) reported that shoot and root but not shoot dry weight significantly decreased in the nodal cutting challenged with salt, compared with the control's growth. This finding has been consistent with the present finding.

Significant micropropagation demands the development of every part of the plant body. The future development of a plant is very much dependent on the emergence and development of leaves. Keeping this perspective in mind, the effect of different concentrations of NaCl on the emergence of the leaf was undertaken. Effect of different concentration of NaCl on the emergence of leaf in Mentha sp

A similar kind of experiment subcultured stem of Mentha was transferred to test tube containing MS media supplemented with different NaCl concentrations. After 7, 14, 21, and 28 days of incubation at the desired temperature, the emergence of leaf in each tube was recorded and mentioned in figure 5.

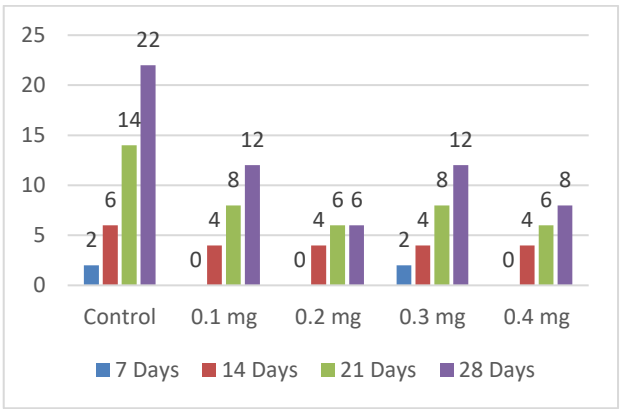

Figure 5. Effect of different concentrations of NaCl on the number of leaves in *Mentha viridis*

On scanning the data described in Fig 5 it appears, that leaf appears to emerge during all the NaCl treatments. The best value has been noticed under the control condition when the number of leaves increases with the lapse of time. Any concentration of NaCl does not seem

to affect severely the emergence of leaf. The number of emergences of leaf increases with lapse of time. NaCl's salinity effect appears to be less in *Mentha viridis* so far as the number of leaf emergences is concerned. A negative impact of different concentrations of NaCl has been reported by Larit & Saiba (2018). A higher concentration inhibits the emergence of leaves in *Solanum tuberosum*.

Conclusion

Mentha is a wild plant having the capacity to grow in a variety of ecological niche. The plant has great economic importance as it produces wide variety of commercial products such as oil, insecticides, pesticides, and food additive. Natural hybridization is a common feature of this plant. Strain improvement using modern biotechnological principles appears to be necessary for this plant to improve the quality and quantity of this plant's produce. One of the most important technological approaches has been *in vitro* method of propagation. Less knowledge is available regarding the impact of salinity on this plant. During the present study effect of different concentrations of NaCl has been evaluated. Shoot length increases at o.1mg/ml and 0.2 mg/ml but get inhibited at 0.3 mg/ml and 0.4 mg/ml. The number of shoots continue to increase up to 0.3 mg/ml but get inhibited at o.4 mg/ml of NaCl. The number of roots gets inhibited at 0.4 mg /ml of NaCl; however, other concentration favours the growth. A similar trend has been noticed in determining root length. The number of leaves increases during all the treatments but less than control. The knowledge achieved from present study can be suitably used in designing an experiment on micropropagation under salt stress conditions on this test plant. This knowledge can also be used in establishing a salt-tolerant variety.

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