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In vitro Salinity Evaluation Studies in Golden Berry (*Physalis peruviana* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author FBC wrote the protocol and wrote the first draft of the manuscript. Authors FBC and PA performed the experiments and performed the statistical analysis. Author FBC managed the literature searches. Author ANO designed the study, managed the analyses of the study and edited the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Present study, therefore, was conducted in *in vitro* conditions to study effect of NaCl at varied levels on growth and chlorophyll content of golden berry shoot apices grown in *in vitro* conditions.
Place and Duration: The study was carried out in the Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey, between March-June, 2017.
Methodology: In this study *in vitro* salinity of golden berry shoot apex culture were studied within the Murashige and Skoog nutrient medium with 1 mg L^{-1} indole acetic acid (IAA)+ 3% sucrose and 0.7% agar supplemented with NaCl (0; 25; 50; 75 and 100 mM). The explants were incubated at 25±2℃ for 4 weeks and related parameters, such as shoot, leaf and root formation, were measured.
Results: Experimental results revealed that different level of salinity treatments in *in vitro* culture had notable effect on above stated growth parameters. These parameters decreased significantly by increasing salinity level for excluding shoot diameters.

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Keywords: golden berry (Physalis peruviana L.); salinity; in vitro; shoot apex culture.

1. INTRODUCTION

Nowadays, agriculture is becoming increasingly difficult due to climate change. It has been noticed that the warmer regions would be even hotter and rainy regions more humid due to climate changes [1]. One of the effects of climate change is the abiotic stress factors in plants. Salinity stress, is the leading cause of abiotic stress and salinity stress threatens many crop groups in the world.

As the amount of salt increases, the ability of the plant to get water becomes difficult. Increase on Na^+ and Cl^- ratios in roots and in leaves inhibits photosynthesis by causing stomatal closure and decreasing total chlorophyll content [2,3]. Salinity stress can affect adversely plants through morphological, changes in their organs by reducing their growth [4,5].

Goldenberry fruit is producted of 162,390 tonnes in roughly 30,622 ha area in the world [6]. It is referred as cape gooseberry, aguaymanto, topotopo, uvilla, uchuva, physalis, giant ground cherry, rasbhari, pokpok, harankash, Inca berry, African ground cherry, Peruvian ground cherry, Peruvian cherry, Aztec berry or golden berry (*Physalis peruviana* L.) [7,8]. This fruit has gained more importance in recent years due to its high content of vitamins (A,B,C), minerals (phosphorus (P) and iron (Fe)) and antioxidants (β-carotene) as well as its antiinflammatory and anticancer properties which are important for human health [9,10,11]. The golden berry (46g/100 g) contains ascorbic acid (vitamin C), which is almost identical to the orange (50 g/ 100 g) [8].

Golden berry belongs to the genus Physalis of the Solaneceae family [11]. It is known that Solanaceae family plants are moderately sensitive to salinity [12]. The responses of the plants to salinity were noticed to vary according to species and varieties [13]. Species in family Solanaceae show variations in terms of salinity tolerance level due to differences in genome structures. In that sense golden berry provides an opportunity to study abiotic stress tolerance mechanisms [14].

It is reported that obtaining salt tolerant plants is not easy because of the shortage on knowledge about the physiological, biochemical and molecular mechanisms of [15]. *In vitro* techniques are important tools for modern plant breeding programs for introducing new traits into selected plants; offer the advantage of evaluation the stress tolerance, such as salinity, drought and frost, of plant species within short term without being affected by environmental factors [14,16,17]. In that sense several researches conducted *in vitro* salt studies on different crops such as ajwain (*Carum copticum* L.) [18], brbin (*Bacopa monnieri* L.) [19], potato (*Solanum tuberosum* L. cv. Challisha) [20]. They pointed out that with the increase in salinity, plant growth was affected in the negative direction. Present study, therefore, was conducted in *in vitro* conditions to study effect of NaCl Sodium chloride at varied levels on growth and chlorophyll content of golden berry shoot apices grown in *in vitro* conditions.

2. MATERIALS AND METHODS

2.1 Plant Material and Culture Conditions

Golden berry seeds were surface sterilized for 15 min with 40% (w/v) sodium hypochlorite. Then, the seeds were rinsed in sterile deionized water three times. Both of seeds were then germinated *in vitro* on agar solidified Murashige and Skoog (MS) basic medium [21]. Golden berry seeds germinated nearly in fourteen days. The medium pH was adjusted to 5.7±0.1 before the addition of agar and subsequent autoclaving at 121℃. The cultured seeds were incubated in growth chamber under 16 h illuminations (40 µmol m^{-2} s^{-1}) at 25±2℃. When the plantlets had developed three true leaves (3 weeks after germination) explants were collected.

2.2 Shoot Apex Culture under Salinity Treatments

Shoot apices 1 cm in length were cut and put perpendicularly into jar (660 ml) containing 100 ml of medium. Shoot apices were cultured on Murashige-Skoog (MS) medium containing 1 mg L^{-1} indoleacetic acid (IAA), 30 g L^{-1} sucrose, and 7 g L^{-1} plant agar and supplemented with different concentrations NaCl (0-100 millimolar (mM). All media were adjusted to pH 5.7±0.1℃ before autoclaving at 121℃ for 20 min. The cultures were maintained at 25±2℃ temperature, under 16/8 h light/dark regime, 40 µmol m^{-2} s^{-1} light intensity provided by white fluorescent bulbs 36 W (Phillips).Five explants per culture jar and

five replicates per salinity level treatment were employed.

2.3 Growth Measurement

After four weeks, plantlets were removed from the culture jar and plantlets fresh and dry weight was measured weigher. Leaf lenght, leaf diameter, shoot lenght, shoot diameter and root lenght measured with caliper. Amount of chlorophyll content was measured in daylight with Spectrum Technologies FieldScout CM1000 Model Chlorophyll Meter (Fig. 1).

2.4 Statistical Analyses

The results of the measured parameters on golden berry plantlets were evaluated in the SPSS 17.0 statistical package program and significant differences (P<.05) between the averages were determined using One-way ANOVA.

3. RESULTS AND DISCUSSION

Salinity can cause stress at different severity depending on species, salinity kind and stress time in plants. Salt amount increasing around the root can cause Na^+ and Cl^- increase in plant tissues and organelles. This increase causes ion stress and increasing of osmotic pressure bringing instability between K^+ and Ca^{++} ions [22, 23,24,25]. Plant's taking up water and growing slow down together with rising osmotic pressure. It can inhibit photosynthesis by damaging protein, chlorophyll; DNA and cell membrane, as a result, even cause the death of the cells. As a result of this, changes in some morphological qualities of the plants can be observed together with the increment of salt density [26,27,28]. Leaves on the plants stay short, leaf areas narrow and plant length shortens [22,29].

Shoot apex culture which is an *in vitro* technique was used in the study in order to observe just the impact of salinity factor without being affected by environmental factors in a more controlled environment to save time and space in the study. For that purpose, the effects of 5 different salt levels applied on golden berry explants were analyzed.

3.1 Effects of Salinity and Plantlet Growth Parameters

The effect of 5 different salt levels added to MS nutrient media on golden berry plantlets was compared with LSD replicate comparison tests according to ANOVA procedure and differences between measured parameters were shown together with average and standard errors (Table 1).

It has been concluded that the increment in salt concentration in tomato [15], potato [30] and eggplant [29,31], causes decrement in shoot length. In this study, it was appointed that shoot length being among the measured parameters was statistically different from each other. While the difference between shoot lengths in control group (no added salt) and salt applications at 25 mM doses was found unimportant as a result of statistics analysis, the difference between especially 100 mM dose salt applications of the control group was found significant. However, it was observed that the salinity didn't have directly proportional effect on shoot diameter being one of the shoot growth measurements. While a decrement has been observed in parallel with the increment in salt concentrations in shoot lengths, the same thing cannot be said considering shoot diameters. There is an increase in 100 mM application whereas there is a regular decrease in the applications from 0 to 50 mM (Fig. 2).

The study was carried out at 5 replications and repeatedly, the averages of the measurements of small, middle and big leaves of the plantlets of the same application were taken. It was observed that 25 mM application in both leaf measurements didn't make a statistical difference compared to control, however, a decrease was observed in especially 100 mM following the increasing of dose. Moreover, it was seen that 75 and 100 mM applications were not statistically so different from each other (Table 1).

It was reported in previous studies that the salinity decreased root density [15,16]. In root length parameter in this study, the longest root lengths were recorded and subjected to statistics analysis. When Table 1 is looked in consequence of the analysis, it can be said that the root length averages can decrease together with the increase of salt rate in the other applications compared to the control group. However, this was not found statistically great difference in root measurement between control and 25, 75 and 100 mM applications. As a result of the morphological observations, we can say that root density decreased in this study (Fig. 3).

Table 1. Effect of NaCl on root length (the longest lenght of roots), shoot length, shoot diamether, leaf length and leaf width) and shoot fresh and dry weight) of *in vitro* plantlets of golden berry (*Physalis peruviana* L.) after 4 weeks in culture (mean±SE)

NaCl level (mM)	Shoot lenght (mm)	Shoot diamether (mm)	Root lenght (mm)	Leaf lenght (mm)	Leaf width (mm)	Fresh weight (g)	Dry weight (g)
0	129,89±1,46^{a}	2,33±0,07^{a}	61,50 ±2,85^{a}	32,48±1,06^{a}	24,31±0,52^{a}	3,10±0,21^{a}	0,18±0,0101^{a}
25	124,49±2,95^{ab}	2,32±0,08^{a}	61,27±3,85^{a}	31,51±1,82^{a}	23,18±0,80^{a}	2,50±0,18^{b}	0,14±0,0106^{b}
50	111,74±3,35^{b}	2,14±0,06^{ab}	60,74±2,68^{a}	27,49±0,74^{b}	20,48±0,58^{b}	1,44±0,09^{c}	0,08±0,0048^{c}
75	83,85±4,04^{c}	1,91±0,08^{b}	57,49±1,28^{a}	24,18±0,86^{bc}	18,87±0,72^{bc}	0,83±0,06^{d}	0,06±0,0036^{cd}
100	69,59±3,95^{d}	1,96±0,09^{b}	55,83±1,77^{a}	23,30±1,54^{c}	17,61±0,88^{c}	0,78±0,07^{d}	0,05±0,0043^{d}
Total	106,09±2,56	2,14±0,04	59,15 ±1,14	28,03±0,62	21,08±0,38	1,78±0,11	0,11±0,0058

The mean difference is significant at the 0.05 level

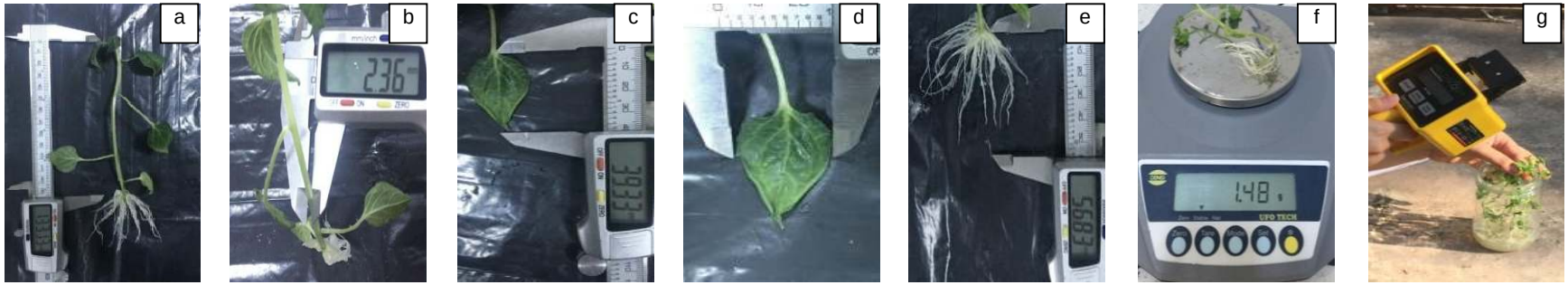
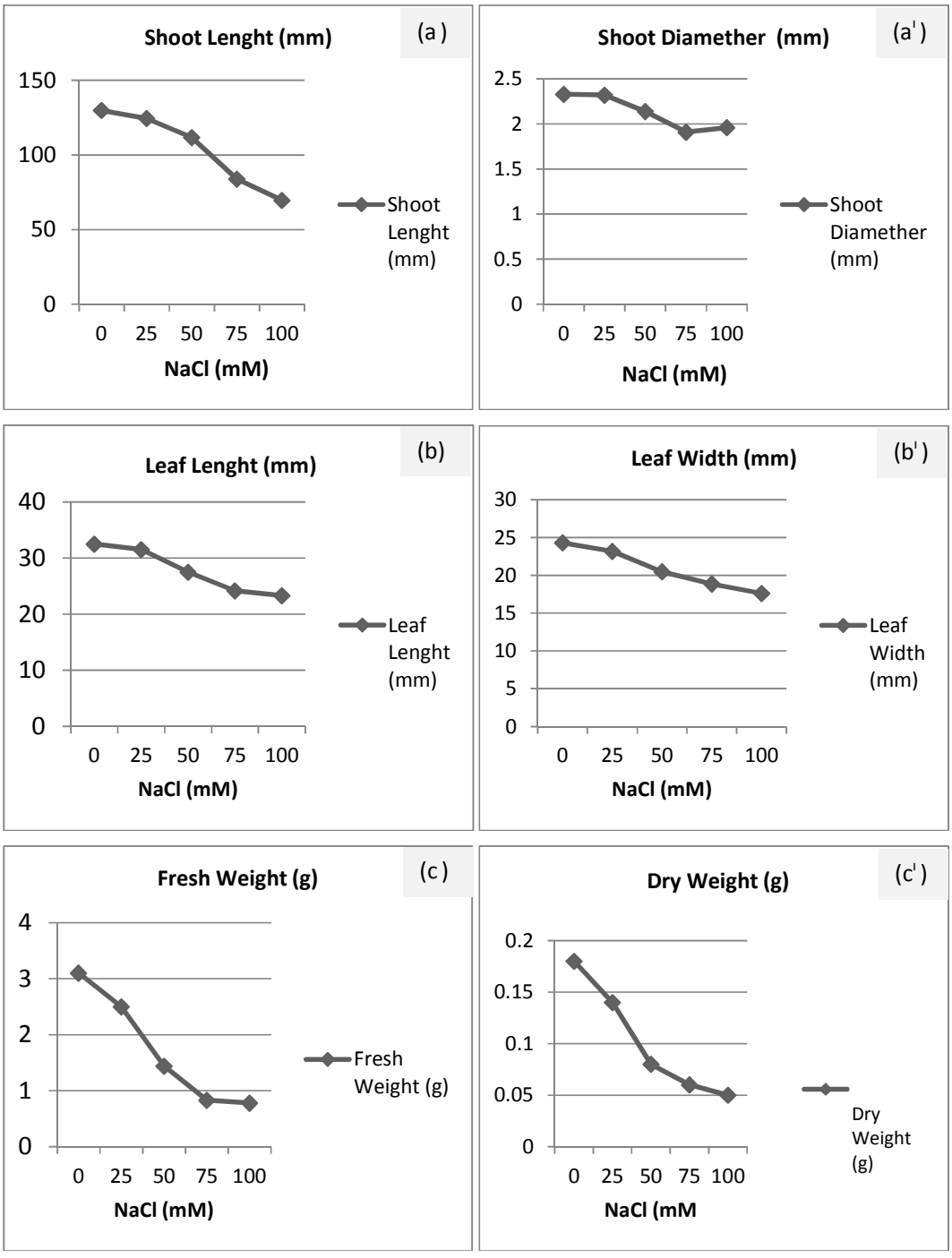


Fig. 1. Measurement of golden berry seedling with caliper shoot length (a), shoot diamether (b), leaf length(c), shoot width(d), root length (e) and measurement of golden berry seedling fresh and dry weight (f), chlorophyll amount (g)

The effect of NaCl percent of shoot and dry weight of plantlets grown *in vitro* from shoot apices is presented in Table 1. In the study, dry weight was weighed on microbalances after being kept at 60° for 18 hours. When all the applications were compared to the control a significant reduction was seen in fresh and dry weight measurements of especially 100 mM compared to the control group. Similar results were reported by [32] and [15] saying that there was a decrement in the increase of salt concentration in tomato and fresh weight of the plants.



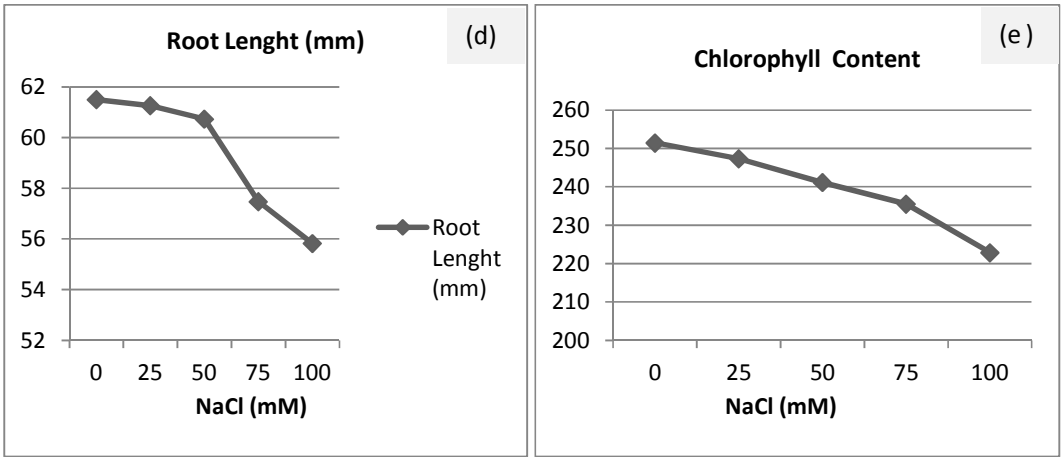


Fig. 2. Effect of NaCl on Shoot length(a), shoot diamether (a^{I}), Leaf length (b) and Leaf width (b^{I}), root length (d) and chlorophyll (e) of golden berry (*Physalis peruviana* L.) shoot apices

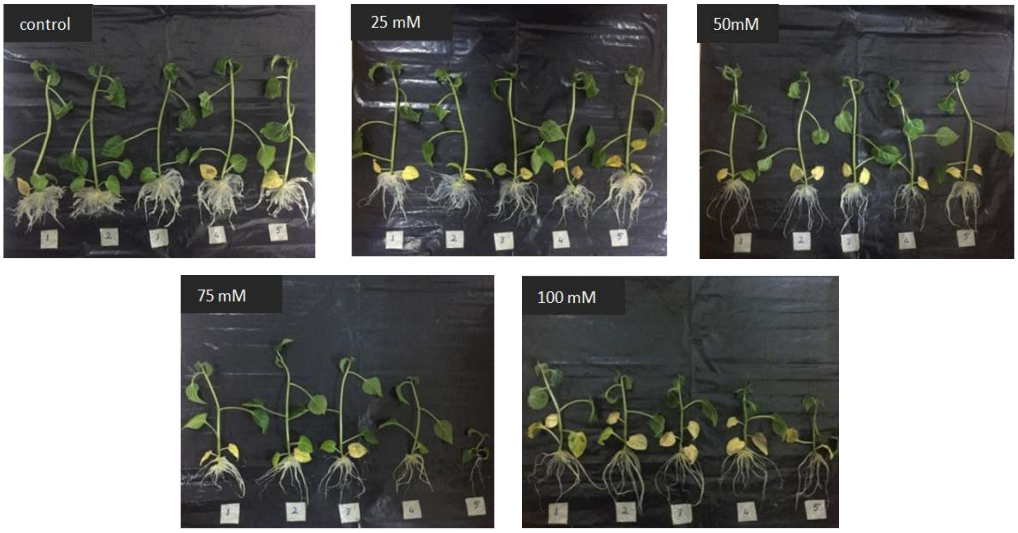


Fig. 3. Shoots were cultured on media supplemented with five different concentrations of NaCl (0, 25, 50, 75 and 100 mM)

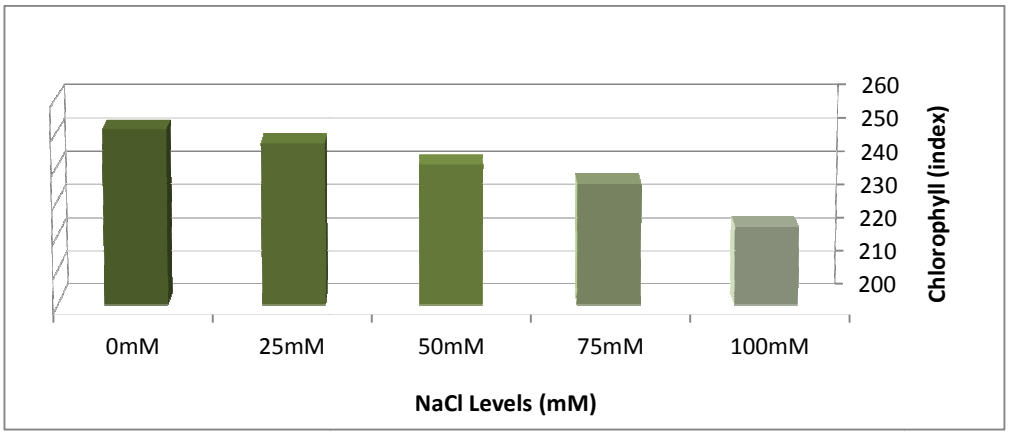


Fig. 4. Change in chlorophyll (index) due to increased salt (NaCl) concentration (mM)

In the study, chlorophyll amount was measured in daylight with Spectrum Technologies FieldScout CM1000 Model Chlorophyll Meter by taking 2 plantlets from each application outside the laboratory. Different salt concentrations applied on golden berry shoot tips were compared according to ANOVA procedure and it was seen that they were statistically different from each other with regards to chlorophyll content. In consequence of statistics analysis, unlike control group, a significant difference was seen at 75 and 100 mM doses ($P<.05$) whereas the differences of salt applications at 25 and 50 mM doses considering chlorophyll content were found insignificant ($P>.05$). It was observed that the salt applications at 25 and 50 mM doses were not different from control group in terms of chlorophyll content, and it can be said that a decrement occurred in chlorophyll value in parallel with the decrement of salt amount (Fig. 4). It is considered that this decrement is related to the increase in the activity of chlorophyllase enzyme decomposing chlorophyll [33].

4. CONCLUSION

As a result, it was seen that control group and 25 mM salt applications did not cause a change at high rate in all the parameters measured in general. However, it was observed that a gradual decrement occurred with the increasing of concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFFERENCES

1. Pachauri R, Meyer L, editors. Climate change. Synthesis report. Contribution of working groups I, II and III to the Fifth assessment report of the intergovernmental panel on climate change. Geneva, Switzerland; 2014.
2. Romero-Aranda R, Soria T, Cuartero J. Tomato plant-water uptake and plant-water relationships under saline growth conditions. Plant Science. 2001;160(2): 265-272.
3. Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI. Plant salt-tolerance mechanisms. In Trends in Plant Science. 2014;19(6):371-379.
4. Al-Karaki GN. Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress. Journal of Plant Nutrition. 2000;23(1):1-8.
5. Roy SJ, Negrão S, Tester M. Salt resistant crop plants. Current Opinion in Biotechnology. 2014;26:115-124.
6. FAOSTAT; 2013. Available:http://faostat.fao.org/faostat
7. Puente LA, Pinto-Muñoz CA, Castro ES, Cortes M. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. Food Research International. 2011;44(7):1733-1740.
8. Ramadan MF, Hassan NA, Elsanhoty RM, Sitohy MZ. Goldenberry (*Physalis peruviana*) juice rich in health-beneficial compounds suppresses high-cholesterol diet-induced hypercholesterolemia in rats. Journal of Food Biochemistry. 2012;37(6): 708–722. DOI:10.1111/j.1745-4514.2012.00669.x
9. Zhang YJ, Deng G-F, Xu X-R, Wu S, Li S, Li H-B. Chemical components and bioactivities of cape gooseberry (*Physalis peruviana*). International Journal of Food Nutrition and Safety. 2013;3(1):15-24.
10. Rabie MA, Soliman AZ, Diaconeasa, ZS Constantin B. Effect of pasteurization and shelf life on the physicochemical properties of physalis (*Physalis peruviana* L.) juice. Journal of Food Processing and Preservation. 2015;39(6):1051–1060. DOI: 10.1111/jfpp.12320
11. Torres J, Pascual-Seva N, San Bautista A, Pascual B, López-Galarza S, Alagarda J, Maroto JV. Growth and nutrient absorption of cape gooseberry (*Physalis Peruviana* L.) in soilless culture. Journal of Plant Nutrition. 2015;38(4):485-496.
12. Jan N, Gautam YK, Vimala Y. Higher salt tolerance of *Physalis peruviana* L. callus with reduced peroxidase activity compared to its *in vitro* raised seedlings. International Journal of Research in Ayurveda Pharmacy. 2015;6(2):272-276.
13. Zayova E, Philipov P, Nedev T, Stoeva D. Response of *in vitro* cultivated eggplant (*Solanum melongena* L.) to salt and drought stress. AgroLife Scientific Journal. 2017;6(1).
14. Taji A, Kumar PP, Lakshmanan P. *In vitro* plant breeding. New York: Food Products Press. 2002;167.
15. Zaki HEM, Yokoi S. A comparative *in vitro* study of salt tolerance in cultivated tomato and related wild species. Plant Biotechnology. 2016;33(5):361-372.

16. Cano EA, Perez-Alfocea F, Moreno V, Caro M, Bolarin MC. Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant Cell Tiss Org Cult. 1998;53(1):19-26.
17. Liza LN, Nasar ANM, Zinnah KMA, Nayem Chowdhury MdAl, Ashrafuzzaman M. *In vitro* growth media effect for regeneration of tomato (*Lycopersicon esculentum*) and evaluation of the salt tolerance activity of callus. Journal of Agriculture and Sustainability. 2013;3(2):132-143.
18. Biswas Md. S, Islam Md. R, Zakaria M. Evaluation of indigenous potato challisha (*Solanum tuberosum* L. Cv. Challisha) somaclonals tolerance to salinity *in vitro*. The Journal of Tropical Life Science. 2017;7(1):77-82.
DOI: 10.11594/jtls.07.01.13
19. Almusawi AHA, Al-Aradi HJ, Hammadi KJ. The impact of salinity stress on morphological and anatomical aspect of water hyssop *Bacopa monnieri* L. Wettst grown *in vitro*. African Journal of Biotechnology. 2017;16(15):801-807.
20. Razavizadeh R, Adabavazeh F, Chermahini MR. Adaptive responses of Carum copticum to *in vitro* salt stress. World Academy of Science, Engineering and Technology. 2017;11(1):37-42.
21. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962;15(3):473–497.
DOI: 10.1111/j.1399-3054.1962.tb08052.x
22. Shannon MC, Grieve CM. Tolerance of vegetable crops to salinity. Scientia Horticulturae. 1998;78(1–4):5-38.
23. Hasegawa M, Bressan R, Pardo JM. The dawn of plant salt tolerance genetics. Trends Plant Sci. 2000;5(8):317-319.
24. Xie JH, Zapata-Arias FJ, Shen M, Afza R. Salinity tolerant performance and genetic diversity of four rice varieties. Euphytica. 2000;116:105-110.
25. Zhu JK. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology. 2002;53:247-273.
26. Daşgan HY, Aktaş H, Abak K, Çakmak İ. Determination of screening techniques to salinity tolerance in tomatoes and investigation of genotype responses. Plant Science. 2002;163(4):695-703.
27. Borsani O, Valpuesta V, Botella MA. Developing salt tolerant plants in a new century: A molecular biology approach. Plant Cell Tiss Org Cult. 2003;73(2):101-115.
28. Amini F, Ehsanpour AA. Soluble proteins, proline, carbohydrates and Na+/Cl− changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under *in vitro* salt stress. Am J Biochem Biotechnol. 2005;1:212–216.
29. Akinci IE, Akinci S, Yilmaz K, Dikici H. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. New Zealand Journal of Crop and Horticultural Science. 2004; 32(2):193-200.
DOI: 10.1080/01140671.2004.9514296
30. Martinez CA, Maestri M, Lani EG. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. Plant Science. 1996; 116(2):177-184.
31. Zayova E, Philipov P, Nedev T, Stoeva D. Response of *in vitro* cultivated eggplant (*Solanum melongena* L.) to salt and drought stress. Agro Life Scientific Journal. 2017;6(1):276-282.
32. Mohamed AN, Ismail MR, Kadir MA, Saud HM. *In vitro* performances of hypocotyl and cotyledon explants of tomato cultivars under sodium chloride stress. African Journal of Biotechnology. 2011;10(44): 8757-8764.
33. Reddy MP, Vora AB. Changes in pigment composition, Hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* S&H) leaves under NaCl salinity. Photosynthica. 1986;20:50-55.

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