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INFLUENCE OF SALT STRESS ON MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF PEACH (*PRUNUS PERSICA* L.) NEMAGUARD ROOTSTOCK *IN VITRO*

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Abstract

This investigation was carried out during the period from 2018 to 2019 to study the effect of NaCl on growth of Peach Nemaguard rootstock. MS media supplemented with 1.0 mg /L and 0.1 mg/L IAA caused the maximum shoot number, shoot length (cm) and shoot fresh weight (g) in starting stage. Adding 1.0 mg /L of both BA and KIN to MS media led to the maximum shoot number and shoot fresh weight (g) in multiplication stage. Salt stress induced by adding sodium chloride (NaCl) at 0.0, 15, 30, 60 and 120 mM into the Murashige and Skooge medium was applied for four weeks. NaCl-induced salt stress reduced the morphological characteristics such as shoot number, shoot length, leaves number, shoot fresh weight (g), shoot dry weight (g) and survival percentage in Peach Nemaguard rootstock. Also, NaCl reduced relative water content, chlorophyll a, b, total chlorophyll and carotenoids. Elements accumulation and proline content in plant leaves in relation to salt stress were increased by increasing salt concentration. Results showed that adding NaCl from 30 to 120 mM to MS media caused the reduction in K, Ca and P elements while this led to an increase in Na and Cl elements in leaf Peach Nemaguard rootstock. Results indicated that adding 30 and 60 mM NaCl to MS media attained a severe reduction in stomata density, length, and width of stomata and inside or outside cell guard in comparison with control. Peach Nemaguard rootstock realized maximum salt tolerance up to 3500 ppm of NaCl.

Keywords **:** *In vitro***,** Salinity, EDX

Introduction

Peach tree is one of the most important deciduous fruit grown in Egypt, while the harvested area reached 95949 faddane and produced 273491 tons FAo, (2017). Peaches, *Prunus persica* L., which belong to the family Rosacea, are originated in China. Peach ranks second to apple among temperate zone deciduous fruit trees from the standpoint of production and value, Childers, (1978). A peach tree is highly demanded by Egyptian consumers. There are many peach varieties growing more widely now throughout the world. Peaches are native to China and their culture dates back to at least 4000 years Prior *et al.* (1992). Demand for stone fruit production is increasing with population growth. Breeding in fruit rootstocks are working around the world as the resulting clonal rootstock that allows replicated in fruit breeding. Fruit cultivation has developed thanks to clonally propagated and dwarfed rootstocks obtained from fruit rooting studies in the world Advantages of Nemaguard peach rootstock exploited as a suitable, compatible rootstock for peach varieties, have high tolerance to nematodes, less fertile soils. Chilling requirements with high. Nemaguard' seedlings are uniform and vigorous, compatible with peach and nectarine cultivars, and impart excellent scion vigor and productivity. It has good resistance to *M. incognita*, *M. javanica* and *M. arenaria* that can reproduce in the roots of 'Nemaguard' Lu *et al.* (2000). Nemaguard' is fairly tolerant to crown gall, but is sensitive to *P. vulnus*, fungal root rots, Verticillium, iron chlorosis and root water logging and may reduce winter hardiness of scion cultivars in cold climates De–Pascale *et al.* (2001) Researchers apply rootstock for development paths to meet the increasing demand and to provide sufficient products to the market that can provide varieties that are better quality, dwarf, early maturing, and resistant to environmental conditions, pests, and diseases mentioned that *Prunus microcarpa* may have commercial potential for rehabilitation works and farming of types of stone fruits as especially dwarfing rootstock Nas *et al.* (2012). The most damaging effects of salinity on plants include ion toxicity, water deficit Liu and Van Staden, (2001) and nutrient imbalance Grattan *et al.* (2001). Salinity limits vegetative and reproductive growth of plants by inducing severe physiological disfunction and causing widespread direct and indirect harmful effects, even at low salt concentrations Shannon and Milgrom, (1994). Tissue injury is induced not only by the osmotic effects of salts but also by specific toxic effects resulting from the accumulation of Cl^- and Na+ Yokoi *et al.* (2002). Therefore, the main goal of this study was to evaluate the effect of induced salinity levels of NaCl (0.0, 15, 30, 60, and 120 mM) on physiological responses and elements accumulation in leaves of Nemaguard peach rootstock cultured *in vitro* conditions.

Materials and Methods

Current shoots of Nemaguard peach rootstock were excised from 1-year-old plants. Shoots were cleaned by under running tap water for 30 min to eliminate dust then immerged in 70% ethanol solution for 30s. Then for sterilization in 0.1 % mercury chloride ($HgCl_2$) for 3 min, then rinsed in distilled water (1x). and sterilized by 10% sodium hypochlorite with droplets of tween 20 for 15 min, then rinsed three times in sterile distilled water, the selected with 3-8 mm length one node cuttings were prepared and cultured in jars containing 30 ml of the Murashige and Skoog (MS) basal medium supplemented with 30 g / L sucrose, 7 agar g / L, 0.0, 0.5,1.0,2.0 and 4.0 mg /L BA and 0.0 and 0.1 mg /L IAA cultures were incubated at 27±2 ^oC with a 16 h photoperiod for four weeks in starting stage . After 30 days, uniform developed explants were excised and re-cultured on

the multiplication stage where micro-shoots of Peach Nemaguard rootstock were cultured on MS media supplemented with 30 g / L sucrose, 7 agar g / L, 0.0, 0.5,1.0 and 2.0 mg /L of both BA and KN. After another 30 days, uniform developed explants were selected and transferred to the MS media containing 30 g / L sucrose, 7 agar g / L , 1.0 mg /L , 0.01 mg / L IAA and different concentrations of sodium chloride (NaCl) (0.0, 15, 30, 60, and 120 mM). The incubation conditions were the same as described above. At the end of experiments period (after 30 days), Chlorophyll a, b, total Chlorophyll and Carotenoids contents of the leaves were measured according to Lichtenther, (1987). The rapid colorimetric method of Bates *et al .,* (1973) was followed to estimate proline contents. Elements accumulation such as Na, Cl, K and Ca leaf were determined using X- ray microanalyzer (EDX).Leaf relative water content (LRWC) was calculated on the basis of Yamasaki and Dillenburg, (1999) method. Growth of axillary shoots under salt stress evaluated after thirty days by measuring the increase in shoot number, shoot length (cm), leaves number, shoot fresh weight (g), shoot dray weight (g), survival percentage Survival percentage was calculated according the following equation by

$$\text{Survival}(\%) = \frac{\text{Number of explants alive at end of time period}}{\text{Number of explants alive at start of time period}} \text{x}100$$

A complete randomized block design was followed and analysis of variance (ANOVA) was performed using two ways ANOVA Co-stat software according to Stern, (1991).

Results and discussion

Effect of plant growth regulators on morphological characteristics Peach Nemaguard rootstock

Starting stage: Data in figures from 1 to 4 Showed the effect of MS media supplemented with 0.0, 0.5, 1.0, 2.0 and 4.0 mg /L BA plus 0.0 and 0.1 mg /L IAA on morphological characteristics Peach Nemaguard rootstock. It was clear that shoot length (cm), leaves number and shoot fresh weight (g) as parameters indicating the morphogenesis and growth as well as shoot number that expressed the multiplication rate were dependent to a great extent on concentration of BA and IAA. The results indicated that the effect of BA on enhancing morphogenetic characteristics was dependent to a great extent on the both BA and IAA concentration via individually or in combination and genotype used where MS media complemented with 0.5 mg /L BA or 0.1 mg /L IAA plus 0.5 mg /L possessed the highest shoot length (cm) and leaves number compared with those of control and other treatments. on the other hand, the maximum shoot number and shoot fresh weight (g) achieved when axillary shoots of Nemaguard rootstock were cultured on MS media supplemented with 2.0 mg /L or 2.0 mg /L BA plus 0.1 mg /L IAA compared with those of control and other treatments. The results going line with Aghaye and Yadollahi, (2012) who found that MS media supplemented with BA at 1.0 mg /L and 0.6 mg /L possessed the maximum shoots number and the highest shoot length (cm) respectively of GF 677 Peach rootstock compared with those of control and other treatments. Also, Sotiropoulos, (2007) reported that the maximum shoots number achieved when single node cutting of Peach, (*Prunus persica* L.) BATSCH. cv. Garnem were cultured on MS media supplemented with 2.0 mg /L BAP individually compared with control and other treatments while the highest shoot length and the maximum leaves number attained when explants were culture on MS media complemented with 0.5 mg / L of BAP compared with any concentrations of 1BA combined with IBA and GA_3. Cytokinin stimulates the initiation and activity of axillary meristems which result in Shoot formation Dobránszki and da Silva, (2010). Cell division, shoot multiplication and axillary bud formation can be promoted by the cytokinin BAP Dobránszki and da Silva, (2010). The influence of cytokinins on tissue or organ cultures can be differed based on the kind of culture, the variety of plant and the age of explant Thorpe *et al.* (2008). It is also reported that BAP is required at low concentrations ranging from 0.5 to 2.5 mg /L Thorpe *et al.* (2008).

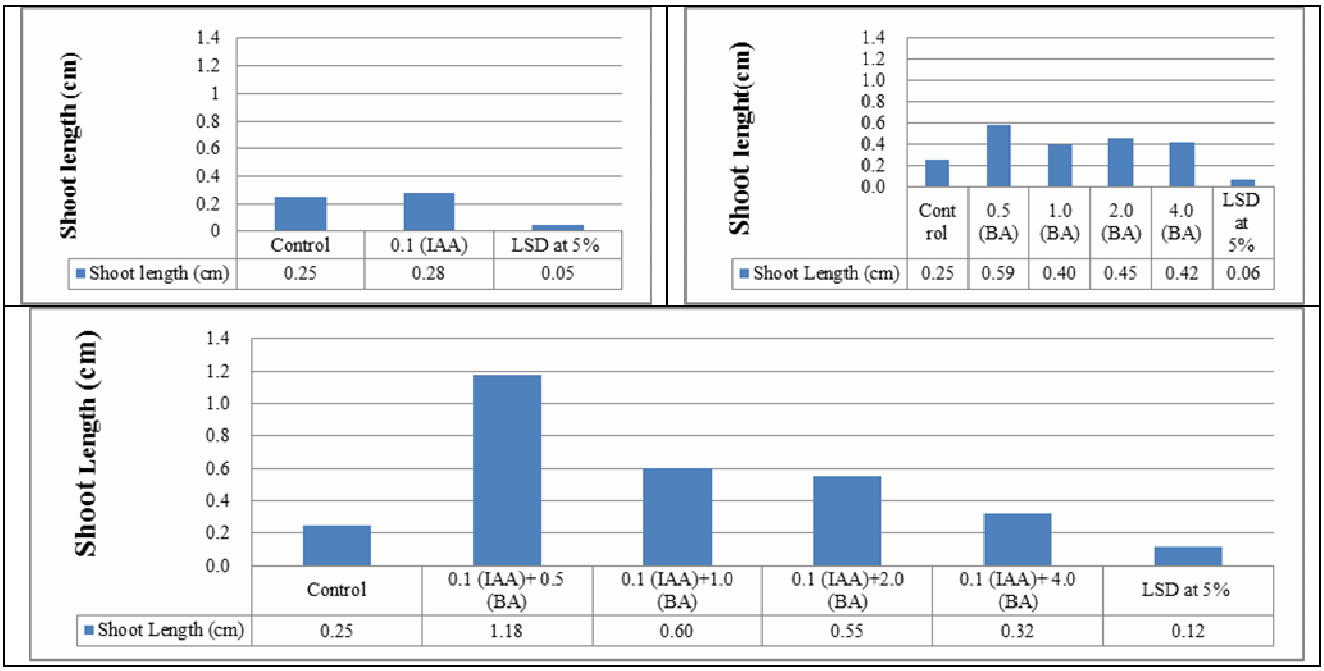

Fig.1 : Effect of MS media supplemented with BA and IAA either individually or in combination on shoot length (cm) of Peach Nemaguard rootstock at starting stage

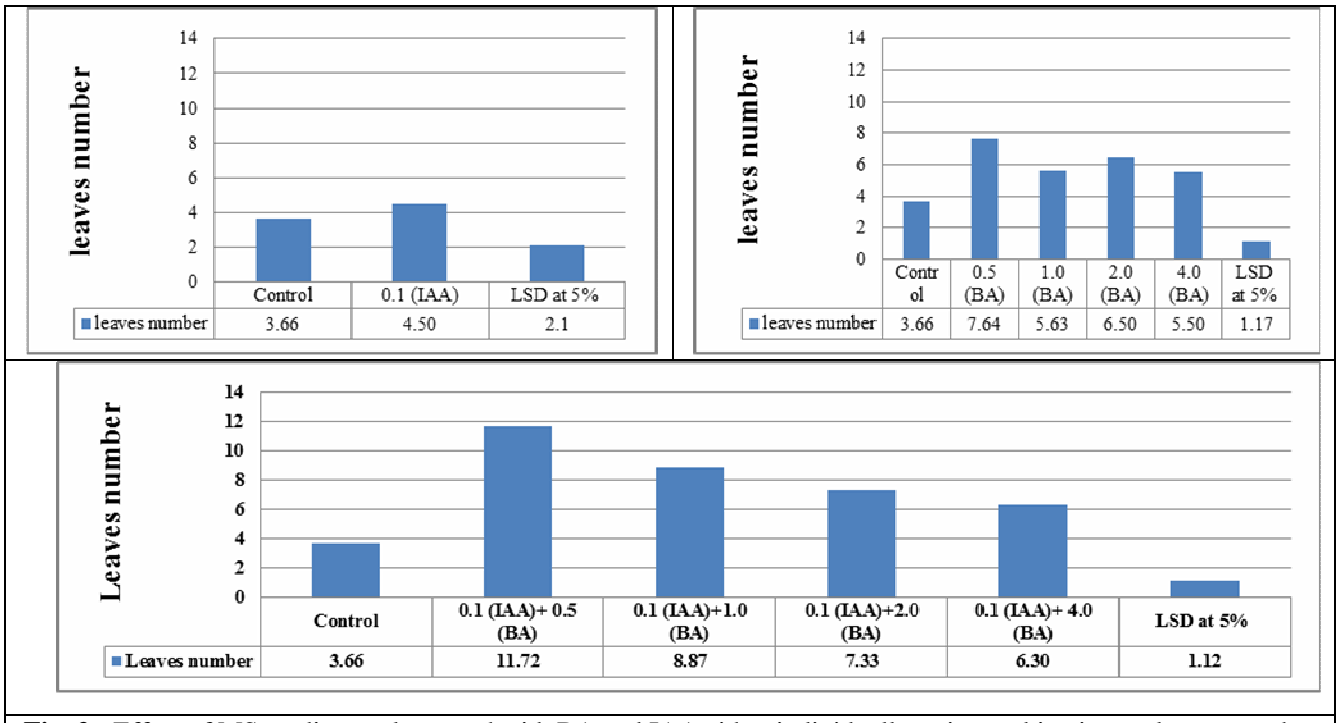

Fig. 2 : Effect of MS media supplemented with BA and IAA either individually or in combination on leaves number of Peach Nemaguard rootstock at starting stage

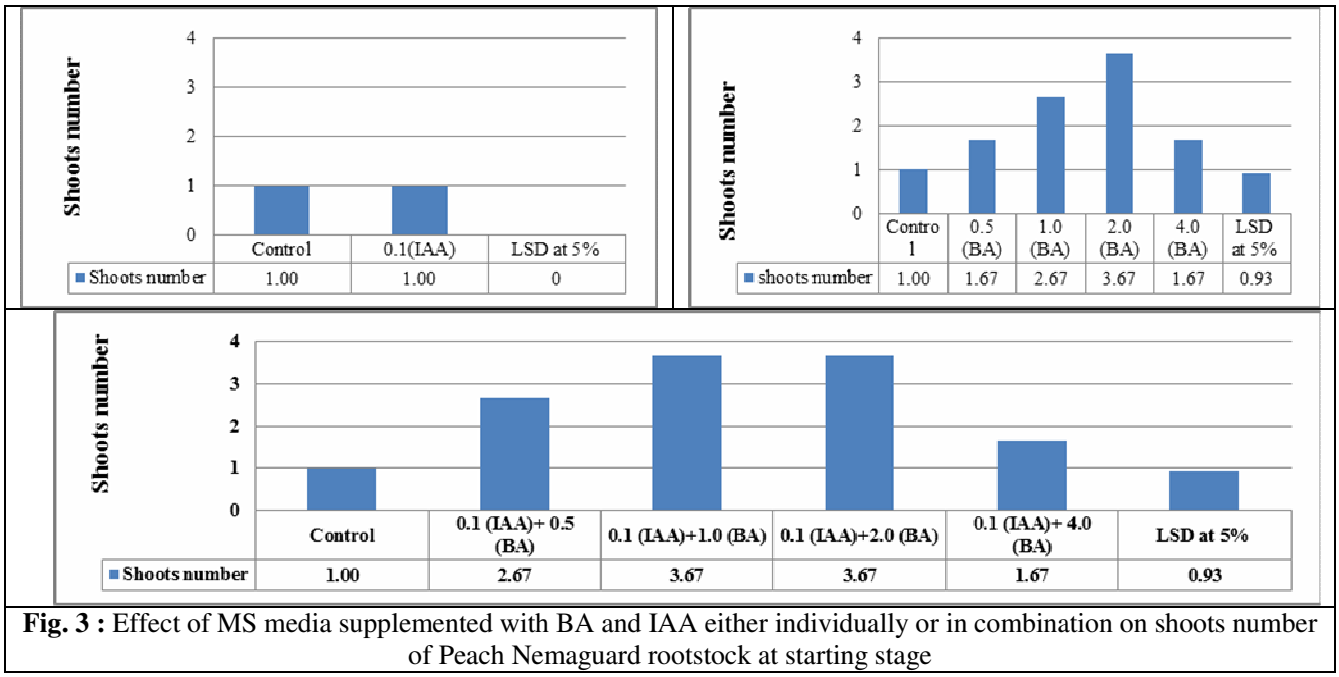

Fig. 3 : Effect of MS media supplemented with BA and IAA either individually or in combination on shoots number of Peach Nemaguard rootstock at starting stage

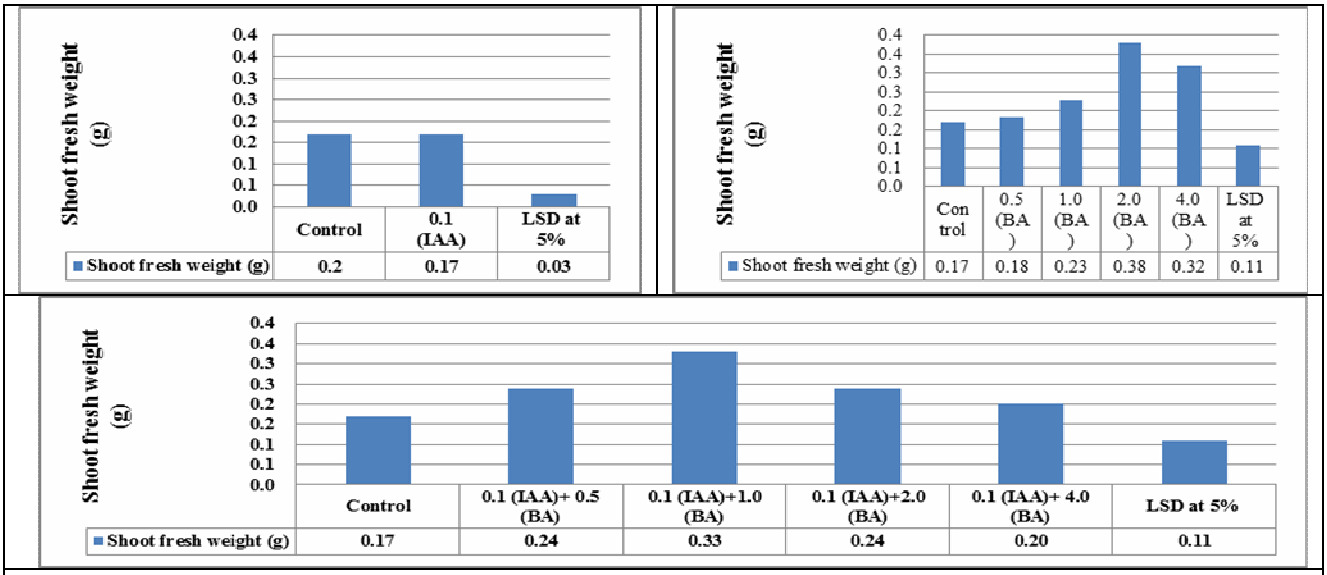

Fig. 4 : Effect of MS media supplemented with BA and IAA either individually or in combination on shoot fresh weight (g) of Peach Nemaguard rootstock at starting stage

3.1.2. Multiplication stage:

Data presented in Figures from 5 to 8 showed the effect of two types of cytokinins namely BA and KIN on the morphogenetic characteristics of Peach Nemaguard rootstock. It was clear that the shoot length (cm) leaves number and shoot fresh weight (g) as parameters indicating the morphogenesis and growth as well as shoot number that expressed the multiplication rate were dependent to a great extent to kind and concentration of the applied cytokinins. The results indicated that shoot length (cm) and leaves number recorded the highest values when the micro-cultures of Peach Nemaguard rootstock were cultured on MS media supplemented with 0.5 mg / L of both BA and KIN individually or in combination between of them in comparison with those of control and other treatments. On the other hand, the maximum shoots number and shoot fresh weight (g) obtained when micro-culture were cultured on MS media supplemented with 1.0 mg /L of both BA and KIN individually or in combination compared with those of control and other treatments. The results are in agreement with those of Ahmed *et al.* (2015) reported that MS media supplemented with 2.0 mg /L of both BA or KIN individually gained the maximum shoots number, shoot length (cm) and leaves number of Nemaguard Peach rootstock compared with those of control and other treatments. Also, Radmann *et al.* (2011) found that re-culture the micro-shoots of Flordaguard' Rootstock on MS media supplemented with 4.0 BAP possessed the highest shoots number compared with control and other treatments. Cytokinins have a stimulatory or an inhibitory role in different developmental processes, such as control of apical dominance in the shoot, root growth and branching, leaf senescence, and chloroplast development Mok, (1994). Cytokinins influence plant shape depending on environmental factors, such as light, water and nutrition Hirose *et al.* (2008). There is also evidence that CKs are involved in the modulation of metabolism and morphogenesis during environmental stress Hirose *et al.* (2008). Endogenous CKs have been shown as active molecules involved in seed germination, leaf senescence, nutrient mobilization, apical dominance, formation and activity of shoot apical meristem and development of vasculature Choi *et al.* (2011). They also promote seed germination, starch and chlorophyll production, bud differentiation and branching also, Cytokinins increase cell division by stimulating the production of proteins needed for mitosis Dolezal *et al.* (2007).

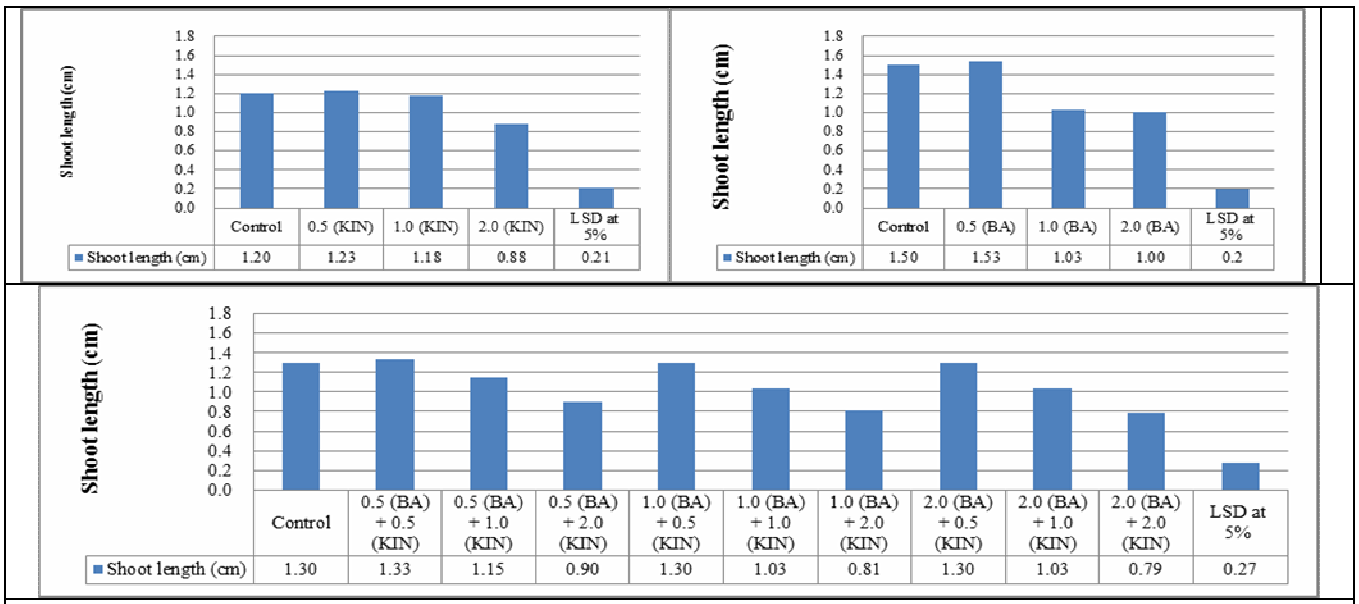

Fig. 5 : Effect of MS media supplemented with BA and KIN either individually or in combination on shoot length (cm) of Peach Nemaguard rootstock at multiplication stage

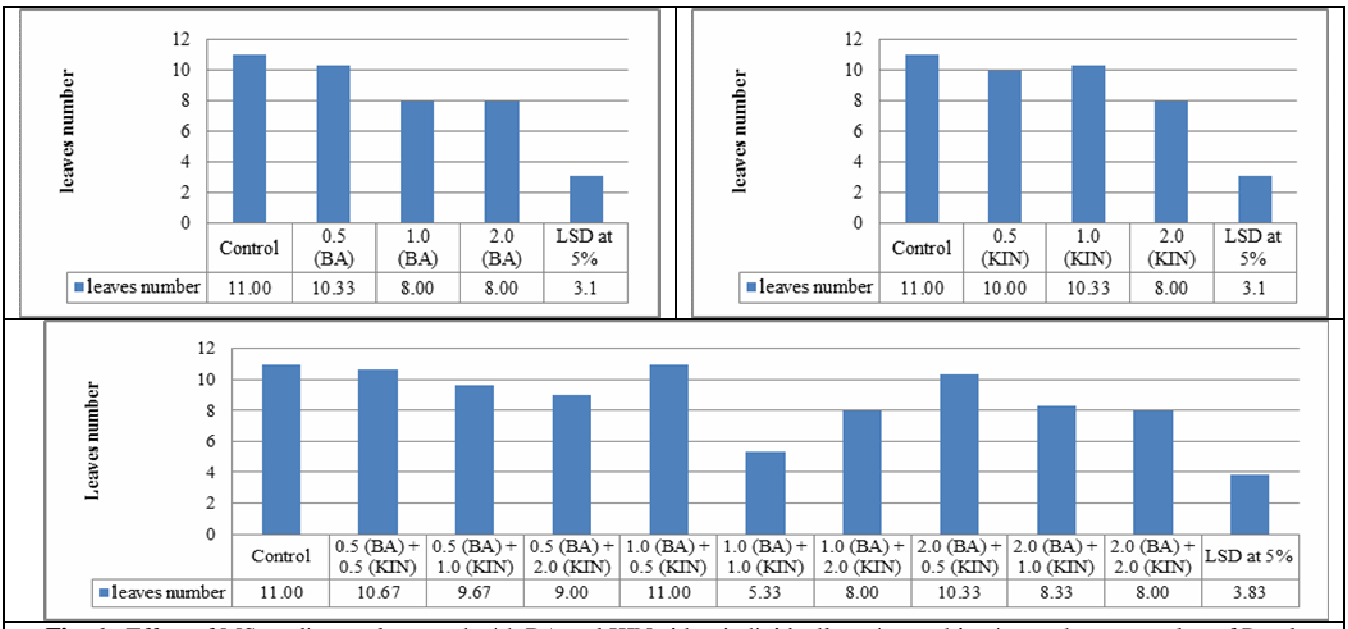

Fig. 6 : Effect of MS media supplemented with BA and KIN either individually or in combination on leaves number of Peach Nemaguard rootstock at multiplication stage

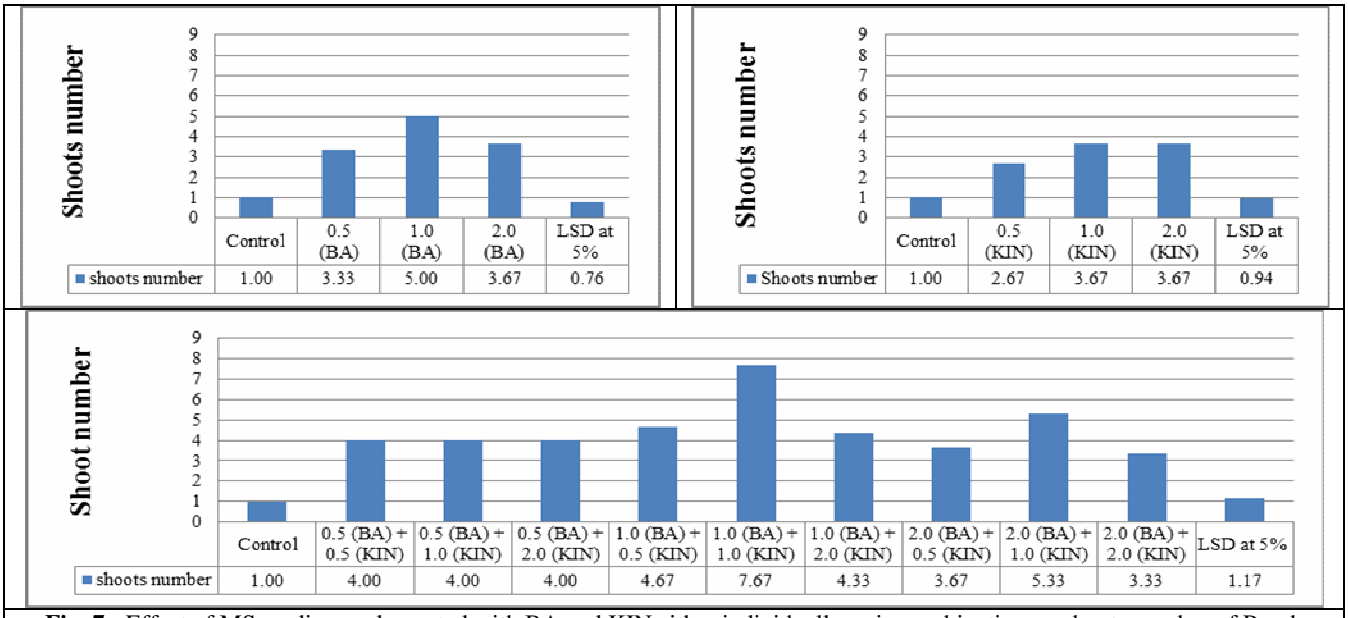

Fig. 7 : Effect of MS media supplemented with BA and KIN either individually or in combination on shoots number of Peach Nemaguard rootstock at multiplication stage

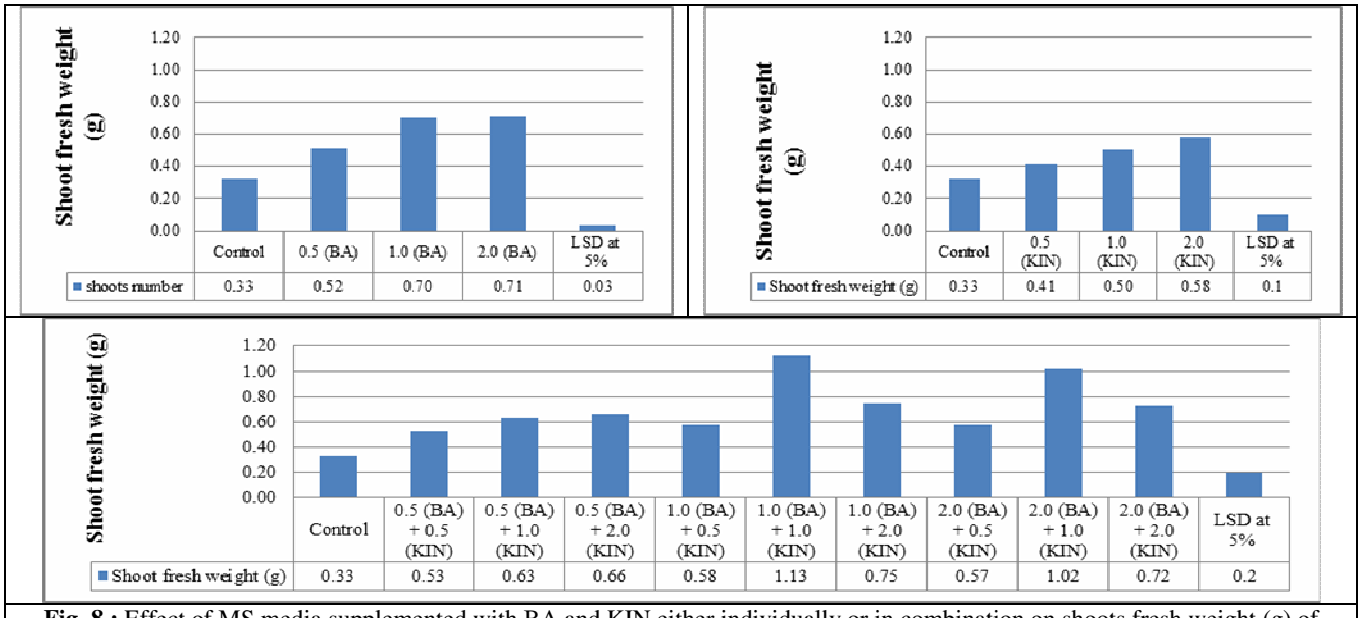

Fig. 8 : Effect of MS media supplemented with BA and KIN either individually or in combination on shoots fresh weight (g) of Peach Nemaguard rootstock at multiplication stage

3.2. Salt stress:

3.2.1. Effect of NaCl on morphological characteristics:

Shoot growth parameters such as shoot number, shoot length, leaves number, shoot fresh weight (g), shoot dry weight (g) of peach Nemaguard rootstock were significantly affected by NaCl treatments. Data in Figure. 9 showed the effect of MS media supplemented with different concentrations of NaCl mM on some morphological characteristics of peach Nemaguard rootstock where supplementation MS media with 15 mM NaCl enhanced all previous parameters compared with control and other treatments. On the other hand, adding NaCl from 30 up to 120 mM to the growth media caused a decrease in all previous parameters in comparison those of control and low level of NaCl. The results are in agreement with Sotiropoulos *et al.* (2006). reported that adding KCL from 0 to 40 mM to MS media not affected on growth of two Prunus rootstocks while an increase in KCl from 40 up to 80 mM led to a reduction in growth of tow Prunus rootstocks compared with those of control and other treatments. The decline in leaf growth is the earliest response of glycophytes exposed to salt stress Munns and Tester, (2008). Likewise, the adverse effects of salinity on growth parameters may be attributed to ionic imbalance, altered availability and uptake of other ions, accumulation of ions in leaf cell vacuoles, decline the photosynthetic rate, and reduced carbon fixation Prior *et al.* (1992). Furthermore, the complex effect of salinity causes a reduction in growth which is due to Osmotic effects or reduction in water absorption and specific effect of ion such as sodium and chlorine that particularly have toxic effects on fruit trees Jalili-Marandi *et al.* (2009). Otherwise, inhibition of shoot growth has been considered a whole plant adaptation to salt stress Qaderi *et al.* (2006). Moreover, these negative effects of salt stress may be attributed reduction of both cell division and cell enlargement Yasseen *et al.* (1987). Too, Plant growth inhibition under salt stress is primarily due to the osmotic effect, whereas toxicity produced by excessive salt accumulation in the plant cells becomes evident at the later stages of growth Munns, (2002).

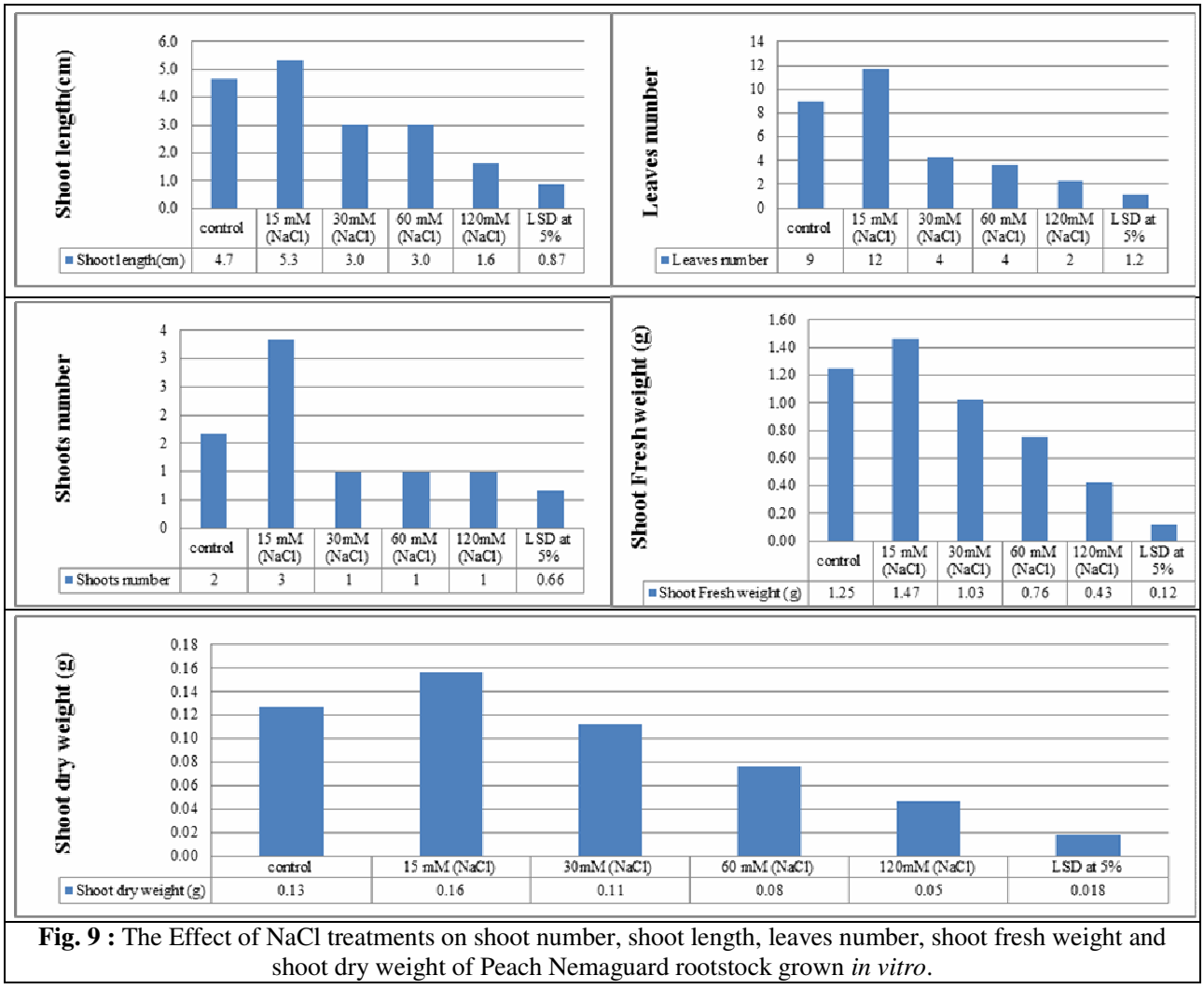

Fig. 9 : The Effect of NaCl treatments on shoot number, shoot length, leaves number, shoot fresh weight and shoot dry weight of Peach Nemaguard rootstock grown *in vitro*.

3.2.2. Effect of NaCl on biochemical characteristics:

Data in Figures.10 showed the effect of NaCl on biochemical characteristics such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, proline and RWC contents of peach Nemaguard rootstock. It was clear that, adding 15 mM NaCl to the growth media enhanced chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and proline contents in comparison with high level of NaCl and control while caused a decrease in RWC. On the other hand, increasing NaCl from 30 up to 120 mM decreased of all previous parameters except proline content which increased with increase of NaCl from 15 mM up to 120 mM compared with control and other treatments. Change in chlorophyll content due to salinity is the most obvious biochemical response Rao *et al* .,(2007). Chlorophyll contents were decreased under salinity stress due to the conversion of glutamate (which is the prefabricate matter of chlorophyll and proline) to proline therefore, occur a lack in chlorophyll content Molazem *et al.* (2010). Carotenoids have antioxidant properties and play an important role in scavenging reactive

oxygen species (ROS) and also acting as accessory light-harvesting pigments De–Pascale *et al.* (2001).Thus the reduction of chlorophyll contents in abiotic stress plants might possibly be due to changes in the lipid protein ratio of pigment-protein complexes or increased chlorophylase activity enzyme Parida *et al.* (2004). or probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions Chutipaijit *et al.* (2008). A number of suggestions have been proposed for proline roles in salinity tolerance. One possibility is that it acts as a store of energy that can be rapidly broken down and used when the plant is relieved of stress. Another is that it acts as an osmolyte and reduces the osmotic potential of the cell, thus reducing toxic ion uptake. In this case, the latter is more likely, with the salt tolerant plants not only producing more proline when stressed, but also having (in most cases) no significant drop in the chlorophyll content. This indicates that the increase in proline is reducing the physiologically detrimental effects of the salt also, under salinity stress, glutamate ligase enzyme is activated and playing an important role in conversion of glutamine to proline. Kafi and Damghani, (2003) Osmoregulation through the accumulation of cellulose solutes, such as proline, which has been proposed as a possible means for overcoming salt stress conditions Sotiropoulos, (2007). A number of suggestions have been proposed for proline roles in salinity tolerance. One possibility is that it acts as a store of energy that can be rapidly broken down and used when the plant is relieved of stress. Another is that it acts as an osmolyte and reduces the osmotic potential of the cell, thus reducing toxic ion uptake. In this case, the latter is more likely, with the salt tolerant plants not only producing more proline when stressed, but also having (in most cases) no significant drop in the chlorophyll content. This indicates that the increase in proline is reducing the physiologically detrimental effects of the salt Hare *et al.* (1998). Under salinity stress, glutamate ligase enzyme is activated and playing an important role in conversion of glutamine to proline Kafi and Afghani, (2003.)Osmoregulation through the accumulation of cellulose solutes, such as proline, which has been proposed as a possible means for overcoming salt stress conditions Sotiropoulos, (2007). Relative water content is mostly correlated with the leaf area, dry weight of the leaf, amount of chlorophyll and other indicators of growth rate Kafi and Afghani, (2003) Maintaining high RWC in leaves is one of the mechanisms that use to tolerate the salt stress that maintain the turgid state of plant cell Walke *et al.* (2003).

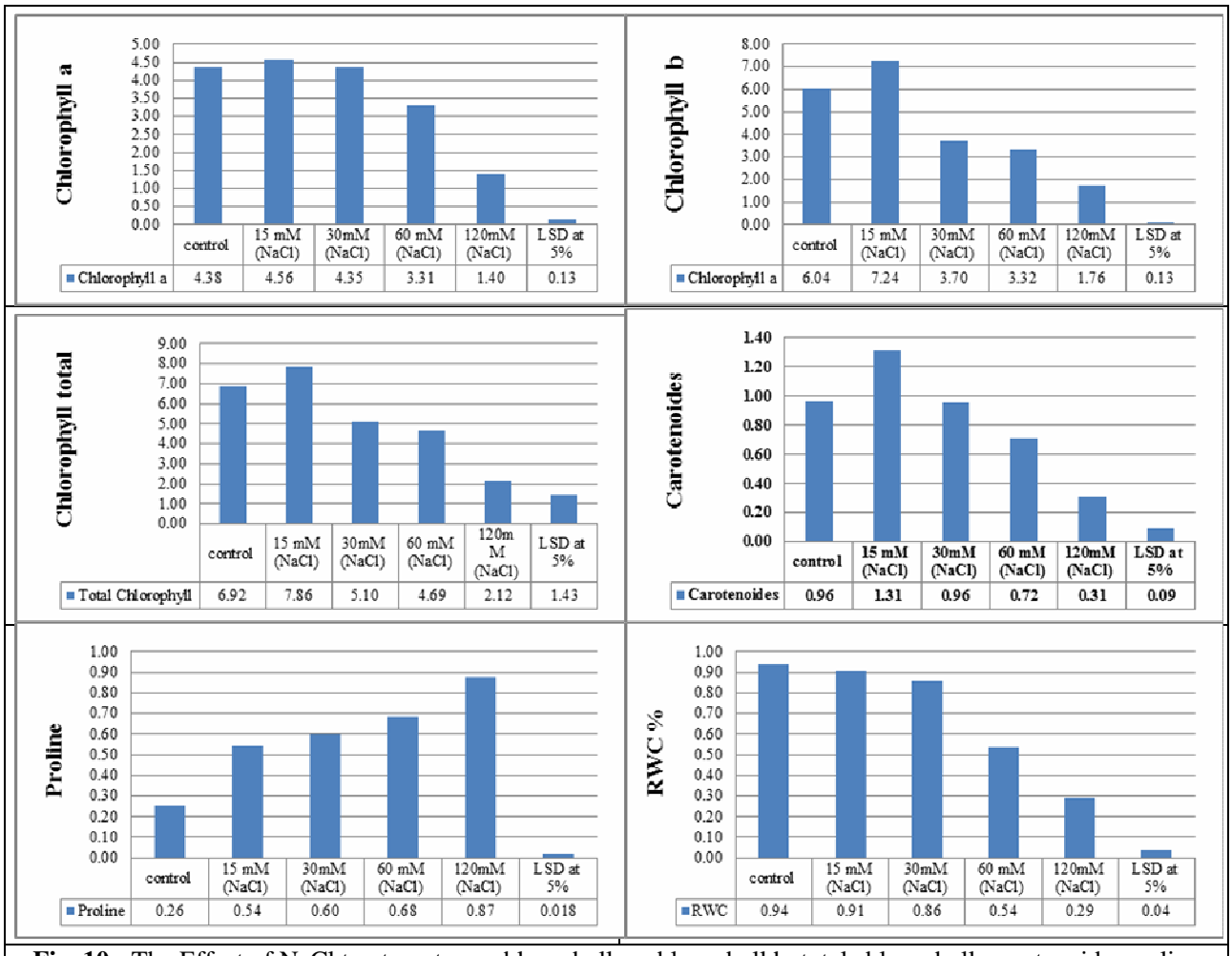

Fig. 10 : The Effect of NaCl treatments on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, proline and RWC contents of Peach Nemaguard rootstock grown *in vitro*.

3.2.3. Effect of NaCl on accumulation of elements:

Results in Figure. 11 showed the effect of MS media supplemented with NaCl 30 and 60 mM and without NaCl on Na, CL, K, Ca, P, Cu, Mn and Zn accumulation in leaves of peach Nemaguard rootstock growing *In vitro*. It was cleared that increasing level of NaCl from 30 up to 60 mM caused an increase of Na and Cl contents in leaf of peach Nemaguard rootstock while led to a decrease of K, Ca, P, Cu, Mn and Zn accumulation in leaves of peach Nemaguard rootstock in

comparison with those of control. The results are in agreement with Sotiropoulos *et al.* (2006) found that Sodium, Fe, Mn, and Zn concentration in tissues of Nemaguard rootstock were significantly higher than the respective values of GF 677 obtained when, micro-shoots of tow Prunus rootstocks: GF 677 *(Prunus persica* × *Prunus amygdalus*), and Nemaguard (*Prunus persica*) were cultured on MS media supplemented with KCl from 0 up to 80 mM. Also, Sotiropoulos and Dimassi, (2004) reported that Fe, Mn and Zn concentrations of kiwifruit shoots *in vitro* were not significantly affected in the presence of 0-80 mM NaCl. The effect of K^+ in osmotic potentials balance depends on several factors such as water content and cell wall elasticity {30}. Moreover, potassium is the most important element which plays an important role in protein synthesis and stimulates photosynthesis. The interaction between K and Na might represent a key factor in determining the salinity tolerance of plants Buschmann *et al.* (2000). Salt tolerance requires not only adaptation to Na^+ toxicity but also the acquisition of K^+ so, the uptake of K^+ affected by Na^+ due to the chemical similarities between them where, K^+ is an essential nutrient in the most terrestrial plants. K^+ transport systems involving good selectivity of K^+ over than Na^+ is considered as an important salt tolerant determinant Amini and Ehsanpour, (2005). Moreover, Salt tolerance in glycophytes is associated with the ability of a plant to limit uptake and/or transport of saline ions (mainly Na^+ and Cl^-) from the root zone to aerial parts of plant Greenway and Munns (1980).

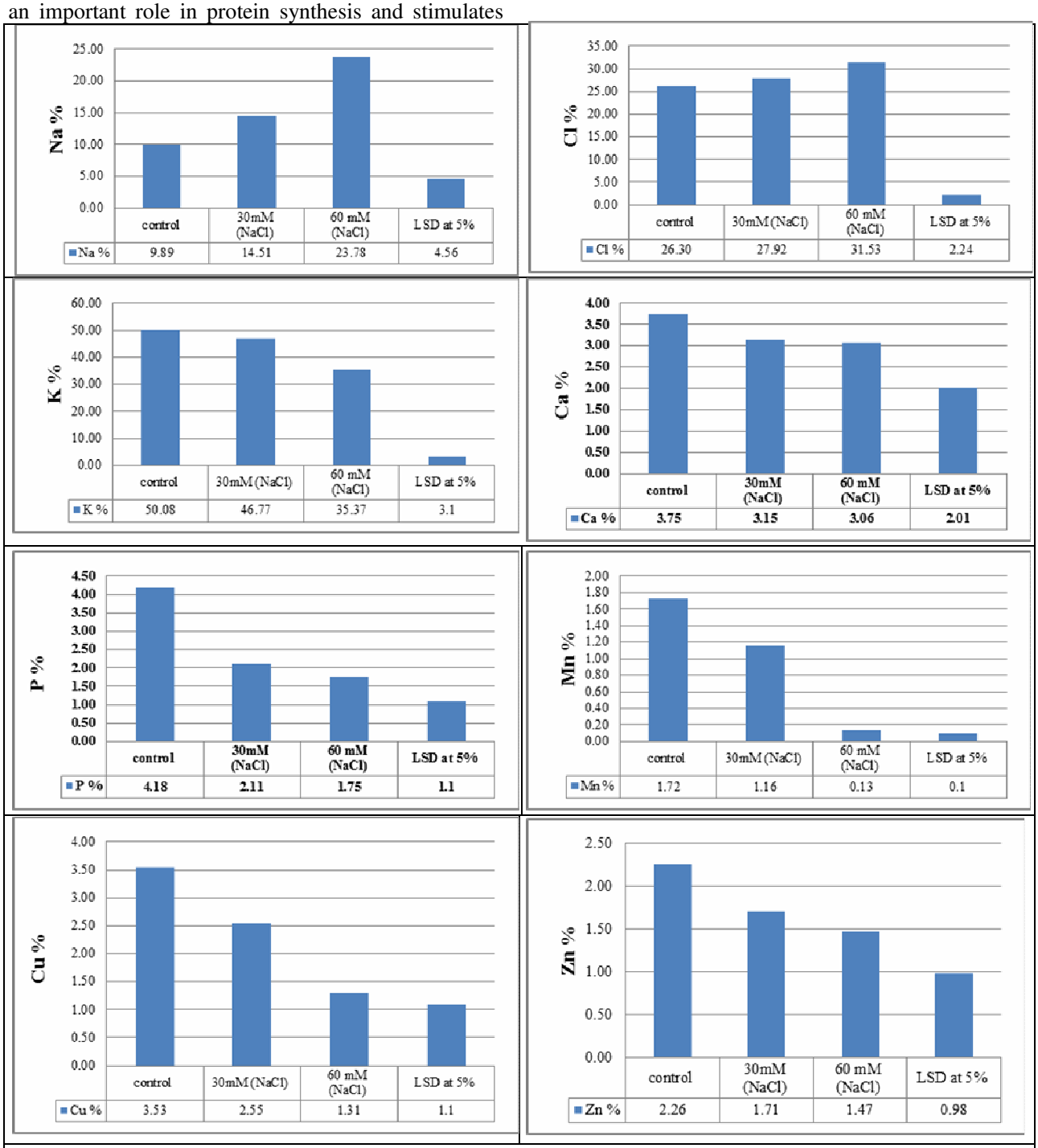

Fig. 11 : The Effect of NaCl treatments on some elements accumulation of Peach Nemaguard rootstock grown *in vitro*.

3.2.4. Effect of NaCl on stomata behavior:

Data in Figure.12 indicated the effect of salt stress induced by NaCl on stomata density, length, and width of stomata and inside or outside cell guard of peach Nemaguard rootstock growing *In vitro*. MS media supplemented with 30 and 60 mM NaCl caused a severe reduction in stomata density, length, and width of stomata and inside or outside cell guard in comparison with control. Stomata conductivity decreases with decreasing relative water content (RWC) due to salt stress and finally the actual amount of CO_2 assimilation decreases leading to photosynthesis reduction Lawlor and Cornic, (2002). Reduced stomata conductivity in grape leaves has been associated with a high salinity level and vice versa Ben-Asher *et al.* (2006). Moreover, under severe conditions, stomata closure seems to be the earliest response to prevent cell dehydration and damaging plant survival Chedlia *et al.* (2007). The main reason for decreasing photosynthesis rate under osmotic stress condition has also been reported by many workers as stomata closure Neocleous and Vasilakakis (2007). In fact closing stomata in response to salinity stress is a regulative mechanism to decrease water loss from tissues. If stomata closure and reduction in transpiration last long, carbon dioxide assimilation by leaf tissues decreases followed by reduction in photosynthesis and plant growth Tardieu, (2005). Stomata closure limits water loss, especially in plants suffering from a water deficit associated with high solute concentrations in the nutrient medium that reduce transpiration and either prevent or minimize the accumulation of toxic ions. However, stomata closure can also lead to diminished photosynthesis if CO_2 cannot enter the plant tissues.

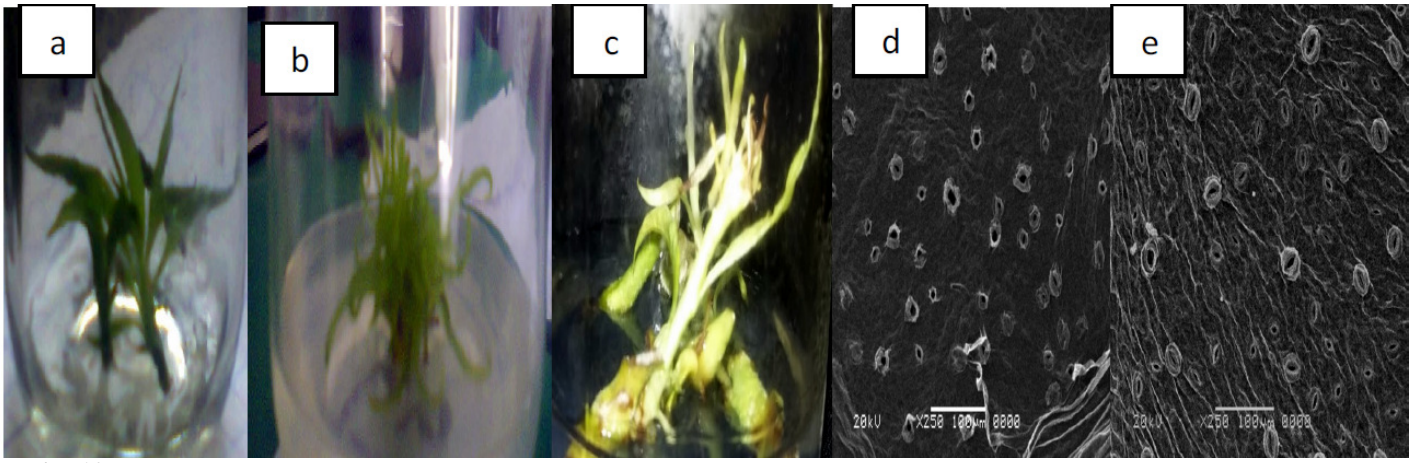

Fig. 12: Effect of NaCl treatments on stomata behavior of Peach Nemaguard rootstock grown *in vitro*. Where, a initiation stage ; b multiplication stage ; c and e NaCl at 60 mM while d . control.

Conclusion

Adding 1.0 mg /L and 0.1 mg/L IAA to MS media caused the maximum shoot number, shoot length (cm) and shoot fresh weight (g) in starting stage. MS media supplemented with 1.0 mg /L of both BA and KIN to MS media possessed the highest shoot number and shoot fresh weight (g) in multiplication stage. Adding NaCl from 30 to 120 mM to MS media caused the reduction in K, Ca and P elements while this led to an increase in Na and Cl elements in leaf Peach Nemaguard rootstock. In addition, adding 30 and 60 mM NaCl to MS media attained a severe reduction in stomata density, length, and width of stomata and inside or outside cell guard in comparison with control. Peach Nemaguard rootstock realized maximum salt tolerance up to 3500 ppm of NaCl.

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