

Impact of indole-3-butyric acid and indole-3-acetic acid on the lateral roots growth of *Arabidopsis* under salt stress conditions

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Abstract

The abiotic severe conditions are known to exert a depressive and harmful effect on the growth and development of higher plants. In the present study, we demonstrated that phytohormones provide a benefit effect to the lateral root growth of *Arabidopsis* under salt stress conditions. Results illustrated herein have shown that the harmful effect of salt expressed as a reduction on roots and shoots growth of *Arabidopsis* plants. Furthermore, the salt stress reduces the lateral root expansion and extension. The latter effect could be alleviated in the presence of IBA than in the presence of IAA. Phytohormones (IAA and IBA), used in this study, cause the capacity to stimulate the lateral roots growth and formation at various degrees. Even, in the presence of salt stress, IAA and IBA preserved the ability to stimulate the lateral root growth but the growth in length of the main seminal root remains affected by the salt. The overall growth of root system was reduced particularly in length. This may be owing to the effect of salt stress on the growth of seminal roots or the stimulating effect of phytohormones on the growth of lateral roots, which finished by dominating the general growth of the root system of the higher plant. In the presence of IAA only, the rate of inhibition of the growth in length of the roots is 20%, whereas this rate is about 38% and 63% if IAA was added with 50 mM and 100 mM of salt, respectively. In total, application of both salt and phytohormones inhibited the growth of the seminal roots in length.

Keywords: *Arabidopsis thaliana*, growth regulation, lateral roots, phytohormone, salt stress.

Abbreviations: EDTA_ethylene-diamine-tetra-acetic acid; DW_dry matter; IAA_indol-3-acetic acid; IBA_indol-3-butyric acid; IR_Initial recolte; LRs_lateral roots; NaCl_sodium Chloride; FW_fresh matter.

Introduction

Plants are often subjected, in nature, to environmental stresses and respond to avoid the harmful effect by an implication and activation of protective reactions. Salinity of soils and irrigation waters constitute the limiting factor for plant productivity, particularly, in Tunisia where many cultivable grounds are in the semi-arid zones (Essemine et al., 2010). However, Tunisia grants to the salinity study an essential importance in the national research programs in order to struggle against the turning into a desert and to identify more tolerant higher plants to salt. In spite the establishment of different investigations on the physiological and biochemical mechanisms of the salt effects on plants (Yamagushi and Blumwald, 2005), the mechanism of tolerance to salt remain exactly unclear and needs more studies to get more insights. All metabolic processes of plants are limited by salt stress (Levigneron et al., 1995). The threshold sensitivity to salt varied according to the development stage (germination or growth). The first effect of salinity was manifested for the most of plants by a decline in the germination capacity of seeds and delayed the rate of germination. This was documented in various species, such as tomato (Torres-Schumann et al., 1989), *Atriplex prostrata* (Katembe et al., 1998) and *Chenopodium quinoa* (Prado et al.,

2000). Salinity may affect also the roots growth. Anatomical and structural changes have been reported in the case of excised pea roots (Salomon et al., 1986). Previous investigations performed by Hodson and Mayer (1986), demonstrated that meristematic cells of pea roots under salt treatment show a precocious vacuolization and mitochondria with clear matrix and few invaginations. In our laboratory, Jbir and co-workers (2003) demonstrated that salt affected the size of the main root meristem (mitotic index, the process of cell division and the size of cortical cells) in wheat roots of two species differed in their sensitivity to salt. As recognized in previous study performed by Fitter (1991), the root system of the most "higher" plants consists of three types of roots: the main (also known as tap or primary), lateral and adventitious. The meristem of the main root is formed throughout embryogenesis. In contrast, the meristems of lateral and adventitious roots are formed post-embryogenically during the lifetime of a plant and their numbers vary according to the age and the growth conditions (Malamy and Benfey, 1997a; Van den Berg et al., 1998). Earliest work on walls of tobacco seedlings, of *Mesembryanthemum crystallinum* or *Arabidopsis thaliana* subjected to salt stress has shown, mainly, structural

modifications, notably, at the pectin level (McCann et al., 1994; Yang and Yen, 2002). Conversely, few data are available concerning the changes in wall-proteomic in response to salt stress. *Arabidopsis thaliana* is a dicotyledonous from the crucifer family, was selected as model plant among different vegetables species. This plant was characterized by a very short life cycle (about 6 to 7 weeks). It is possible to use this model plant to create transgenic plants, as different database of mutants can be established and their specific genome has completely been sequenced and documented. *Arabidopsis thaliana* is described as sensitive glycophyte (Wu et al., 1996), although it may contain genes of tolerance to salt like in halophytes (Zhu, 2000). *Arabidopsis thaliana* is, mainly, used in previous investigations to determine the threshold of tolerance to salt in plants (Zhu, 2000; Labidi et al., 2002). Two forms of auxin act differently on the lateral root formation and their effects segregate among plant species. IBA is the preferred auxin for the induction of root formation, as it is much more potent than IAA or synthetic auxins (Ludwig-Muller, 2000). IBA and IAA can be interconverted (Bartel et al., 2001), which has led to the suggestion that IBA may act as a precursor to IAA. IBA and IAA are transported in a polar fashion in roots and hypocotyls of *Arabidopsis* at similar rates and amounts, while in the inflorescence stem IBA transport is much lower than IAA transport (Rashotte et al., 2003). *In vivo* studies on the function of IBA are rather limited (Ludwig-Muller, 2000; Bartel et al., 2001). IBA has been identified in a number of plant species from maize (*Zea mays*) and pea (*Pisum sativum*) to *Arabidopsis*, and concentrations of free IBA approach the levels of free IAA in a number of plants (Ludwig-Muller, 2000). IBA, like IAA, is also found in conjugated forms, yet at significantly lower levels than IAA (Ludwig-Muller et al., 1993). In the present investigation, we focus on the study of the differential effects of exogenous IBA and IAA on the lateral root formation under salt stress conditions in *Arabidopsis thaliana* seedlings.

Material and methods

Plant material and germination conditions

Seeds *Arabidopsis thaliana* were placed at 4 °C for 15 days, and then imbibed with distilled water throughout 3 hours and returned to 4 °C for 4 supplementary days. Seeds were pre-treated at 4 °C to promote germination before placing at the specified growth conditions. *Arabidopsis* seeds used for our experiments were sown into soil and were then transferred to the growth chamber at 22/18 °C (day/night) with a 16-h photoperiod at a photon flux density of 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 70% humidity. Germination was followed for 3 weeks in soil, under growth room conditions, where seeds were irrigated with distilled water during the first week, and then with Hoagland and Arnon (1950) nutrient solution during the two other weeks.

Growth conditions

Arabidopsis seedlings sown into soil during 3 weeks were thinned to one plant per container and watered only with nutrient medium. One week after thinning, plants were irrigated with modified compositions of nutrient solution as described by Hoagland and Arnon (1950). The nutrient solution may

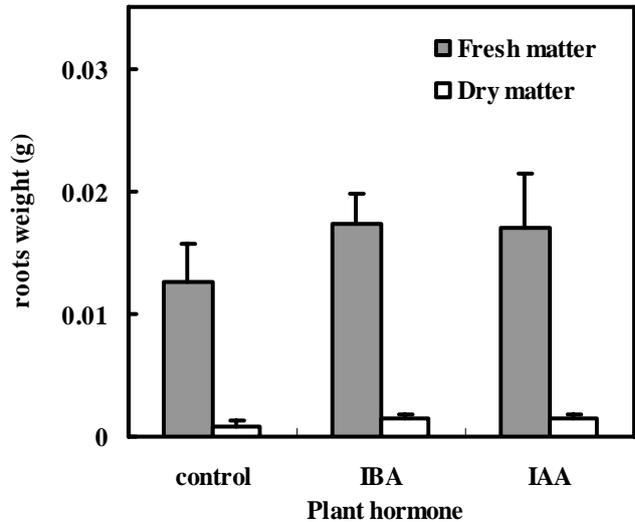


Fig 1. The initial collection of dry and fresh roots matter of three-week-old seedlings. As indicated in the histogram, roots weight was taken for control plants, and stimulated with plant hormones (IAA or IBA). Mean (\pm SE) of ten plants were taken.

containing salt at different concentrations (50 mM and 100 mM), and the nutrient medium was changed every three days. After 10 days of treatment with chloride salt plants were collected and separated into roots and shoots, and different parameters were taken; water content, principal root length, dry and fresh matter weight.

Iron Stock solution required for nutrient medium

Iron stock solution was prepared as follows: 26.1 g of EDTA was dissolved in 268 mL of KOH (1N), and then we added 24.9 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Total volume was adjusted to of 950 mL with distilled water and kept one night under shaker. Finally, we adjusted the pH to 5.5 with KOH solution. The iron solution was restored in the dark to avoid any oxidation process. The iron solution was added media avoid iron depletion or limitation in the basic media. We paid attention to this iron availability in the media owing to its crucial role in the growth of lateral roots. Indeed, any deficiency in iron can cause a decrease in the normal growth of lateral roots (Moog et al., 1995).

Preparation of IAA and IBA Stock solutions

In this study we would like appreciate the effect of the exogenous phytohormones on the growth and development of the root system of *Arabidopsis*, particularly, on the lateral root architecture and extent. According to previous literatures, we have chosen to work with these phytohormones (IAA and IBA) because they were known to have the ability to stimulate root growth. IAA was prepared in the dark to avoid oxidation phenomenon and dissolved in hot distilled water. On the other hand, IBA was dissolved previously in the methanol, and then we adjusted the volume with distilled cold water.

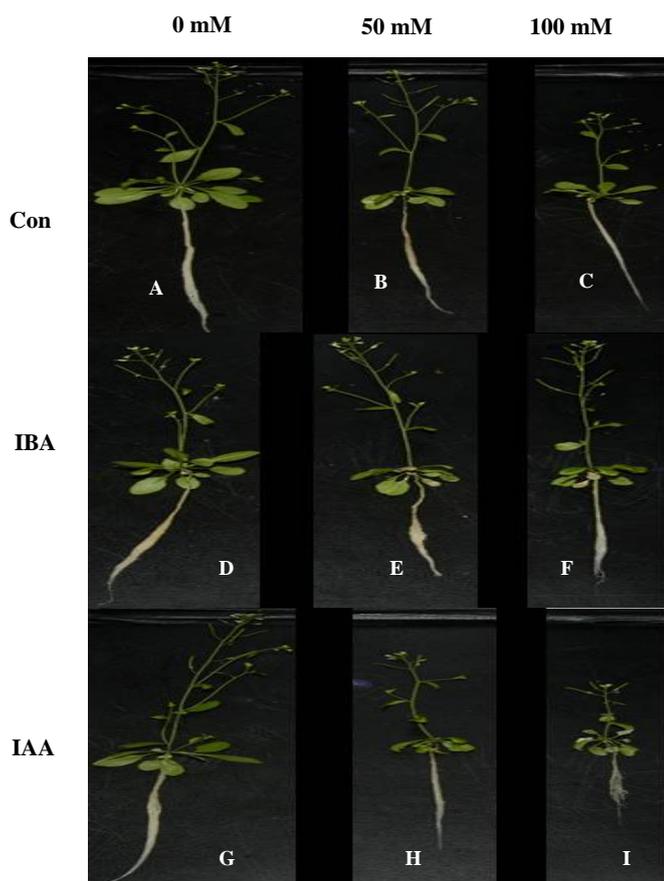


Fig 2. Comparison of root growth in *Arabidopsis* plants submitted to different treatments: (A) control without salt, and (B) with 50 mM NaCl and (C) 100 mM NaCl. In the second range (middle) of data roots are exposed to a combined effect of plant hormones. (D) IBA was added only, (E) 3 days before 50 mM NaCl, or (F) 3 days before 100 mM NaCl. The same procedure, as for IBA, was followed with IAA in the case of G (IAA only), H (3 days before 50 mM NaCl), and I (3 days before 100 mM NaCl) as shown in the photo.

Growth root Stimulation

After transfer of *Arabidopsis* seedlings, plants continued to grow one week on Hoagland medium with nutrient diluted 4 times to avoid ionic toxicity. Two forms of auxin (IAA or IBA) were added to the nutrient solution at a concentration of 10^{-6} M to stimulate the roots growth and subsequently observed the influence of plant hormones on the architecture of roots. The phytohormones played a crucial role by attenuating the detrimental effect of salt on the development especially of lateral roots. The auxin forms were added separately 3 days before the initial collection has been performed.

Parameters of root growth

Roots growth is expressed as a percentage of the control according to the formula:

$$(\Delta DW_{\text{treated}} / \Delta DW_{\text{control}}) \times 100$$

$$\begin{cases} \Delta DW_{\text{treated}} = DW_{\text{finale}} - DW_{\text{initial}} \\ \Delta DW_{\text{control}} = DW_{\text{finale}} - DW_{\text{initial}} \end{cases}$$

Water content was expressed with ml g^{-1} DW and estimated as follows: Water content = $(FW - DW) / DW$

Statistics

Statistical data analysis was performed by analysis of variance (ANOVA). Duncan tests were carried out to test significance of differences between treatment means using the SPSS for Windows, release 12.0.

Results and discussion

The general root system architecture

The roots growth is triggered at the early growth of seedlings in the length trend and overall root system of plants derived from the primary seminal root. Fig 2 shows that the application of sole salt exerted a depressive effect on lateral roots growth (compare A with B and C in Fig. 2). Plant hormones alleviate the effect of salt on the lateral root growth and allowing the formation of more LR. This may be owing to the effect of the phytohormone to reduce the sequestration of toxic ions such as Na^+ and Cl^- . However, as reported by Tarakcioglu and Inal (2002), excess of NaCl in plant cells induces major physiological and biochemical changes and eventually harmful effects to the growth and overall productivity of the plant. The root system originates from a primary root develops during embryogenesis. This primary root produces secondary roots, which in turn produce tertiary roots. All secondary, tertiary, quaternary and further roots are referred as lateral roots. The root system seems to be much affected in the presence of even a low NaCl concentration but the addition of IAA and IBA alleviate the harmful effect of salt on the root development. A similar study made by Younis and co-workers (Younis et al., 2008), in which they demonstrated a reversal harmful NaCl effects in lettuce plants by foliar application with urea.

General remarks on the lateral root growth and orientation

The rate of cell division in the meristem determines the growth rate of the new organ. Therefore, most hormones and genes monitoring the cell cycle have been implicated in meristem activity, and influence lateral root growth rates. In addition to cell division, the rate of cell elongation controls also the overall growth. For instance, in maize the *slr1* and *slr2* mutants elongate the lateral root slowly, and this has been ascribed to decreased cell elongation (Hochholdinger et al., 2001). The trend of root growth is determined by various tropisms (i.e. phototropism, gravitropism, and hydrotropism), which would intuitively be considered to be regulated by the environmental challenges. Lateral roots in most plants start by growing horizontally, and ultimately turn to grow vertically. According to literature IBA may act either directly as a distinct auxin or indirectly through its conversion to IAA. This interconversion of IBA and IAA has been demonstrated to occur in several plants (Epstein and Ludwig-Müller, 1993), but there is no direct evidence that conversion of IBA to IAA is necessary for IBA action.

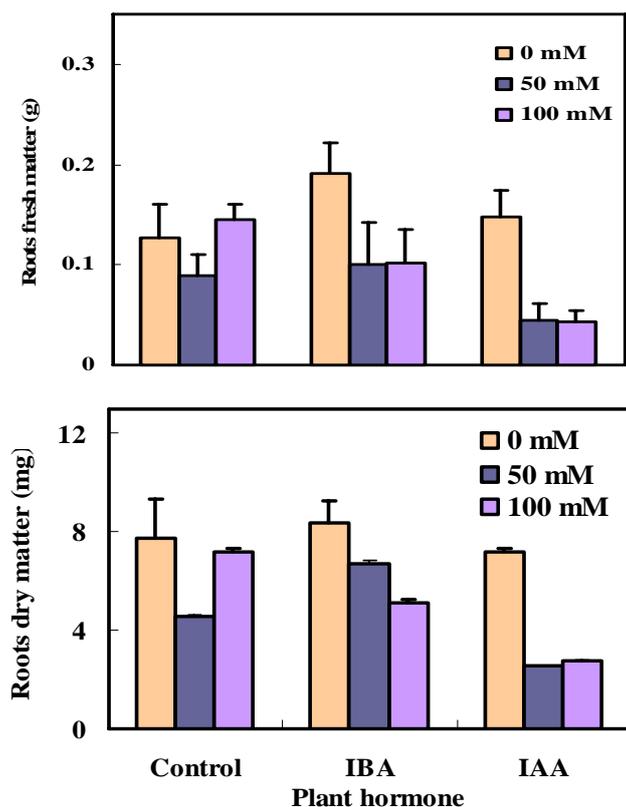


Fig 3. Dry and fresh matter of roots at the final collection of six-week-old plants of *Arabidopsis*. Plants were separated to different batches. One batch of control plants without salt stress, another batch subjected to the effect of NaCl only (50 mM and 100 mM). Other batches, in which roots growth was stimulated, previously, by plant hormones (IAA or IBA at 10^{-6} each) for 3 days, and then salt stress was applied as indicated above (see material and methods for more details).

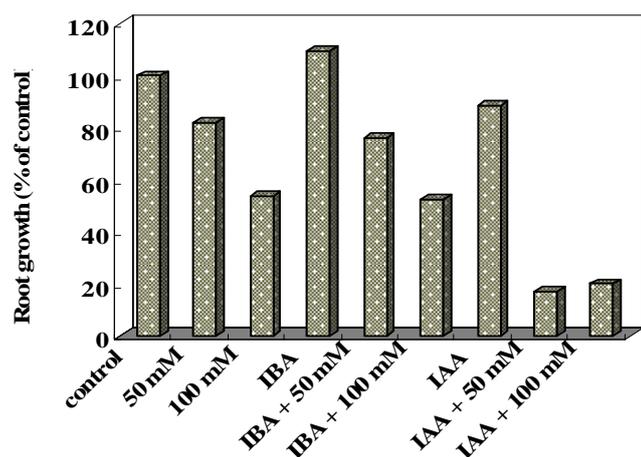


Fig 4. The root growth expressed as percentage of the control (for details see material and methods). Dry matter used in the quantification of the growth was harvested from both the initial and final collects.

Effect of phytohormones on the root emergence during early growth

Arabidopsis plants were photographed just before the initial collect of roots and shoots separately. Careful examination of different organs of plants demonstrated that the growth of the roots is mainly stimulated in length (data not shown). To get more insight about the role of phytohormones (IAA and IBA) on the root growth, we applied the plant hormones to the media separately and then growth plants for 10 supplementary days before harvesting roots and shoots. Results consigned in figure 1 showed an increase in the weight of roots fresh matter after the addition of these plant hormones. This is a proof in line with the fact that phytohormones stimulate the root growth. The nutrient status of the plant is very important as it has a strong effect on plant growth and development. Thus, nutrients have been shown to work with hormones to modulate plant growth and development. Our results are in agreement with the study made by Wang and co-workers (Wang et al., 2003), in which they showed that both exogenous IAA and IBA caused inhibition of seminal root elongation and promote the lateral root growth. A similar study was made, in which, authors demonstrated that at low concentrations IAA promoted stem elongation and inhibit it at high, presumably due to induction of ethylene (Goodwin and Mercer, 1983; Giush Uddin Ahmed and Lee, 2010), and concentration that promoted growth in one part of a plant may inhibit it in another. In our case, we found inhibition at high concentration of IAA both for shoots and roots of *Arabidopsis* plant. This may be owing to the toxic effect exerted by the salt. However, IBA seems to be opposite to salt effect and exerted a benefit effect on the overall plant growth and specially that of the root.

Effect of salt stress on the root growth

Fig 2 A-C showed the effect of salt stress on both root and shoot growth of *Arabidopsis*. High salt concentration was accompanied by a decrease in the overall growth of plant, mainly; the lateral roots (see Fig. 2 A-C). The decline in root growth under salt stress conditions is more pronounced at the level of lateral roots emergence and root hair (Fig. 2 B-C). The effect of salt only without combination with phytohormones on the root and shoot development (Fig. 3) expressed as percentage of the control, was manifested by a decrease in the overall plant growth and particularly, roots. In a study made by Naheed et al., (2007), they obtained a decline in the growth of rice (*Oryza sativa* L.) and harmful effect of salt on the productivity. It is possible that the decrease in the shoot and root growth under salt stress conditions could be due to several reasons. One possibility is that salinity reduces photosynthesis, which in turn, limits the supply of carbohydrates needed for growth (Alam et al., 2004). A second possibility is that salinity reduces shoot and root growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium (Alam et al., 2004). A third possibility is that the root response to salinity was to down-regulation of shoot growth (and root as well) via a long distance signal (Alam et al., 2004). Fourth, a disturbance in mineral supply, either on excess or deficiency, induced by changes in concentrations of specific ions the

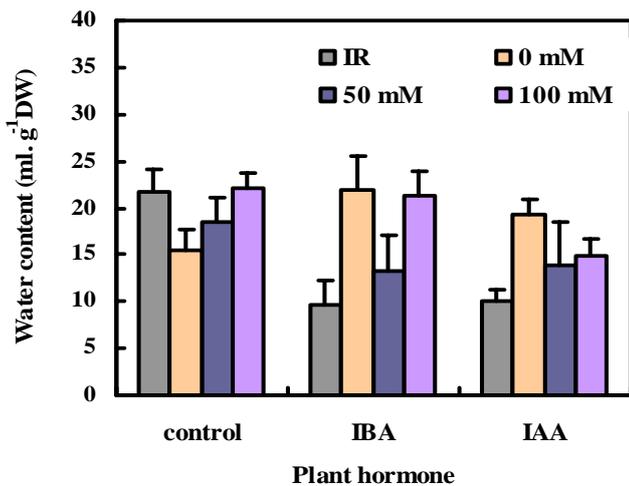


Fig 5. Water content of roots assessed at both the initial and final collect. This parameter was estimated as indicated in material and methods for control and plants exposed to salt stress at 50 mM or 100 mM NaCl.

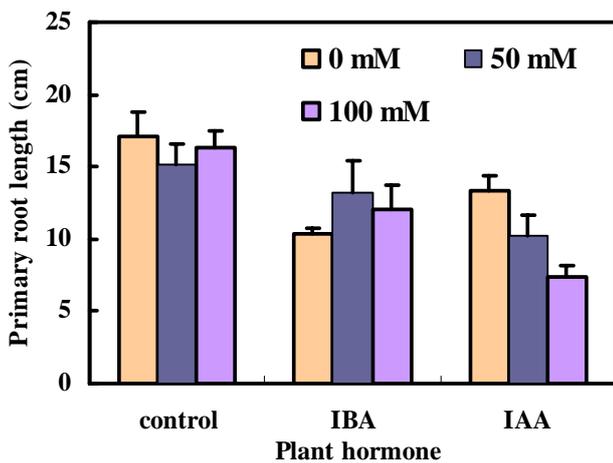


Fig 6. The histogram depicts the primary root length of *Arabidopsis* six-week-old plants grown on nutrient medium without (0 mM) or with (50 mM or 100 mM) NaCl.

growth medium might have directly affect the growth (Lazof and Bernstein, 1998).

Combined effect of salt stress and phytohormones on the root growth

Figure 2 showed both the effect of NaCl solely and NaCl along with plant hormones on root growth. Plants irrigated with media supplemented with salt and plant hormones (Fig. 2 E-F and H-I) grow better than in the case where plants were irrigated only by media containing salt only (Fig. 2 B-C). Thus, phytohormones can alleviate effect of salt on the lateral root growth and allow the increase of their number and elongation by stimulating the rate of cell division and differentiation. Notably, IBA has a better capacity to stimulate the root growth than IAA.

Reversely, in various studies made by different authors (Bassil et al., 1991; Eşitken et al., 2003) they associated the rooting enhancing capacity of bacterial strains is thought to be related to IAA production. Root growth is also expressed as percentage of the control estimated from produced dry matter in control and treated samples during a determined time period. Results obtained in Fig. 4 have shown a stimulation of the growth after addition of IBA compared to the control and after application of IAA. However, we conclude that IBA can avoid the toxic effect of NaCl and alleviate its effect better than IAA. As an evidence, in the presence of IBA, the decline of the root growth is around 20% and 50% at 50 mM and 100 mM of salt, respectively. The drop in root growth is very drastic in the presence of IAA together with salt. It is about 80% even at lowest concentration of salt (50 mM).

Water content of roots under combined effect of salt stress and plant hormones

As shown in figure 5, during the initial collect, the water content of overall roots system is about 22 ml g⁻¹ DW in the control. This amount in the presence of both plant hormones IAA and IBA decreases to about 10 ml g⁻¹ DW. This imply that phytohormones decrease the water uptake by roots. In *Arabidopsis* roots, harvested in the final collection, the water content was about 15 ml g⁻¹ DW in the control treatment (without salt). However, The water content increases to 18 ml g⁻¹ DW and 22 ml g⁻¹ DW at 50 mM and 100 mM NaCl, respectively. The effect of IBA in combination with salt does not much disturb the water conten, However, when IAA was applied along with strong NaCl concentration (100 mM), the water conentn declined from 19 ml.g⁻¹ DW to around 14 ml g⁻¹ DW. This decrease might be correlated with an increase of Na⁺ and Cl⁻ sequestration in the root cells. This finding is in agreement with Yang et al., (2002), in which, they demonstrated a negative correlation between relative water content (RWC) and Na⁺ content by about 2% decrease after 24 h of stress.

Combined effect of salt stress and plant hormones on the primary root length

Results in histogram of Fig 6 demonstrated that phytohormones (IBA or IAA), supplemented alone or accompany with salt, at different concentrations to the media, influence the primary root length. In the presence of IAA at 10⁻⁶ M solely, the rate of inhibition of the seminal root growth in length, compared to control treatment, is 20%. While, this rate is about 38% and 63%, whether IAA was applied with 50 mM and 100 mM of salt, respectively. In the presence of IBA, the rate of inhibition of the the primary root length without salt is 37%, while this rate is about 20% and 25% when IBA was added together with 50 mM and 100 mM of salt, respectively. We concluded when phytohormones were applied without salt stress, the rate of inhibition of the primary root length is more pronounced in the presence of IBA than in the presence of IAA (about two times of IAA effect). This reverse is correct for the lateral roots growth. Thus, the IBA form of auxin has a benefit effect on the lateral roots growth and stimulte their development better than IAA. Our results are in agreement with Wang and co-workers (Wang et al., 2003), in which, they show that both IAA and IBA inhibited seminal root elongation in rice (*Oryza Sativa* L.),

but IAA can only reach to the same inhibition degree as that of IBA at a more than 20-fold concentration.

Conclusion

The response of plants to excess NaCl is complex and involves changes in their morphology, physiology and metabolism. Therefore, salt stress is considered as a major environmental factor that limits the plant fitness and productivity. Investigations reported herein have shown that the salt stress affects the overall plant growth and specially, the lateral roots growth. However, this effect was explained by the fact that the salt stress may degrade the mitotic index, the process of cell division and the size of cortical cells. The effect of salt on the lateral root development might be alleviated when salt was added together with growth regulator hormones. Nevertheless, these two forms of auxin here act differently on the lateral roots formation and cause a decrease in the main seminal root elongation and triggered the expansion of lateral roots. The degree of lateral root stimulation varied between these two phytohormones. Thus, the best lateral roots formation was recorded for IBA. Also, the highest number of lateral roots recorded in media supplemented with IBA as compared to the one supplemented with IAA. Media supplemented with IBA caused significant increase in the number of lateral root formation. Therefore, IBA is thought to play an important role in the cell division and expansion. It seems to be arisen from the fact that IBA cause a stimulation of the lateral root metabolism to a level better than that found after addition of IAA to the media.

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