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# Performance of Faba Bean Varieties Grown under Salinity Stress and Biofertilized with Yeast

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**Abstract:** A pot trial was executed on certain faba bean varieties (Giza Blanka, Giza 461, 634, 674 and 717) were grown in sandy soil at the green house of National Research Centre, for investigating to what extent biofertilization with a soil yeast *Rhodotorula glutinis* may lower the deleterious effect of salinity stress. Increasing salinity level was accompanied by decreasing dry weight particularly with the high concentration (6000 ppm). Inoculation with *Rhodotorula* augmented plant dry weight in all tested varieties in comparison with the uninoculated ones with increasing percents ranged from 17.78 to 8.18 being the highest with variety Giza 461 and the lowest with Giza 717. Each of protein level, soluble carbohydrate, potassium content and catalase activity in plant leaves of various varieties greatly affected by increasing salinity level. Inclusion of *Rhodotorula glutinis* to salinity treatments mitigated their adverse effects. On the other hand, each of sodium, proline and hydrogen peroxide contents in plant leaves was increased with increasing salinity levels. Bio-treatment of *Rhodotorula glutinis* resulted in reducing values of the previous parameters for all tested faba bean varieties either the salt tolerant or the sensitive ones.

**Key words:** Faba bean varieties, salinity, yeast strain, germination, protein, ions, soluble carbohydrate, proline, hydrogen peroxide, catalase activity

## INTRODUCTION

Salinity is the most serious water quality problem in agriculture. Water salinity is an environmental stress factor that inhibits growth and yield of glycophytic crop plants in many regions of the world[1-3]. As more land becomes salinized through poor local irrigation practice, the regional impact of salinity on crop production is becoming increasingly important world-wide[4,5] creating a pressing need for improved salt tolerant plants. Inhibitory effect of salinity on seed germination, plant growth, nutrient uptake and metabolism was studied by[6-11]. Improving salinity and tolerance of crop plants using biofertilization has been an important but largely unfulfilled aim of modern agricultural development. Plant salt tolerance varies according to species, also different responses to salinity can be observed due to growth stages of the same plant[3].

Therefore, the present study was conducted to detect the effect of different salinity levels on different faba bean varieties, estimating more specific details about sensitive and tolerant varieties of the same genus. Also using biofertilization, yeast strain of *Rhodotorula* sp. to enhance the degree of plant tolerance .

## MATERIALS AND METHODS

A pot experiment was executed twice during the two successive seasons of 2001 and 2002 at the greenhouse of the National Research Centre, Dokki, Cairo, Egypt to investigate the influence of different salinity levels and biofertilization with soil yeast (*Rhodotorula glutinis*) on growth and chemical composition of certain faba bean varieties (Giza Blanka, Giza 674, Giza 717 m Giza 461 and Giza 634). Plastic pots of 30 cm diameter were filled with 10 kg uncultivated low fertile sandy soil of the following characteristics: clay, 8.19%; silt, 6.40%; sand, 84.30%; organic matter, 0.09%; total nitrogen 0.015%; total phosphorus; 0.038%; pH, 7.41; E.C., 3.7 mmhos $cm^{-3}$.

Faba bean seeds were sown at the rate of five seeds $pot^{-1}$. According to the respective treatments, inoculation was carried out with a liquid culture of *Rhodotorula* sp. containing $1.8 \times 10^6$ CFU $ml^{-1}$, where each pot received 50 ml. Inoculation was done directly after sowing of faba bean seeds where each pot received 50 ml of the freshly prepared liquid culture. Pots were irrigated with Hoagland nutrient solution for two weeks, then seedlings were subjected to different salinity levels of NaCl solution from 1000 to 6000 ppm in addition to control (without using

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salinity). Each treatment was replicated four times in a complete randomized block design.

Also, germination data were recorded in lab under natural conditions at temperature of 27±5°C. Seeds were placed in Petri dishes 9cm in diameter on two layers of 90 mm filter paper moistened with tap water or NaCl solution. The germination experiment was carried out for five varieties and six salinity levels in addition to control, whether inoculated with yeast strain or uninoculated. Each treatment was replicated in three Petri-dishes and five seeds were placed in each. Germination was inspected daily and seedling emergence recorded as the appearance of the cotyledons. Total number of emerged seedlings in each replicate was determined and evaluated as percentage, in calculation of total emergence.

Seedlings (stems+leaves) were harvested and evaluated for their response to salinity. Samples were dried for 48 h at 70°C to determine dry weight and analyze ion concentration (Na and k) spectrophotometrically following nitric-perchloric acid digestion.

Protein, soluble carbohydrate, hydrogen peroxide ($H_2O_2$) and catalase enzyme activity were estimated according to the method of Bradford[12], Mc Cready *et al.*[13], Mac Nevin and Uron[14] and Arnon[15].

The combined data of the two seasons were statistically analyzed according to the procedures of Snedecor and Cochran[16] where the means of studied treatments were compared using L.S.D test at 0.05 significance level.

## RESULTS AND DISCUSSION

In general, increased NaCl salinity decreased total emergence of seedlings derived from inoculated and uninoculated faba bean varieties (Fig. 1). However, total emergence percentages in seedlings of inoculated groups with yeast strain were higher compared with those of the unionculated ones under different salinity treatments. The most inhibiting salinity level for total emergence was 6000 ppm, also it was clear that some of the varieties were sensitive to salinity as that of Giza 461 and Giza 643, moderately affected as that of Giza 717 and resistant varieties as that of Giza Blanka and Giza 674. Similar findings were supported by Ayres and Westcot[17]. They deduced that salinity delays germination but does not appreciably reduce the final percentage of germination. The effect of salinity on plant growth is related to the stage of plant development at which salinity is imposed. Also, Rahoades[18] reported that some plants are generally relatively tolerant during germination, but become more sensitive during emergence and early seedling stage.

Similarly, dry weight was reduced under saline condition mostly at 6000 ppm. It was reduced by 57.3, 62.0, 59.0, 63.9 and 67.4% in inoculated samples of Giza Blanka, Giza 674, 717, 461 and 634, respectively (Fig. 2). Greenway and Munns[19] reported that the effect of salinity on leaf area was greater than on dry weight, as salt accumulation in the shoot occurs via transpiration stream, which is highest in old leaves killing them. Salt stress induced injuries which can occur not only due to osmotic and oxidative effects, but also toxic and nutrient deficiency effects of salinity. This was almost clear in obtained results. Table 1 indicates the effect of various salinity levels and inoculation with soil yeast (*Rhodotorula* sp.) on protein content in leaves of different faba bean varieties. Leaves protein content of salinity tolerant varieties i.e., Giza Blanka and Giza 674 in addition to the moderately salinity tolerant variety (Giza 717) was significantly decreased at moderate salinity levels (3000 and 4000 ppm). On the other hand, the sensitive faba bean varieties to salinity, namely Giza 461 and Giza 634, revealed significant decrease in their leaves protein content at both low levels of salinity (1000 and 2000 ppm) in comparison with the control treatment. With exception of few cases, biofertilization with *Rhodotorula* sp. augmented leaves protein content of the different tested faba bean varieties when compared with the non-biofertilized treatments at the diverse salinity levels. This could be attributed to the growth hormones produced by the yeast strain. Irrespective of salinity and biofertilization treatments, the tolerant and moderately tolerant faba bean varieties contained leaves protein content more than the sensitive varieties[20].

The influence of various salinity levels and biofertilization with *Rhodotorula* sp. on leaves soluble carbohydrate content of different varieties of faba bean (Table 2). Regardless of the divers treatments, it was observed that the salinity tolerant faba bean varieties contained more leaves soluble carbohydrate than either moderate or the sensitive varieties. Nevertheless, it seems that leaves soluble carbohydrate content greatly affected by salinity level, where the significant decrease were revealed at 2000 ppm salinity level for the majority of faba bean varieties either tolerant or sensitive to salinity. With exception of variety Giza 674, inoculation with *Rhodotorula* sp. increased soluble carbohydrate content of leaves incomparison with the uninoculated treatments. Similar results were obtained by Bahr and Gomaa[21].

Levitt[3] stated that all resistant plants must possess adaptation originating from osmoregulation, by

Table 1: Effect of different salinity levels in addition to yeast inoculation on protein content (mg $100^{-1}$ mg DW) in leaves of faba bean varieties

| Salinity | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| Levels (ppm) | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 15.21 | 12.01 | 13.08 | 12.83 | 14.81 | 14.11 | 12.11 | 12.11 | 12.31 | 12.11 |
| 1000 | 14.90 | 14.71 | 14.11 | 12.92 | 14.91 | 14.17 | 11.76 | 11.14 | 11.22 | 11.91 |
| 2000 | 14.88 | 14.34 | 14.25 | 13.10 | 14.96 | 13.95 | 10.35 | 10.91 | 10.54 | 11.44 |
| 3000 | 14.56 | 13.53 | 11.41 | 12.86 | 14.11 | 13.40 | 10.33 | 10.21 | 9.85 | 10.91 |
| 4000 | 12.23 | 11.51 | 11.21 | 12.12 | 13.71 | 11.32 | 9.58 | 9.11 | 9.33 | 9.86 |
| 5000 | 10.63 | 10.33 | 10.76 | 10.57 | 11.14 | 10.44 | 8.79 | 8.52 | 9.11 | 8.98 |
| 6000 | 10.50 | 8.38 | 9.35 | 9.11 | 9.15 | 9.11 | 8.33 | 8.12 | 8.73 | 8.11 |
| L.S.D. at 0.05 | 0.85 | 0.71 | 0.75 | 0.63 | 0.73 | 0.61 | 0.51 | 0.50 | 0.61 | 0.41 |

In : Inoculated with yeast and Un : Uninoculated

Table 2: Effect of different salinity levels in addition to yeast inoculation on soluble carbohydrate content (mg $100^{-1}$ mg DW) in leaves of faba bean varieties

| Salinity | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| Levels (ppm) | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 15.1 | 14.43 | 15.23 | 15.20 | 13.91 | 13.85 | 13.67 | 13.51 | 12.62 | 12.53 |
| 1000 | 14.97 | 14.43 | 15.33 | 15.22 | 13.92 | 13.11 | 13.25 | 13.16 | 12.31 | 11.94 |
| 2000 | 13.96 | 13.32 | 15.11 | 14.91 | 13.56 | 12.75 | 12.76 | 12.53 | 11.77 | 10.86 |
| 3000 | 13.11 | 12.11 | 13.61 | 14.14 | 12.30 | 12.11 | 10.91 | 11.72 | 10.55 | 9.79 |
| 4000 | 12.06 | 11.87 | 13.42 | 13.49 | 11.81 | 11.52 | 9.62 | 10.84 | 9.66 | 8.32 |
| 5000 | 11.86 | 11.55 | 12.15 | 13.11 | 10.20 | 10.10 | 9.11 | 8.91 | 8.85 | 8.31 |
| 6000 | 10.41 | 10.72 | 11.52 | 12.67 | 9.51 | 9.34 | 8.85 | 8.31 | 8.11 | 8.11 |
| L.S.D. at 0.05 | 0.85 | 0.72 | 0.86 | 0.96 | 0.81 | 0.75 | 0.56 | 0.55 | 0.49 | 0.47 |

In : Inoculated with yeast and Un : Uninoculated

Table 3 : Effect of different salinity levels in addition to yeast inoculation on $K^+$ content (mg $g^{-1}$ DW) in leaves of faba bean varieties

| Salinity | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| Levels (ppm) | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 1.06 | 1.06 | 0.93 | 0.91 | 1.05 | 0.98 | 0.96 | 0.94 | 0.95 | 0.94 |
| 1000 | 1.08 | 0.96 | 0.95 | 0.92 | 0.97 | 0.91 | 0.95 | 0.94 | 0.96 | 0.94 |
| 2000 | 0.98 | 0.96 | 0.94 | 0.91 | 0.95 | 0.88 | 0.91 | 0.91 | 0.85 | 0.86 |
| 3000 | 0.98 | 0.91 | 0.89 | 0.87 | 0.91 | 0.76 | 0.89 | 0.81 | 0.83 | 0.73 |
| 4000 | 0.96 | 0.85 | 0.81 | 0.79 | 0.81 | 0.73 | 0.76 | 0.71 | 0.79 | 0.63 |
| 5000 | 0.94 | 0.83 | 0.74 | 0.66 | 0.81 | 0.73 | 0.71 | 0.63 | 0.77 | 0.61 |
| 6000 | 0.91 | 0.81 | 0.71 | 0.66 | 0.79 | 0.64 | 0.65 | 0.63 | 0.71 | 0.61 |
| L.S.D. at 0.05 | 0.06 | NS | 0.06 | 0.05 | 0.07 | 0.11 | 0.12 | 0.08 | 0.13 | 0.17 |

In : Inoculated with yeast and Un : Uninoculated and NS : Not significant

Table 4: Effect of different salinity levels in addition to yeast inoculation on $Na^+$ content (mg $g^{-1}$ DW) in leaves of faba bean varieties

| Salinity | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| Levels (ppm) | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 1.26 | 1.27 | 1.28 | 1.37 | 1.31 | 1.29 | 1.24 | 1.27 | 1.21 | 1.25 |
| 1000 | 1.26 | 1.28 | 1.30 | 1.45 | 1.31 | 1.31 | 1.25 | 1.27 | 1.24 | 1.27 |
| 2000 | 1.27 | 1.41 | 1.31 | 1.63 | 1.36 | 1.34 | 1.28 | 1.30 | 1.39 | 1.44 |
| 3000 | 1.27 | 1.56 | 1.35 | 1.72 | 1.42 | 1.46 | 1.55 | 1.63 | 1.53 | 1.59 |
| 4000 | 1.33 | 1.59 | 1.35 | 1.79 | 1.66 | 1.72 | 1.68 | 1.78 | 1.75 | 1.81 |
| 5000 | 1.33 | 1.63 | 1.40 | 1.81 | 1.79 | 1.83 | 1.94 | 1.95 | 1.93 | 2.11 |
| 6000 | 1.36 | 1.63 | 1.40 | 1.85 | 1.85 | 1.91 | 1.96 | 1.98 | 1.98 | 2.11 |
| L.S.D. at 0.05 | NS | 0.09 | 0.10 | 0.08 | 0.06 | 0.09 | 0.08 | 0.04 | 0.06 | 0.06 |

In : Inoculated with yeast and Un : Uninoculated and NS : Not significant

dehydration avoidance which is the basis of their tolerance of the salt induced osmotic stress.

Osmoregulation can occur in plants by active uptake of inorganic ions (such as Na, K and Cl) or synthesis of organic solutes (such as sugars, organic acids, free amino acids and proline) depending on species. Previous results and conclusions were also supported by Hasegawa *et al.*[22].

Table 5: Effect of different salinity levels in addition to yeast inoculation on proline content (µ mole/g F.wt) in leaves of faba bean varieties

| Salinity Levels (ppm) | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 2.56 | 2.78 | 2.12 | 2.37 | 2.47 | 2.36 | 2.38 | 2.41 | 2.39 | 2.41 |
| 1000 | 2.57 | 2.75 | 2.14 | 2.40 | 2.49 | 2.37 | 2.36 | 2.43 | 2.40 | 2.40 |
| 2000 | 2.63 | 2.79 | 2.15 | 2.40 | 2.58 | 2.61 | 2.38 | 2.57 | 2.49 | 2.52 |
| 3000 | 2.84 | 2.99 | 2.47 | 2.66 | 2.73 | 2.82 | 3.33 | 3.47 | 3.66 | 3.10 |
| 4000 | 3.12 | 3.11 | 3.53 | 3.72 | 4.21 | 4.17 | 4.43 | 4.71 | 4.67 | 4.91 |
| 5000 | 3.92 | 3.76 | 3.88 | 3.91 | 4.55 | 4.91 | 4.92 | 5.11 | 4.96 | 5.35 |
| 6000 | 4.11 | 4.98 | 5.11 | 5.23 | 5.10 | 5.28 | 5.25 | 5.32 | 5.33 | 5.41 |
| L.S.D. at 0.05 | 0.38 | 0.41 | 0.49 | 0.51 | 0.47 | 0.56 | 0.45 | 0.47 | 0.41 | 0.45 |

In : Inoculated with yeast strain and Un : Unioculated

Table 6: Effect of different salinity levels in addition to yeast inoculation on hydrogen peroxide content (n moles $100^{-1}$ mg D.W) in leaves of faba bean varieties

| Salinity Levels (ppm) | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 114.2 | 113.9 | 117.1 | 110.1 | 130.5 | 106.8 | 122.1 | 114.2 | 125.5 | 126.3 |
| 1000 | 118.1 | 125.1 | 136.3 | 124.9 | 132.1 | 129.7 | 119.8 | 114.1 | 124.9 | 126.1 |
| 2000 | 130.1 | 134.6 | 157.1 | 14.3 | 145.6 | 148.3 | 117.3 | 123.5 | 137.3 | 141.6 |
| 3000 | 138.9 | 143.1 | 166.2 | 156.1 | 149.7 | 157.1 | 127.1 | 133.6 | 156.6 | 159.3 |
| 4000 | 151.0 | 156.5 | 171.1 | 173.6 | 151.0 | 165.6 | 158.9 | 161.7 | 166.9 | 175.1 |
| 5000 | 164.1 | 168.5 | 179.5 | 176.5 | 164.7 | 181.7 | 173.5 | 183.5 | 181.3 | 188.2 |
| 6000 | 175.7 | 176.6 | 131.6 | 179.3 | 183.5 | 186.7 | 187.6 | 188.7 | 186.6 | 191.1 |
| L.S.D. at 0.05 | 10.9 | 9.7 | 11.1 | 9.8 | 11.12 | 11.7 | 12.1 | 13.6 | 12.9 | 13.7 |

In: Inoculated with yeast strain and Un : Uninoculated

Table 7: Effect of different salinity levels in addition to yeast inoculaton on catalase enzyme activity (units $100mg^{-1}$ DW) in leaves of faba bean varieties

| Salinity Levels (ppm) | Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Giza Blanka | | Giza 674 | | Giza 717 | | Giza 461 | | Giza 634 | |
| | In | Un. | In | Un. | In | Un. | In | Un. | In | Un. |
| Control | 134.0 | 133.0 | 133.0 | 130.0 | 120.0 | 118.0 | 125.0 | 118.0 | 123.0 | 120.0 |
| 1000 | 130.0 | 125.0 | 131.0 | 130.0 | 115.0 | 118.0 | 127.0 | 120.0 | 128.0 | 120.0 |
| 2000 | 125.0 | 120.0 | 125.0 | 122.0 | 98.0 | 96.0 | 110.0 | 101.0 | 115.0 | 110.0 |
| 3000 | 120.0 | 118.0 | 116.0 | 120.0 | 96.0 | 91.0 | 93.0 | 88.0 | 95.0 | 95.0 |
| 4000 | 98.0 | 95.0 | 110.0 | 95.0 | 88.0 | 85.0 | 78.0 | 72.0 | 85.0 | 80.0 |
| 5000 | 94.0 | 94.0 | 94.0 | 84.0 | 84.0 | 84.0 | 76.0 | 68.0 | 78.0 | 68.0 |
| 6000 | 93.0 | 91.0 | 87.0 | 81.0 | 78.0 | 78.0 | 68.0 | 68.0 | 68.0 | 68.0 |
| L.S.D. at 0.05 | 8.5 | 8.1 | 7.5 | 7.3 | 6.8 | 6.5 | 6.1 | 5.8 | 5.7 | 5.6 |

In : Inoculated with yeast strain and Un : Uninoculated

As for leaves potassium content, the salt tolerant variety Giza Blanka surpassed the other varieties in leaves K-content (Table 3). Biofertilization of faba bean varieties with yeast (*Rhodotorula* sp.) increased K-content for all varieties when compared to the uninoculated treatments. The same trend was recorded by Bahr and Gomaa[21] where they found that inoculation of triticale with yeast increased nutrient elements content. Further, leaves K-content was decreased with the increasing salinity level where the most pronounced decreases were recorded with the salt sensitive varieties Giza 461 and Giza 634. Regarding sodium content of faba bean leaves, Na-content recorded its highest values is salt sensitive varieties (Table 4). An observed decrease in leaves Na-content due to inoculation with *Rhodotorula* sp. compared to the uninoculated treatments. It was found that increasing salinity level was accompanied by an increase in leaves Na-content to reach its maximum value with 6000 ppm.

Grattan and Grieve[23] reported that the reduction of K concentration in plant tissue observed with increased salinity could be due to the interaction Na x K at the uptake and transport level. Reduction of K uptake in plants by Na is a competitive process.

Proline content in leaves (Table 5) indicates that the salt sensitive varieties accumulated more proline than the salt tolerant ones. Biofertilization of faba bean with *Rhodotorula* sp. resulted in decreasing leaves content of proline, regardless of salinity level in comparison with the non-biofertilized treatments. Also, plants under high

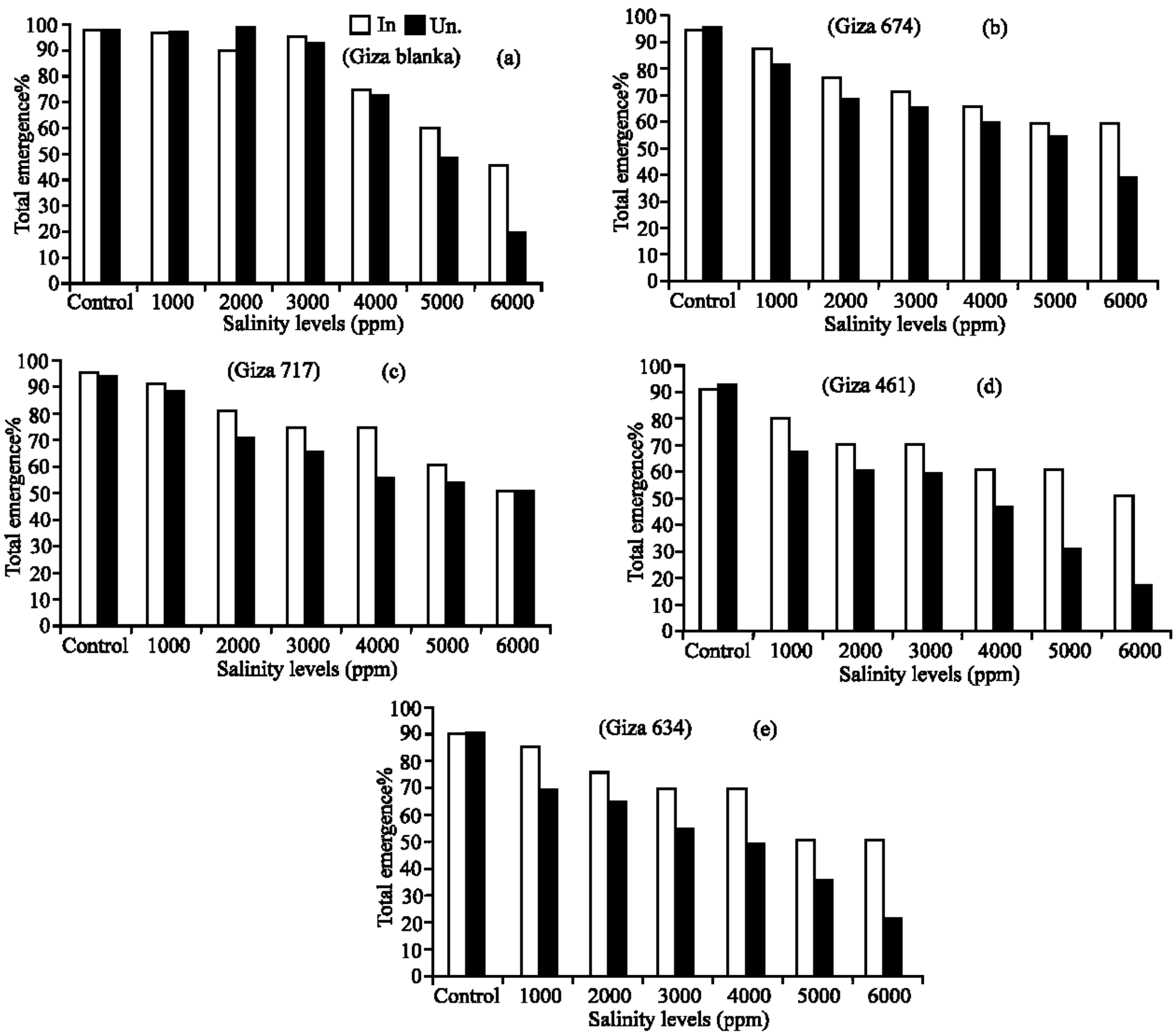


Fig. 1a-e: The effect of different salinity levels in addition to yeast inoculation on total emergence (%) of faba bean varieties

salinity level revealed proline accumulation in their leaves more than the low salinity levels, treated plants irrespective of both varieties and biofertilization with *Rhodotorula* sp. Previous results were supported by Levitt[3], Hasegawa *et al.*[22] and Hathout[24] suggested that proline seemed to have additional function other than osmoregulation because of its poor ability to resist the toxic effect of salinity. Demir and Kacacaliskan[25] suggested that proline may play a role as enzyme stabilising agent under NaCl salinity.

The impact of diverse salinity levels in the presence or absence of biofertilization with yeast (*Rhodotorula* sp.) on hydrogen peroxide content of faba bean leaves was presented in Table 6. Hydrogen peroxide content was increased with increasing salinity level. In general, the nonbiofertilized treatments recorded results higher than the biofertilized ones. Besides, the salt sensitive faba bean varieties contained more ($H_2O_2$) than the salt tolerant and moderately tolerant varieties. Lin and Kao[26] supported previous results and added that increasing concentrations of NaCl reduce root growth. This reduction is closely correlated with the increase in $H_2O_2$ level.

Catalase enzyme activity in leaves of faba bean varieties was affected by various salinity levels and biofertilization with yeast (*Rodotorula* sp.) was demonstrated in Table 7. Obvious decrease in catalase activity due to increasing salinity levels was observed regarding the different faba bean varieties, where the control treatments recorded the highest significant values in comparison with different salinity treatments.

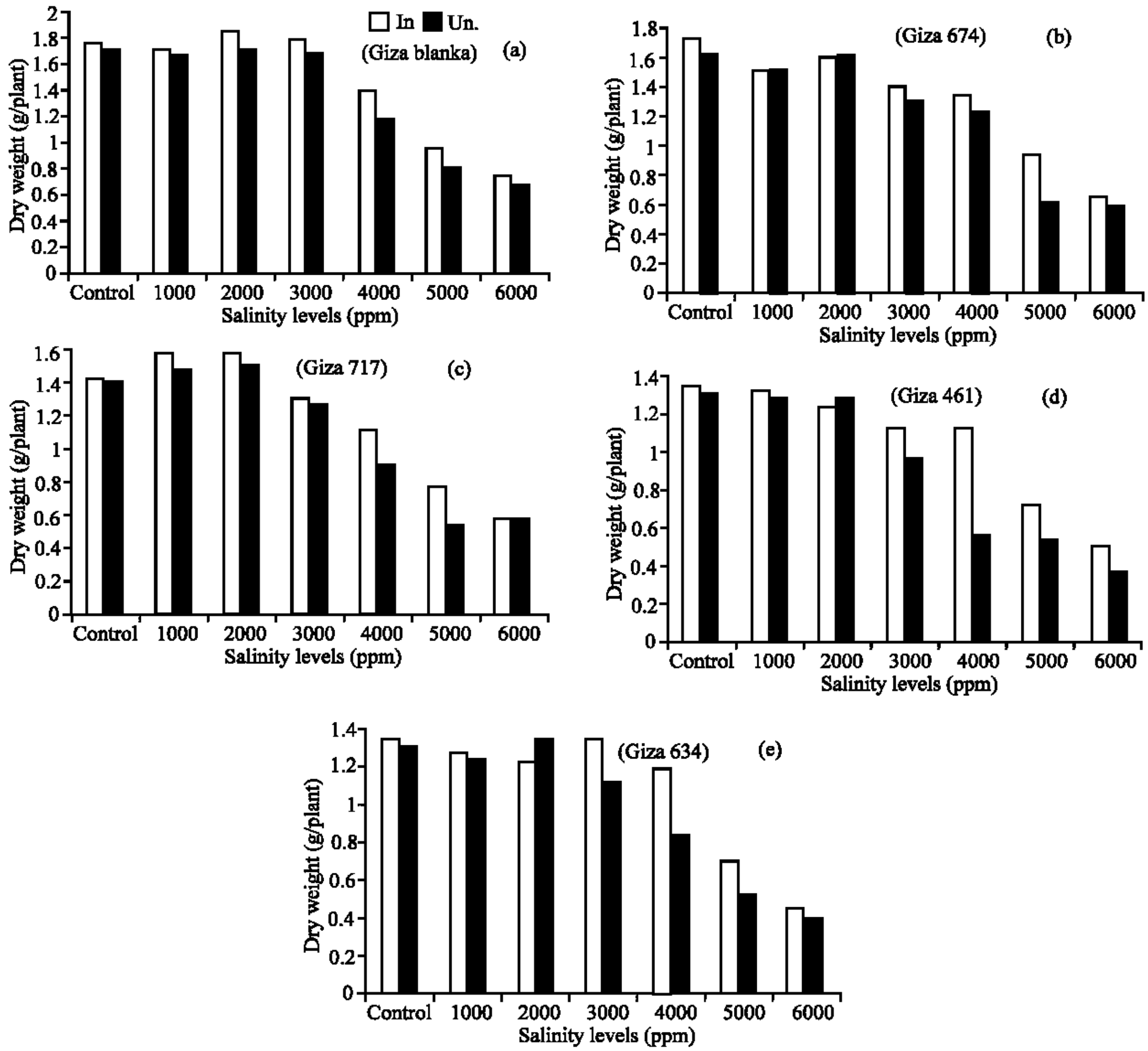


Fig. 2a-e: The effect of differen salinity levels in addition to yeast inoculation on plant dry weight (g $plant^{-1}$) of faba bean varieties

Little increase in catalase enzyme activity owing to biofertilization with *Rhodotorula* sp. were observed compared to the non-biofertilized treatments. Furthermore, the fababean varieties of slat tolerant characteristic showed catalase enzyme activity more than the salt sensitive ones. Shukry *et al.*[27] and Heikal *et al.*[28] summarized the relation between plant growth, nutrients and enzyme activity and concluded that germination and water uptake of *V. faba* seeds were suppressed in response to salinity stress which lead to an increase in osmotic potential, $Na^+$, $Cl^-$, proline and decrease in $K^+$, $K^+$/Na ratio and catalase activity.

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