# Response of salt stressed barley seedlings to phenylurea

**R.M. Ali, H.M. Abbas**

*Faculty of Science, Fayoum, Cairo University, Egypt*

**ABSTRACT**

The effect of phenylurea with reported cytokinin-like activities on seed germination, seedling growth, activities of antioxidant enzymes, polyphenol, peroxidase, indoleacetic acid oxidase, and total phenolic compounds, flavonoids was investigated in stressed barley seedlings. The application of phenylurea decreases the activity of peroxidase, indoleacetic acid oxidase and increases the activity of polyphenol oxidase with decrease in total phenolic compounds and flavonoids and consequent increase in growth rate. Saline (NaCl) stress in barley seedlings causes an increase in total phenolic compounds, flavonoids and enhancement of peroxidase and indoleacetic acid oxidase activities and consequent decrease in growth rate. The adverse effect of salt stress on germination, antioxidant enzymes, phenolic compounds, flavonoids can partially be rectified by phenylurea.

**Keywords:** barley; phenylurea; salt stress; antioxidant enzymes; polyphenol oxidase; peroxidase; indoleacetic acid oxidase; phenolic compounds; flavonoids

Environmental stresses are thought to result in the production of active oxygen in plant species, causing oxidative stress (Smirnoff 1993, Gossett et al. 1994, Hernandez et al. 1999). The ability of higher plants to scavenge the toxic active oxygen seems to be a very important determinant of their tolerance to environmental stress. The primary compounds of components of this antioxidant system include carotenoids, flavonoids, phenolic compounds as well as antioxidant enzymes such as catalase, peroxidase, polyphenol oxidase and indoleacetic acid oxidase.

Phenolic compounds and flavonoids are among the most influential and widely distributed secondary products in the plant kingdom. Many of them play important physiological and ecological roles, being involved in resistance to different types of stress (Delalonde et al. 1996, Rice et al. 1998, Ayaz et al. 2000).

Salinity stress can reduce cytokinin export from the root to the shoot in many plants (Rao and Rao 1985, Kuiper et al. 1988, 1990). An adequate cytokinin supply is essential for normal plant development; it can explain why exogenous applications of cytokinin can overcome the effect of salinity stress on the growth of wheat seedlings (Naqui et al. 1982, Roth 1987).

In our study we used phenylurea because we believed that phenylurea application is likely to be a commercially viable way of reducing the effects of salinity stress compared with other synthetic cytokinins or kinetin. It is water soluble but it is little known about the response of phenylurea metabolism during stress. Therefore, the present study was undertaken to examine the effect of different levels of phenylurea on germination, seedling growth, phenolic compounds, flavonoids and antioxidant enzymes in barley grown under salinity stress.

## MATERIAL AND METHODS

The plant material was *Hordeum vulgare* L. (Giza 123 variety), an important crop grown in Egypt. Seeds were kindly supplied by the Egyptian Ministry of Agriculture. Selected seeds of uniform size were sterilised with 0.1% mercuric chloride for 3 min and thoroughly washed with distilled water. Seeds were soaked in distilled water or phenylurea solutions (0.1, 1, 3 and 5 ppb) for 4 hrs before they were sown in distilled water or NaCl solution (50 and 100mM). These seeds were then transferred to sterile Petri dishes containing two sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water or NaCl solution. Each Petri dish containing 20 seeds and each treatment was replicated three times. The seeds were allowed to germinate at 25°C in darkness. Seeds were considered to germinate after a radicle emerged from the testa. Seedlings were allowed to grow for 10 days. 3 ml of distilled water or NaCl solution were added to each Petri dish on days 3 and 6 from the beginning of imbibition. Rate of germination was recorded at intervals of 24 hrs for 72 hrs. The length and fresh mass of shoots and roots of 10 days seedlings were also recorded.

**Extraction and quantification of total phenols.** Phenolic compounds of the plant material were extracted with methanol. Total phenolic content was assayed quantitatively by $A_{765}$ with Folin-Cocalteau reagent (Singleton and Rossi 1965, Singleton et al. 1985). The results were expressed as µg of p-hydroxycinnamic acid (g fresh mass).

**Extraction and quantification of total flavonoids.** Flavonoids of the plant material were extracted with 80% methanol at 60°C, shaken for 20 min and filtered. The filtrate was diluted at 1:3 and 100 µl of reactive solution (1% 2 amino-ethyldiphenylborate) was added (Hairi et al.

Table 1. Effect of phenylurea and NaCl salinity on percentage of germination of barley seeds

| Treatment | | Duration of germination (hours) | | |
|---|---|---|---|---|
| NaCl (mM) | phenylurea (ppm) | 24 | 48 | 72 |
| 0 | 0 | 76.47 | 94.12 | 100 |
| | 0.1 | 76.47 | 88.24* | 100 |
| | 1 | 79.41 | 88.24* | 100 |
| | 3 | 79.41 | 88.24* | 100 |
| | 5 | 76.71 | 82.35** | 94.24 |
| 50 | 0 | 64.70** | 73.52** | 79.41** |
| | 0.1 | 70.59** | 82.35** | 88.24** |
| | 1 | 76.47 | 88.24* | 90.27** |
| | 3 | 76.47 | 88.24* | 92.16** |
| | 5 | 70.59** | 73.52** | 88.35** |
| 100 | 0 | 62.74** | 70.59** | 76.95** |
| | 0.1 | 64.70** | 76.47** | 82.35** |
| | 1 | 64.70** | 79.41** | 82.35** |
| | 3 | 60.00** | 76.47** | 82.35** |
| | 5 | 58.13** | 70.59** | 76.47** |
| *LSD* 5% | | 3.43 | 4.29 | 5.21 |
| *LSD* 1% | | 5.19 | 6.49 | 7.88 |

* significant differences as compared with the control
** highly significant differences as compared with the control

1991). Spectrometric measurements were done at the maximum wavelength of 404 nm. Extract absorption was compared with that of a standard (Luteolin) resulting in the calculation of total amount of flavonoids.

**Assays of total antioxidant enzyme activities.** For assaying the activities of polyphenol oxidase, indoleacetic acid oxidase (IAA-O) and peroxidase, the plant material was extracted according to the method of Kar and Mishra (1976) with some modifications (Fathouh 1991). IAA oxidase activity was assayed following the method described by Darbyshire (1971). The activities of polyphenol oxidase and peroxidase were measured according to the method of Kar and Mishra (1976). The activity of the above-mentioned enzymes is expressed as follows: IAA oxidase µg IAA oxidised by the enzyme mg F.W./h. Peroxidase changes in the optical density g F.W./min. Polyphenol oxidase changes in the optical density g F.W./min.

## RESULTS AND DISCUSSION

The final germination percentage and growth of barley seedlings estimated as length and fresh-dry mass of shoots and roots increased significantly with increased concentration of phenylurea (Tables 1 and 2). The results also suggest that NaCl salinity inhibits seed germination and seedling growth of barley like in other seeds. The adverse effect of NaCl has been attributed to changes in osmotic potential resulting from reduced water (Bewley and Black 1994, Bradford 1995, Ali 2000). Phenyl urea pretreatment led to a marked increase in the percentage of germination and seedling growth under stressed conditions. It also appears that a decrease in germination and growth is related to salinity induced disturbances of the metabolic process leading to an increase in phenolic compounds (Dhingra and Varghese 1985, Ayaz et al. 2000) and oxidative enzymes (Darbyshire 1971, Gossett et al. 1994, Thipyapony et al. 1995, Dionisio-Sese and Tobita 1998, Kennedy and Filippis 1999). Barley seedlings exposed to NaCl suffer from an oxidative stress as seen from its effect on phenolic compounds, flavonoids and oxidant enzymes assayed in shoots and roots (Table 3). The first possibility seems likely since the compounds that were found to accumulate flavonoids belong to the class of phenolics that are potential stimulators of peroxidase and indoleacetic acid oxidase (Gossett et al. 1994, Dionisio-Sese and Tobita 1998, Kennedy and Filippis 1999). Thus, it can lower the auxin level in the tissues and consequently cause growth retardation. A large number of studies have also demonstrated that the polyphenol oxidase enzyme increases in response to biotic and abiotic stress (Thipyapony et al. 1995).

The results of this study indicate that the phenylurea application led to a significant increase in the growth rate of shoots and roots and marked reduction in the total phenolic compounds and flavonoids in shoots, but increase in roots. The results also pointed out clearly that the phenylurea application led to an increase in polyphenol oxidase activity and a decrease in peroxidase and indoleacetic acid oxidase activities (Tables 2 and 3). In this respect, the regulation of auxin levels in plants is known to be controlled mainly by the enzymes auxin oxidase and

Table 2. Effect of phenylurea and NaCl salinity on growth parameters of barley after 10 days of germination

| Treatment | | Shoots | | | Roots | | | Water content (%) |
|---|---|---|---|---|---|---|---|---|
| NaCl (mM) | phenylurea (ppm) | length (cm) | fresh mass (mg/plant) | dry mass (mg/plant) | length (cm) | fresh mass (mg/plant) | dry mass (mg/plant) | |
| 0 | 0 | 13.85 | 79 | 10 | 12.67 | 74 | 18 | 81.70 |
| | 0.1 | 14.20 | 92** | 11** | 13.20 | 73 | 18 | 82.24 |
| | 1 | 13.98 | 90** | 10 | 12.90 | 74 | 18 | 82.93 |
| | 3 | 13.98 | 90** | 10 | 13.00 | 78 | 16 | 84.45 |
| | 5 | 14.50** | 107** | 11** | 13.65** | 88** | 15 | 86.67 |
| 50 | 0 | 6.67** | 55** | 8** | 7.80** | 54** | 10** | 80.80 |
| | 0.1 | 14.57* | 101** | 12** | 9.00** | 64** | 14** | 84.24 |
| | 1 | 13.50 | 89** | 10 | 9.10** | 64** | 14** | 84.31 |
| | 3 | 13.30 | 88** | 10 | 9.30** | 64** | 14** | 84.21 |
| | 5 | 12.66** | 78 | 9** | 10.30** | 68** | 12** | 85.62 |
| 100 | 0 | 6.40** | 49** | 9** | 6.61** | 40** | 8** | 79.54 |
| | 0.1 | 8.50** | 65** | 7** | 6.70** | 40** | 9** | 86.67 |
| | 1 | 7.90** | 62** | 8** | 7.00** | 50** | 10** | 83.93 |
| | 3 | 7.60** | 60** | 5** | 6.00** | 50** | 10** | 86.36 |
| | 5 | 7.50** | 58** | 5** | 6.00** | 40** | 8** | 86.73 |
| *LSD* 5% | | 0.61 | 4.90 | 0.49 | 0.86 | 5.88 | 3.19 | |
| *LSD* 1% | | 0.92 | 7.42 | 0.74 | 1.36 | 8.9 | 4.43 | |

* significant differences as compared with the control
** highly significant differences as compared with the control

peroxidase (Reineck and Bandurski 1988). Phenylurea displays a clear cytokinin-like activity (Cavender et al. 1988). However, it is possible that phenylurea increases cytokinin activity by inhibiting cytokinin oxidase. Laloue and Fox (1989) showed that phenylurea inhibited cytokinin oxidase from wheat. Even though phenylurea increases cytokinin

Table 3. Effect of phenylurea and NaCl salinity on phenolic compounds, flavonoids and antioxidant enzymes after 10 days of germination

| Treatment | | Shoots | | | | | Roots | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 0 | 0 | 0.47 | 0.36 | 1.96 | 131.81 | 6.13 | 0.09 | 0.21 | 2.42 | 144.70 | 6.68 |
| | 0.1 | 0.34** | 0.28** | 2.46** | 88.44** | 1.99** | 0.11 | 0.40** | 2.61 | 118.90** | 5.30** |
| | 1 | 0.25** | 0.29** | 2.38** | 79.82** | 2.20** | 0.14** | 0.46** | 2.81 | 123.73** | 3.52** |
| | 3 | 0.14** | 0.26** | 2.69** | 78.79** | 2.61** | 0.25** | 0.52** | 3.55** | 130.38* | 5.57** |
| | 5 | 0.11** | 0.26** | 2.58** | 87.82** | 2.75** | 0.35** | 0.42** | 3.11* | 129.57** | 3.61** |
| 50 | 0 | 0.49 | 0.40* | 2.01 | 156.83** | 7.38* | 0.13* | 0.22 | 2.35 | 94.45** | 7.25 |
| | 0.1 | 0.29** | 0.38 | 3.90** | 60.71** | 7.17 | 0.27** | 0.25* | 2.30 | 106.17** | 4.27** |
| | 1 | 0.26** | 0.36 | 3.83** | 90.08** | 6.38 | 0.39** | 0.35** | 3.24* | 123.88** | 4.30** |
| | 3 | 0.20** | 0.35 | 3.20** | 93.50** | 4.58** | 0.17** | 0.36** | 3.64** | 109.39** | 3.89** |
| | 5 | 0.11** | 0.28** | 2.83** | 65.43* | 3.50** | 0.14** | 0.23 | 2.40 | 89.00** | 3.89** |
| 100 | 0 | 0.50 | 0.44** | 2.06* | 186.04** | 7.73* | 0.20** | 0.24 | 2.72 | 116.94** | 7.04 |
| | 0.1 | 0.50 | 0.33 | 3.41** | 112.63** | 7.71* | 0.19** | 0.27** | 2.66 | 176.36** | 5.87* |
| | 1 | 0.52* | 0.28** | 3.71** | 108.50** | 8.92** | 0.17** | 0.28** | 2.87 | 177.29** | 5.32** |
| | 3 | 0.38** | 0.20** | 4.70** | 118.90* | 9.18** | 0.18** | 0.29** | 3.34* | 151.45 | 5.11** |
| | 5 | 0.16** | 0.16** | 3.00** | 129.29 | 6.27 | 0.14** | 0.27** | 3.77** | 108.55** | 4.86** |
| *LSD* 5% | | 0.05 | 0.04 | 0.70 | 8.58 | 1.10 | 0.03 | 0.04 | 0.74 | 0.86 | 0.64 |
| *LSD* 1% | | 0.07 | 0.06 | 0.11 | 12.99 | 1.67 | 0.04 | 0.06 | 1.11 | 10.39 | 0.96 |

* significant differences as compared with the control
** highly significant differences as compared with the control
A = NaCl (Mm) , B = phenylurea (ppm)
1 = total phenolics (mg/g F.M.), 2 = flavonoids (mg/g F.M.), 3 = phenol oxidase optical density (min/g F.M.),
4 = peroxidase optical density (min/g F.M.), 5 = IAA.O.µg IAA oxidised (mg F.M./min)

activity by inhibiting cytokinin oxidase, it will still be expected to increase the growth rate of barley.

On the other hand, in seedlings originating from seeds soaked in phenylurea of different concentrations and treated with 50mM NaCl, the content of phenolic compounds, flavonoids and peroxidase enzyme decreased in shoots, but it increased in roots. The activity of indoleacetic acid oxidase also decreased but polyphenol oxidase activity increased both in shoots and in roots, and consequently the growth rate increased in comparison with that of salinised seedlings (Tables 2 and 3).

At 100mM NaCl and treated with phenylurea, phenolic compounds, flavonoids and peroxidase enzyme decreased in shoots, which was accompanied by an increase in polyphenol oxidase and indoleacetic acid oxidase. However, in the case of roots the content of phenolic compounds and indoleacetic acid oxidase decreased, which was accompanied by an increase in flavonoids, polyphenol oxidase and peroxidase and decrease in the growth rate compared to nonsalinised plants.

The results presented in this paper suggest that phenylurea can increase the growth rate of salinity stressed barley to some extent although the growth rate increase is small and does not fully alleviate the effect of salinity stress.

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**ABSTRAKT**

**Reakce mladých rostlin ječmene vystavených solnému stresu na fenylmočovinu**

U mladých rostlin ječmene vystavených stresu jsme sledovali vliv fenylmočoviny s aktivitou podobnou cytokininům na klíčivost obilek a růst a na aktivitu antioxidačních enzymů, polyfenolu, peroxidázy, oxidázy indoloctové kyseliny, celkových fenolických látek a flavonoidů. Aplikace fenylmočoviny snižuje aktivitu peroxidázy a oxidázy indoloctové kyseliny a zvyšuje aktivitu oxidázy polyfenolů při poklesu celkových fenolických látek a flavonoidů a následném zvýšení rychlosti růstu. Solný (NaCl) stres působí u mladých rostlin ječmene zvýšení celkových fenolických látek a flavonoidů a zvýšení aktivity peroxidázy a oxidázy indoloctové kyseliny a následně snižuje rychlost růstu. Nepříznivý vliv solného stresu na klíčivost, antioxidační enzymy, fenolické látky a flavonoidy lze částečně kompenzovat aplikací fenylmočoviny.

**Klíčová slova:** ječmen; fenylmočovina; solný stres; antioxidační enzymy; oxidáza polyfenolů; peroxidáza; oxidáza kyseliny indoloctové; fenolické látky; flavonoidy

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*Corresponding author:*

Dr. Refaat Mohamed Ali, Botany Department, Faculty of Science, Fayoum Branch, Cairo University, P.O. Box 63514 Fayoum, Egypt
e-mail: hesham_m_abbas@hotmail.com

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