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Alleviation of Osmotic Stress on Seed Germination and Seedling Growth of Cotton, Pea and Wheat by Proline

By

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With 8 Figures

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Summary

HEIKAL M. M. D. & SHADDAD M. A. 1982: Alleviation of osmotic stress on seed germination and seedling growth of cotton, pea and wheat by proline. — *Phyton (Austria)* 22 (2): 275—287, with 8 figures. — English with German summary.

The rate of water uptake, rate of germination, final germination percentage, as well as seedling growth were considerably lowered with the rise of osmotic stress levels using NaCl or polyethylene glycol 6000 (0—8 bars). More considerable reduction was obtained under polyethylene glycol than under NaCl, presumably due to the greater water stress caused by the former osmotic substrate.

The addition of exogenous proline was very effective in counteracting the effect of osmotic stress on seed germination and seedling growth of the tested species.

Zusammenfassung

HEIKAL M. M. D. & SHADDAD M. A. 1982. Minderung des osmotischen Streß bei Keimung und Keimlingswachstum von Baumwolle, Erbse und Weizen durch Prolin. — *Phyton (Austria)* 22 (2): 275—287, mit 8 Abbildungen. — Englisch mit deutscher Zusammenfassung.

Die Wasseraufnahme, Keimfähigkeit und das Wachstum der Keimlinge wird durch Wasserstreß (hervorgerufen durch NaCl und Polyäthylenglykol 6000, 0—8 bar) vermindert, wobei letzteres stärker wirkt. Zugabe von Prolin vermag die Wirkung des osmotischen Streß auf Keimung und Keimlingswachstum deutlich zu mindern.

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Introduction

Numerous investigations have been done to study the effect of osmotic stress on seed germination and plant growth. It was generally found that, as osmotic stress increased the ability of the seeds to germinate was reduced (MAYER & POLJAKOFF-MAYBER 1963, WILLIAMS & UNGAR 1972, SHANNON & FRANCOIS 1977, AKESON *et al.* 1980 and STOUT *et al.* 1980). Also, some investigators (HUTTON 1971, POLJAKOFF-MAYBER & GALE, 1975 and HEIKAL *et al.* 1980) found that growth of some plants was reduced under the effect of osmotic stress.

In addition, some other authors recorded, an accumulation of the amino acid proline in plants exposed to osmotic stress (STEWART *et al.* 1966, BARNARD & OAKS 1970; STEWART & LEE 1974, TALL *et al.* 1979 and CHAUHAN *et al.* 1980). Also, SINGH *et al.* (1973) mentioned that resistant varieties of barley, exposed to osmotic stress accumulated more proline than non-resistant ones and addition of exogenous proline helped the non-resistant varieties to overcome the stress.

In the view of the role played by proline in counteracting the inhibitory effect of osmotic stress on some plants, the present work was, therefore, undertaken to describe studies on the interaction effect between osmotic stress and proline on seed germination and seedling growth of the economic plants cotton, pea and wheat.

Materials and Methods

The effect of osmotic stress on seed germination and seedling growth of cotton (*Gossypium barbadense* var. Dandara), pea (*Pisum sativum* var. Marvel) and wheat (*Triticum vulgare* var. Giza 156) plants was studied. The following osmotic stresses were used: 2, 4, 6 and 8 bars by dissolving NaCl and/or polyethylene glycol 6000 (PEG) in $\frac{1}{10}$ N HOAGLAND'S solution. Seeds of the control group were germinated using only $\frac{1}{10}$ N HOAGLAND'S solution as substrate.

Seed germination: Twenty-five seeds from the tested species were pretreated with 10% clorox (5.25% sodium hypochlorite) for 4 min. and then were germinated in petri dishes at about 25°C and darkness as described by MAFTOUN & SEPASKHAH (1978). Four replicates petri dishes were prepared from each treatment. Seeds were considered to be germinated after the radicle emerged from the testa.

Another experiment was carried out simultaneously to study the interaction effect between proline and osmotic stress on seed germination. Seeds of the tested species were treated in the same manner using appropriate NaCl or PEG solutions to which the amino acid proline (100 µM/l) was added.

Water absorption: Twenty-five seeds were weighed and treated as in the foregoing parts. After various time intervals seeds were blotted dry

with paper tissues and weighed. The water absorption values were expressed in grams of solution absorbed per gram of dry seeds.

Seedling development: Twenty-five seeds were placed between folded paper towels, covered by a plastic wrap, rolled up, and placed upright

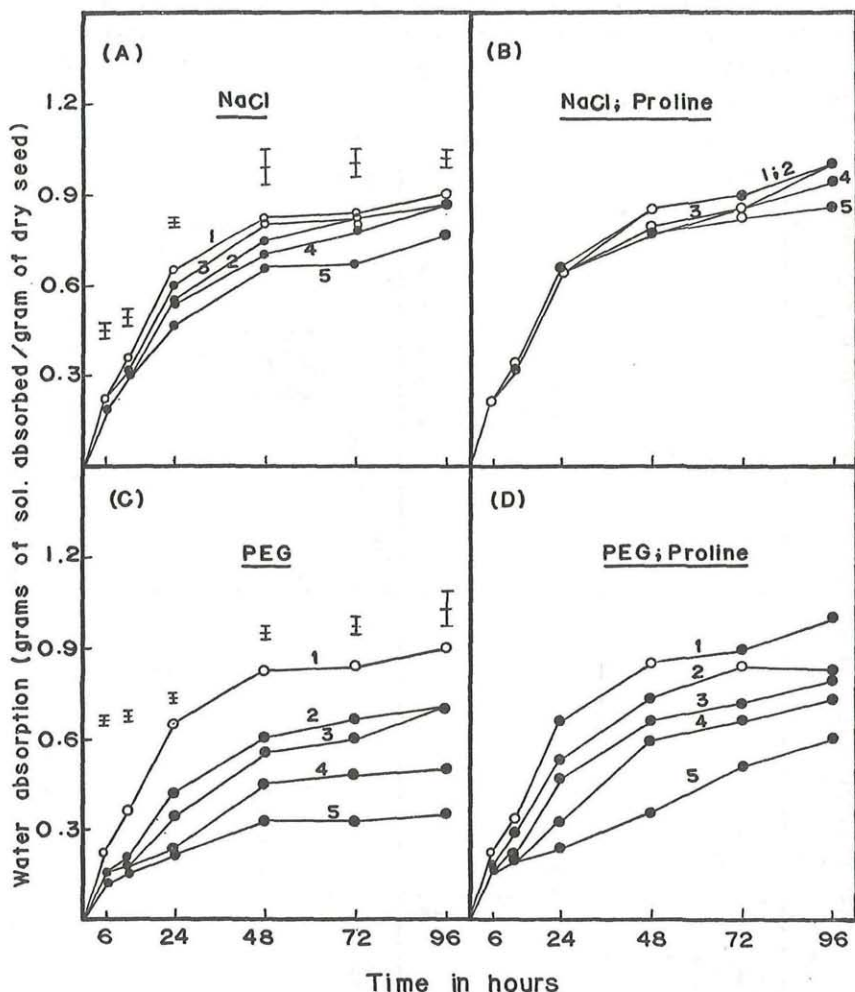


Fig. 1. Effect of proline on average water absorption by cotton seeds at different osmotic stresses induced both by NaCl (A, B) and PEG 6000 (C, D). — Treatments: 1 = control, 2 = 2 bars, 3 = 4 bars, 4 = 6 bars, 5 = 8 bars. Signatures: closed circles mean significant differences between the values indicated and proline untreated control ($P \leq 1\%$). Values marked by half closed circles mean nonsignificant differences as compared with the same control. The least significant differences (L. S. D., $P = 1\%$) are indicated on diagram A and C

in 600 ml beakers. Eighty milliliters of solution were used to saturate the towles in each of the treatments. Seeds were treated in the same manner as described for the germination experiment. Beakers were placed in an incubator at about 25° C and darkness for 10 days. Distilled water was added as needed to compensate for evaporation loss. At the end of the experiment, length of hypocotyls and roots in case of cotton and pea, length of shoots and primary roots in case of wheat were recorded.

Data of all experiments were subjected to analysis of least significant difference test (LSD).

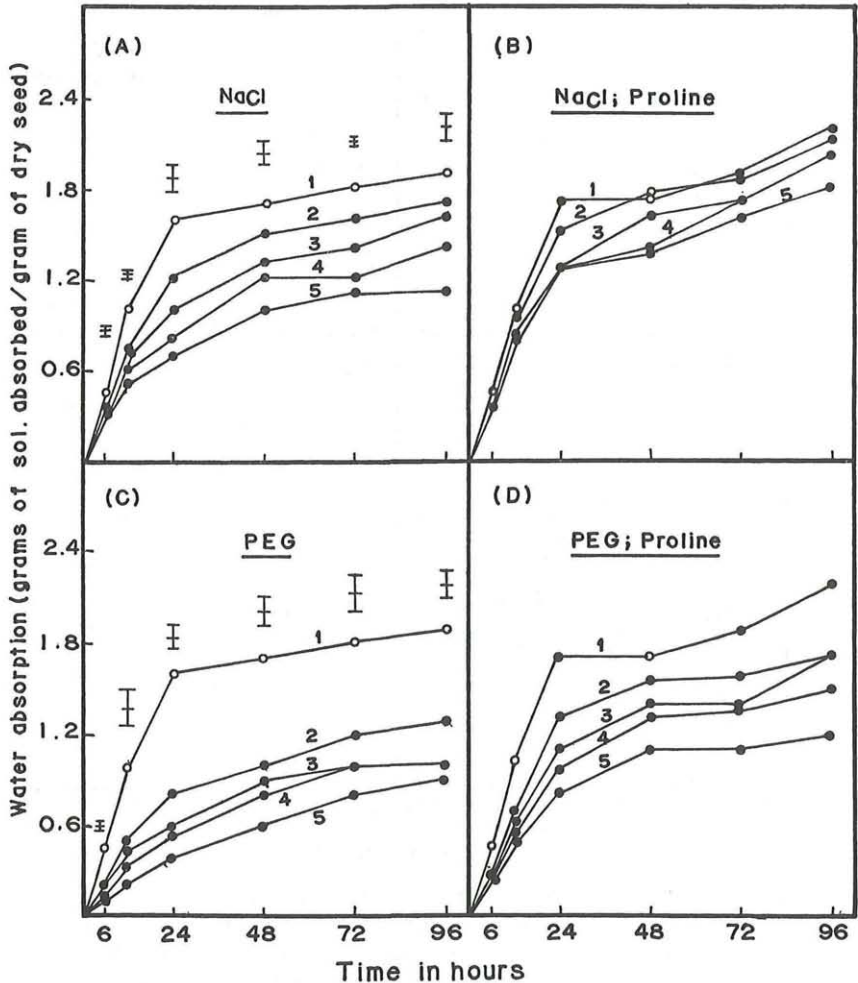


Fig. 2. Effect of proline on average water absorption by pea seeds at different osmotic stresses induced both by NaCl (A, B) and PEG 6000 solutions (C, D). Treatments and signatures see Fig. 1

Results

Water absorption: Generally the average amount of water absorbed (g/g seed dry weight) was significantly decreased with the increase in osmotic stress of the substrate (Fig. 1A, 1C, 2A, 2C, 3A, 3C). Comparison between the effect of NaCl and PEG at iso-osmotic concentrations indicate that the latter had the more inhibitory effect.

Addition of exogenous proline to the substrates increased the average water absorption by the tested seeds at all levels of osmotic stress (Fig. 1B, 1D, 2B, 2D, 3B, 3D).

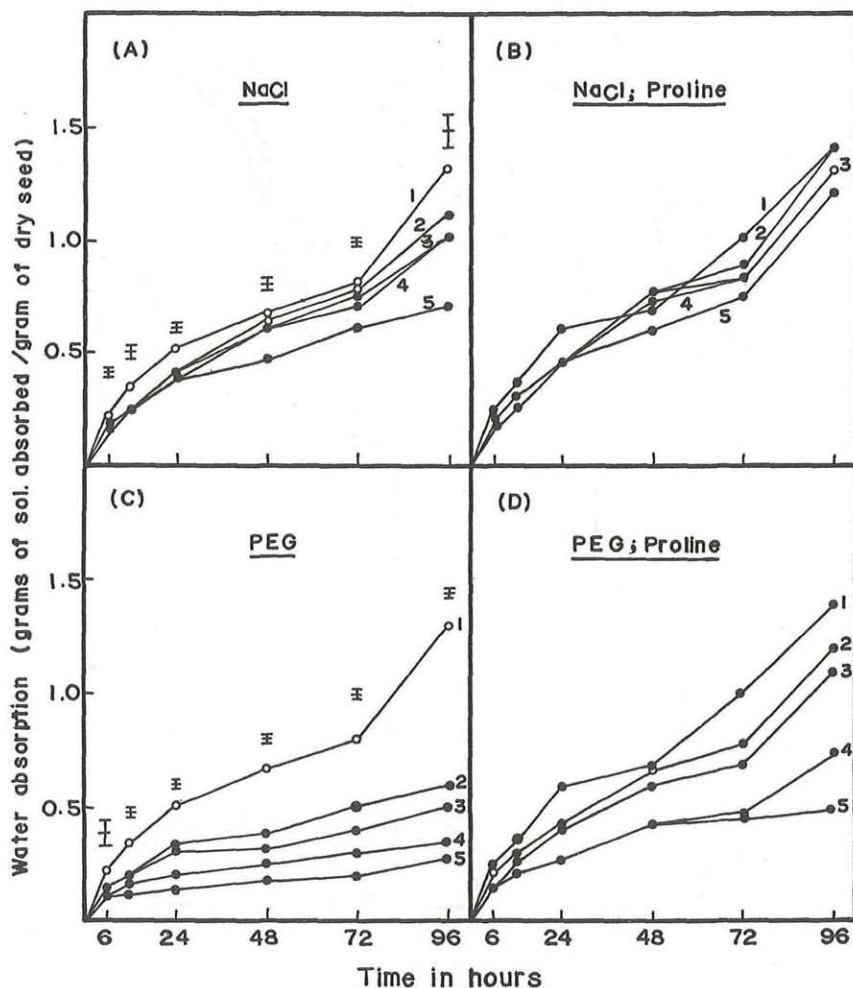


Fig. 3. Effect of proline on the average water absorption by wheat seeds at different osmotic stresses induced both by NaCl (A, B) and PEG 6000 solutions (C, D). Treatments and signatures see Fig. 1

Germination: Using either NaCl or PEG, the germination rates and final germination percentages of cotton, pea and wheat seeds were considerably reduced with the increase in osmotic stress levels (Fig. 4A, 4C, 5A, 5C, 6A, 6C). It can be noticed that the inhibitory effect of PEG on the germination was much more obvious than that of NaCl. Moreover, germin-

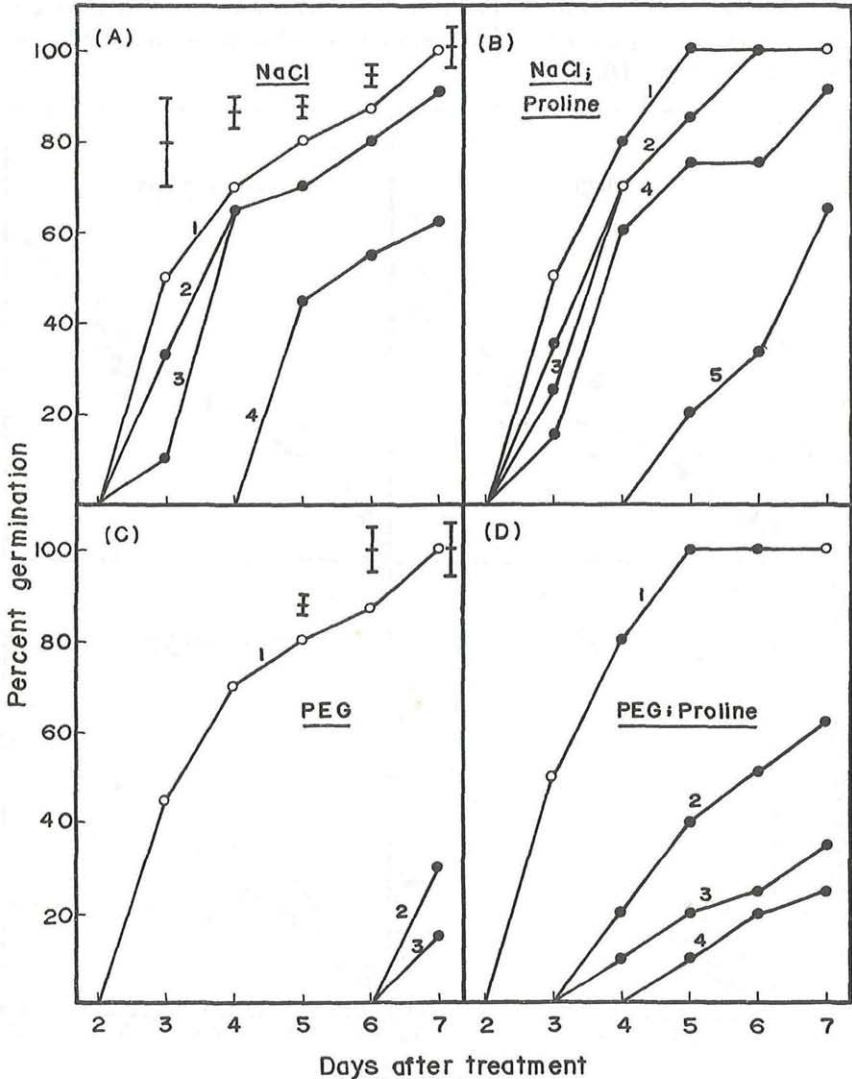


Fig. 4. Effect of proline on average germination of cotton seeds under NaCl (A, B) and/or PEG 6000 (C, D) induced osmotic stress. Treatments and signatures see Fig. 1

ation of cotton and Pea seeds was completely inhibited at 6 and 8 bars PEG and that of wheat seeds at the level 8 bars only.

Addition of exogenous proline increased the germination rates and final germination percentages of the osmotically stressed seeds (Fig. 4B, 4D, 5B, 5D, 6B, 6D, 7B, 7D). In addition proline stimulated germination of cotton and wheat seeds stressed with 8 bars of NaCl and PEG respectively.

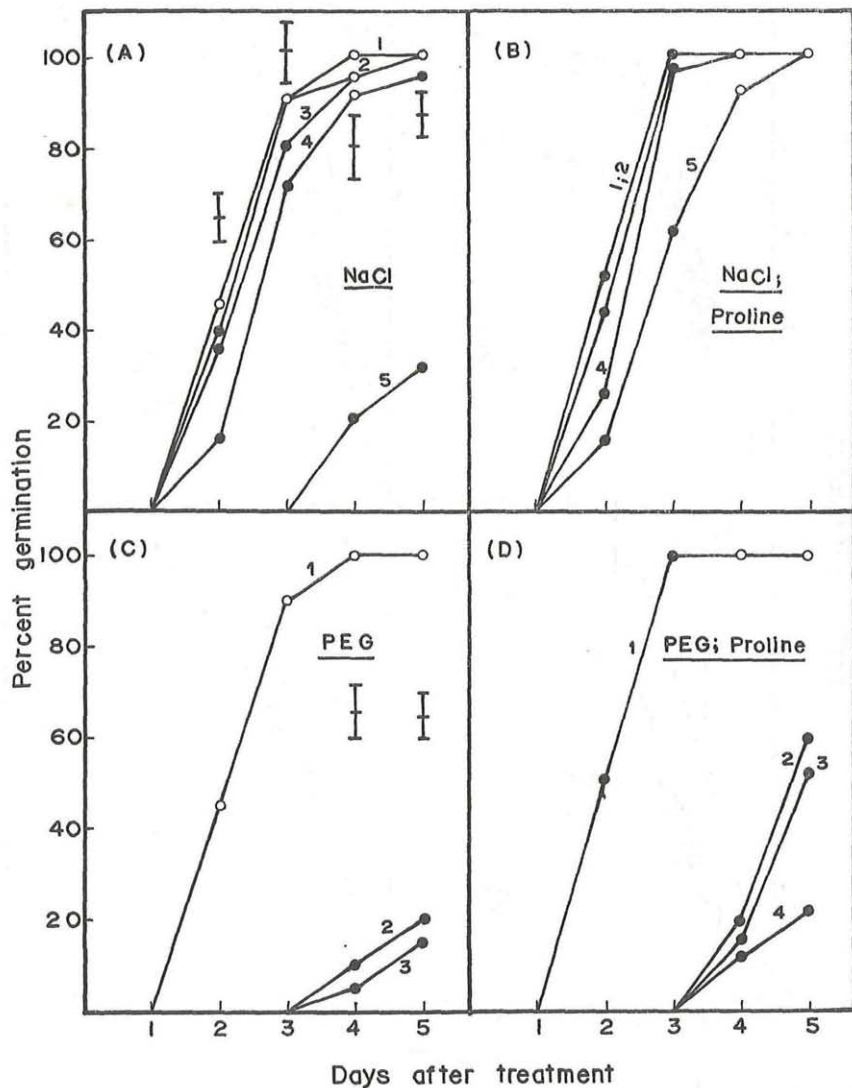


Fig. 5. Effect of proline on average germination of pea seeds under NaCl (A, B) or PEG 6000 (C, D) induced osmotic stress. Treatments and signatures see Fig. 1

In case of cotton and pea seeds stressed with 6 bars PEG, germination was excited in the presence of proline, on the otherhand, proline did not interfere with the inhibitory effect of the level 8 bars PEG.

Seedling development: After 10 days from soaking, measurements of root and hypocotyl length in case of cotton and pea and primary root and shoot in case of wheat were recorded at all the used treatments (Fig. 8).

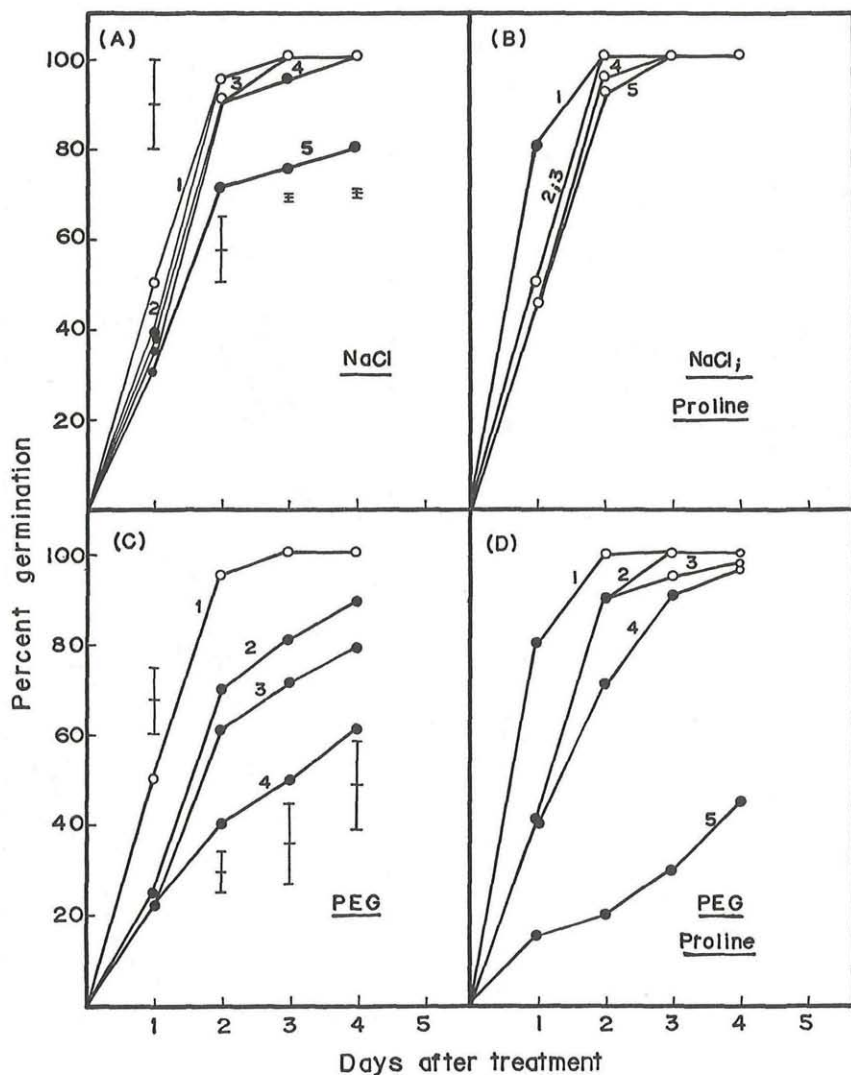


Fig. 6. Effect of proline on average germination of wheat seeds under NaCl (A, B) or PEG 6000 (C, D) induced osmotic stress. Treatments and signatures see Fig. 1

Seedling growth of the tested species was significantly decreased at all the concentrations of both NaCl and PEG (Fig. 8A, C, E and G), except at the level 2 bars NaCl, where the reduction was nonsignificant. When comparison between the effect of NaCl and PEG at iso-osmotic concentrations were made, the latter was more inhibitory than the former.

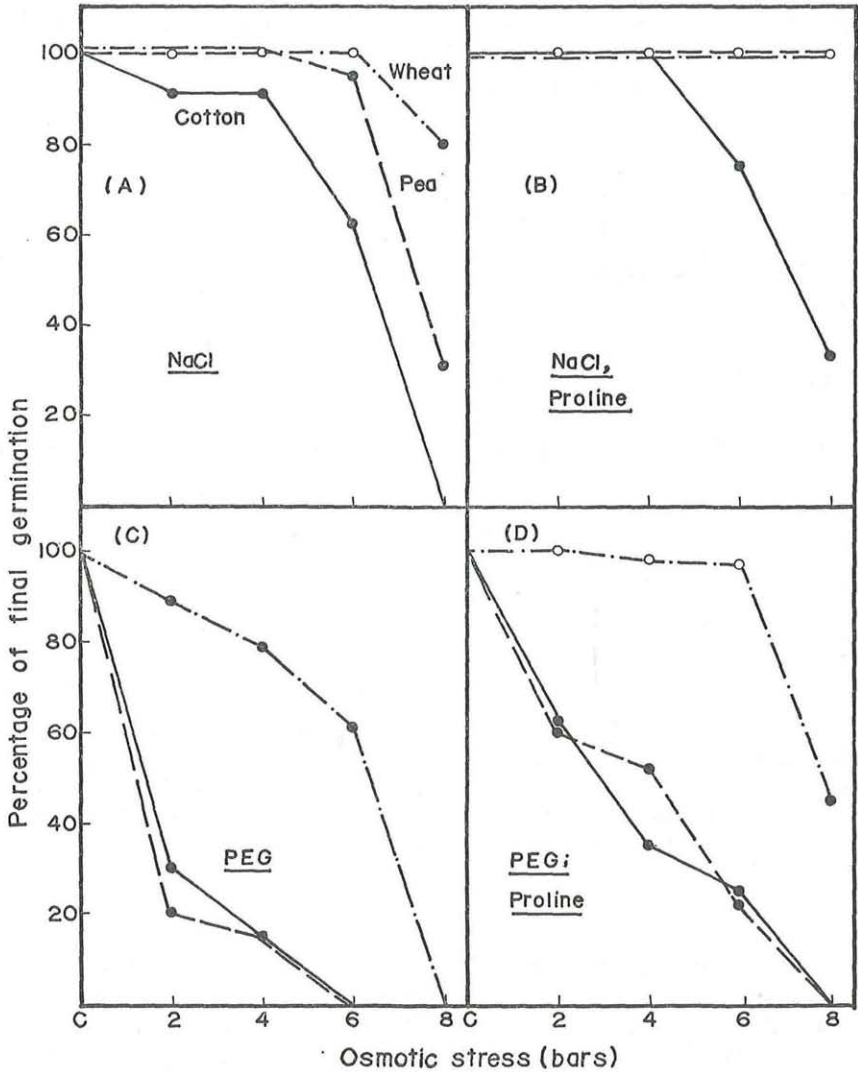


Fig. 7. Effect of proline on final germination of seed of cotton (—————), pea (-----), and wheat grains (-.-.-.-) at different osmotic stresses induced by NaCl (A, B) or PEG 6000 (C, D) solutions. C = control treatment

Proline was significantly induced an increase in seedling growth of the tested species at all the used treatments (Fig. 8B, D, F and H). This increase was more pronounced under NaCl than under PEG stress. However, the increase in seedling growth of the control treatment, induced by proline was nonsignificant, except in case of wheat root and pea hypocotyl, where the increase was significant. Moreover, proline not only overcome the inhibitory effect of NaCl on seedling growth of the three species but also induced a significant increase in their growth as compared with the untreated proline control treatment.

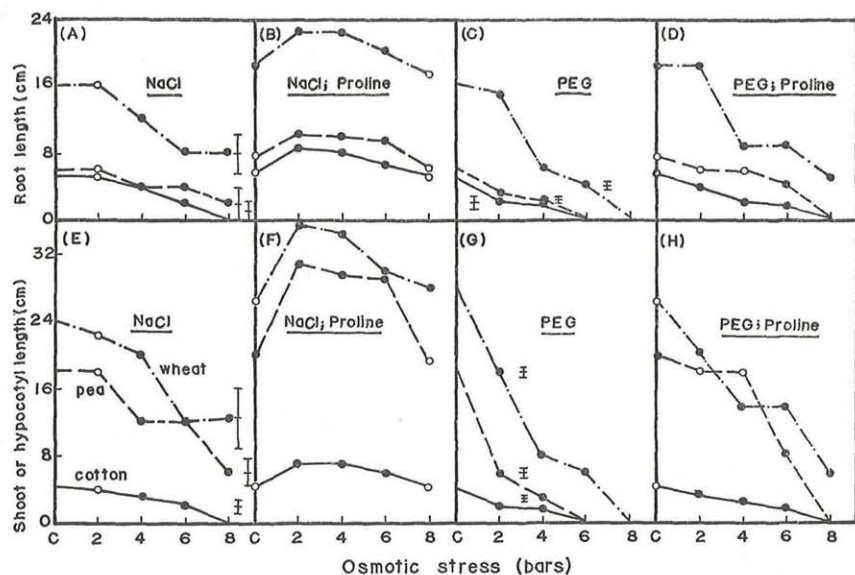


Fig. 8. Effect of proline on the length of root and hypocotyl of seedlings of cotton (—) and pea (---) and on the length of primary root and shoot of wheat seedlings (-.-.-) at different osmotic stresses induced both by NaCl (A, B, E, F) and PEG 6000 (C, D, G, H) solutions at the 10th day from planting. C = control treatment

Discussion

The inhibitory effect of increasing osmotic stress on the rate of water absorption by the used seeds was also observed by UHVITS 1946, STROGOV 1964, PRISCO & O'LEARY 1970. The difference in H₂O uptake by the tested seeds soaked in NaCl or PEG solutions at the iso-osmotic concentrations probably is the result of NaCl being a permeating solute.

The inhibitory effect of osmotic stress on germination rate and final germination was recorded by PARMAR & MOORE 1968; KAUFMANN & ROSS 1970, SIONIT *et al.* 1973, HADAS 1976 and SIEGEL *et al.* 1980. The decrease in

germination rate can be correlated with the decrease in the rate of water absorption. This observation is in agreement with the results obtained by STROGOV 1964, PRISCO & O'LEARY 1970 and HEIKAL & SHADDAD 1981. The higher values for germination in NaCl than in PEG at iso-osmotic concentrations were the result of the greater water absorption by the seeds in the former substrate (PRISCO & O'LEARY 1970).

The reduction in seedling growth with increasing osmotic stress is generally in accordance with the results obtained by PARMAR & MOORE 1968, ODEGBARO & SMITH 1969, PRISCO & O'LEARY 1970, WILLIAMS & UNGAR 1972 and DARRA *et al.* 1973.

The recorded stimulation in germination and seedling growth of the tested species, which induced by the interaction effect between proline and osmotic stress is in accordance with the results obtained by BAR-NUN & POLJAKOFF-MAYBER 1977 working with pea. In addition, our data showed that, proline was generally alleviate the inhibitory effect of osmotic stress induced by NaCl or by PEG on the studied parameters (average water uptake, germination and seedling growth). In other words the addition of exogenous proline helped seeds of the tested speices which are non-resistant to osmotic stress to overcome the effect of osmotic stress. This observation is in agreement with that observed by SINGH *et al.* 1973 working with different varieties of barley.

In view of the role played by proline in counteracting the effect of osmotic stress, it was suggested that proline may be the major source of energy and nitrogen during post-stress metabolism (STEWART *et al.* 1966) or it acts as an osmotic regulator during osmotic stress (STEWART & LEE 1974). Also, PALFI & JUHASZ 1970 suggested that proline is not directly involved in osmotic adjustments but it plays indirect role in osmoregulation in the plants by increasing hydration of the protoplasm. On the other hand, BAR-NUN & POLJAKOFF-MAYBER 1977 suggested that proline has some additional metabolic effect which is not yet understood.

From data recorded in this work and literature surveyed, it is obvious that the role of proline in counteracting the effect of asmotic stress on some plants is more complicated and still an open question which needs further investigations.

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