Research Article

Seed priming enhanced seed germination traits of wheat under water, salt and heat stress

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Abstract

Abiotic or physicochemical stresses affect plant's internal mechanisms as well as external morphology. Seed priming can be an effective tool to minimize effects of the abiotic stress on plant growth and wheat seed priming used as stress tolerance mechanism. The study was conducted to evaluate effects of seed priming on germination of two wheat varieties under drought, high salt levels and high temperature stress. Hydro priming (distilled water), Osmopriming (5milli molar KNO₃, 10 mM KNO₃) and halo priming (5 mM NaCl, 10 mM NaCl) were used. Under drought and heat stress, hydro priming resulted in the highest germination index to increase up to 70 and 65 percent as compared to stressed seeds while under salt stress, 5 mM KNO₃ priming showed highest germination index up to 75 percent. Under salt stress, 5mM KNO₃ priming resulted in highest seedling vigor index with 86% increase as compared to stressed seeds. Under water and heat stress, hydro primed seeds showed highest percentage i.e. 50% and 45% increase in germination stress tolerance index as compared to stressed seeds. Under salinity stress, 5mM KNO₃ primed showed 51% increase in germination tolerance index as compared to stressed seeds. Seed priming was proved as an effective technique to increase abiotic stress tolerance.

Key words: Wheat seed priming; Abiotic stress; Germination.

Introduction

Abiotic stress causes a decline in crop growth and productivity. Drought stress causes loss of water use efficacy, induction of heat stress and reduces stem extension. Biological macromolecules are damaged due to the production of free ions [1]. It was found that salinity stress resulted in a decrease of water content, accumulation of hydrogen peroxide and electrolyte release in wheat seedlings [2, 3]. Enzyme deactivation and change in permeability of membrane are

the effects of heat stress [4]. For high yield in annual crops, even and fast field emergence is the primary requirement [5]. For this purpose, seed priming is a suitable technique. Seed priming is a pre-sowing seed treatment. It is partial hydration of seeds performed in controlled conditions while the emergence of radical is avoided by drying before sowing [6]. Hydro priming is immersing of seeds using distilled water. Halo priming is soaking of seeds using

different salt solutions. Osmopriming is immersing of seeds in different solutions of osmotic concentrations [7]. Seed priming is proved to be a cost effective strategy which is very helpful to increase growth attributes of cereal crops. Soaking of seeds before sowing can enhance the rate of growth and seedling establishment [8, 9]. Keeping above literature in view, the present work was planned to answer the question whether seed priming is effective treatment and which seed priming technique is most effective in relation to respective stress conditions.

Materials and methods

The research was carried out in the Plant physiology lab, Department of Botany at the Arid Agriculture University to study the role of wheat seed priming in relation to abiotic stress tolerance. Two varieties of wheat i.e. NARC- 2009 and NARC- 2011 were sown. Seed surface sterilization was done [10]. Hydro priming was carried out by soaking seeds in distilled water for 24 hours under lab conditions. Redrying of seeds was done under air after soaking [11]. Osmo priming was carried out by soaking seed in an osmotic solution, i.e. Potassium Nitrate (KNO_3) for 24 hours under lab conditions. Two concentrations of KNO_3 i.e. 5mM and 10mM were used. Seeds were washed with distilled water and re-dried their original weight [12]. Halo priming was carried out by soaking seeds in sodium chloride (NaCl) solution for 24 hours under lab conditions. Two concentrations of NaCl i.e. 5mM and 10mM were used. Seeds were washed with distilled water and re-dried to their original weight [13]. Seeds were sown on a moist layer of filter paper in petri plates to monitor germination daily until all seeds were germinated. 5mm radical length was taken as criteria to consider seed germinated [14]. Control seeds were irrigated with distilled water. Germination analysis was done on the basis of following parameters.

Germination percentage

Germination percentage of each treatment was calculated using following formula

$$\text{Germination \% age} = \frac{\text{Total seeds germinated} \times 100}{\text{Total no of seeds planted}}$$

Germination index (G.I.)

Germination index was calculated using following formula [15].

$$\text{Germination Index} = n/d$$

Where n= no of seedlings emerged on day 'd'. d= days after planting.

Promptness index (P.I.)

Promptness index (P.I) was determined using following equation

$$\text{Promptness index (P .I)} = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.50) + nd_8 (0.25); \text{ where } nd_2, nd_4, nd_6, nd_8 \text{ are number of emerging seedlings on day 2, 4, 6, 8 [16].}$$

Seedling vigor index (S.V.I.)

Seedling vigor index was calculated using this equation [17].

$$\text{S. V.I Index} = \text{Seedling length (cm)} \times \text{germination \% age}$$

Germination stress tolerance index (G.S.T.I.)

Germination was studied under stress conditions. For salt stressed treatments, seeds were irrigated with sodium chloride solution (50mM) for 1 day. For drought stressed treatments, irrigation was hold for 1 day. For heat stressed treatments, seeds were treated with high temperature 35 degree Celsius in incubator for 3 min. Germination stress tolerance index (G.S.T.I) was calculated using following equation [16]

$$\text{Germination stress tolerance index (G.S.T.I)} = \frac{\text{Promptness index of stressed seeds}}{\text{Promptness index of control seeds}} \times 100.$$

Results

Germination analysis

Germination Percentage:

Decrease in germination percentage (Fig.1) was observed in seeds exposed to drought, salt and heat stress as compared to control seeds. Under normal irrigation, 5mM KNO_3

was prominently effective among priming treatments with 42% increase as compare to control. Under drought conditions, hydro priming was most effective with 48% increase followed by 5mM KNO₃ with 32%, 10mM KNO₃ with 25%, 5mM NaCl with 12% and 10mM NaCl with 6% increase in germination percentage as compared to drought stressed seeds. Under salt stress, 5mM KNO₃ priming resulted in highest germination percentage with 26% increase followed by 10mM KNO₃ with 11%, hydro priming with 8%, 5mM NaCl with 5% and 10mM NaCl with 4% increase in

germination percentage as compared to salt stressed seeds. Under heat stress, hydro priming was most effective with 33% increase followed by 5mM KNO₃ with 25%, 10mM KNO₃ with 18%, 5mM NaCl with 11% and 10mM NaCl with 7% increase in germination percentage as compared to heat stressed seeds. When subjected to ANOVA, it was found that effect of seed priming remained significant ($p \leq 0.05$). Germination percentage of NARC-2009 variety was higher than NARC- 2011 variety on average.

Fig. 1. Effect of Hydro priming, Osmo priming (5mM, 10mM KNO₃) and halo priming (5mM, 10mM NaCl) on germination percentage of two wheat varieties under drought, salt and heat stress.

T₀= well watered and un primed, T₁= hydro primed and well watered, T₂= Osmo primed (5mM KNO₃) and well watered, T₃= Osmo primed (10mM KNO₃) and well watered, T₄= halo primed (5mM NaCl)and well watered, T₅= Halo primed (10mM NaCl) and well watered, T₆= drought stressed, T₇= salt stressed, T₈= heat stressed, T₉= hydro primed and drought exposed, T₁₀= Osmo primed (5mM KNO₃) and drought exposed, T₁₁= Osmoprimed 10mM KNO₃ and drought exposed, T₁₂= halo primed (5mM NaCl)and drought exposed, T₁₃= Halo primed (10mM NaCl) and drought exposed, , T₁₄= hydro primed and salt exposed, T₁₅= Osmo primed (5mM KNO₃)

and salt exposed, T₁₆= Osmo primed 10mM KNO₃ and salt exposed, T₁₇= halo primed (5mM NaCl)and salt exposed, T₁₈= Halo primed (10mM NaCl) and salt exposed, , T₁₉= hydro primed and heat exposed, T₂₀= Osmo primed (5mM KNO₃) and heat exposed, T₂₁= Osmoprimed 10mM KNO₃ and heat exposed, T₂₂= halo primed (5mM NaCl)and heat exposed, T₂₃= Haloprimed (10mM NaCl) and heat exposed. V₁= NARC-2009 and V₂= NARC-2011

Germination Index:

Germination index (Fig. 2) was drastically reduced under drought, salt and heat stress as compare to control. Under well watered

conditions, 5mM KNO₃ was most effective with 36 % increase as compared to control. Under drought stress, hydro priming showed 70 % increase in germination index followed by 5mM KNO₃ with 61%, 10mM KNO₃ with 45%, 5mM NaCl with 24% and 10mM NaCl with 6 % increase in germination index as compared to drought stressed seeds. Under salt stress, 5mM KNO₃ priming resulted in highest germination index with 75% increase followed by 10mM KNO₃

with 69%, hydro priming with 58%, 5mM NaCl with 20% and 10mM NaCl with 9% increase in germination index as compared to stressed seeds. Under heat stress, hydro priming was most effective with 65% increase followed by 5mM KNO₃ 75%, 10mM KNO₃ with 71%, 5mM NaCl with 23% and 10mM NaCl 7% increase in germination index as compared to stressed seeds. Results showed ($p \leq 0.05$) when subjected to statistical analysis.

Fig. 2. Effect of Hydro priming, Osmo priming (5mM, 10mM KNO₃) and halo priming (5mM, 10mM NaCl) on germination index of two wheat varieties under drought, salt and heat stress.

T0= well watered and un primed, T1= hydro primed and well watered, T2= Osmo primed (5mM KNO₃) and well watered, T3= Osmo primed (10mM KNO₃) and well watered, T4= halo primed (5mM NaCl)and well watered, T5= Halo primed (10mM NaCl) and well watered, T6= drought stressed, T7= salt stressed, T8= heat stressed, T9= hydro primed and drought exposed, T10=

Osmo primed (5mM KNO₃) and drought exposed, T11= Osmo primed 10mM KNO₃ and drought exposed, T12= halo primed (5mM NaCl)and drought exposed, T13= Halo primed (10mM NaCl) and drought exposed, , T14= hydro primed and salt exposed, T15= Osmo primed (5mM KNO₃) and salt exposed, T16= Osmo primed 10mM KNO₃ and salt exposed, T17= halo primed (5mM NaCl)and salt exposed, T18= Halo primed (10mM NaCl) and salt

exposed, T19= hydro primed and heat exposed, T20= Osmo primed (5mM KNO₃) and heat exposed, T21= Osmo primed 10mM KNO₃ and heat exposed, T22= halo primed (5mM NaCl)and heat exposed, T23= Halo primed (10 mM NaCl) and heat exposed. V1= NARC-2009 and V2= NARC-2011

Promptness Index:

Promptness index (Fig. 3) was reduced under drought, salt and heat stressed seeds. Under well watered conditions, 5mM KNO₃ resulted in highest promptness index with 31% increase as compared to control. Under water stress, hydro primed seeds showed 80% increase in promptness index followed by 5mM KNO₃ with 75%, 10mM KNO₃ with 69%, 5mM NaCl with 13% and 10mM NaCl with 4% increase as compared to drought stressed seeds. Under salinity stress, 5mM KNO₃ primed showed.

Fig. 3. Effect of Hydro priming, Osmo priming (5mM, 10mM KNO₃) and halo priming (5mM, 10mM NaCl) on promptness index of two wheat varieties under drought, salt and heat stress.

T₀= well watered and un primed, T₁= hydro primed and well watered, T₂= Osmo primed (5mM KNO₃) and well watered, T₃= Osmo primed (10mM KNO₃) and well watered, T₄= halo primed (5mM NaCl)and well watered, T₅= Halo primed (10mM NaCl) and well watered, T₆= drought stressed, T₇= salt stressed, T₈= heat stressed, T₉= hydro primed and drought exposed, T₁₀= Osmo primed (5mM KNO₃) and drought exposed, T₁₁= Osmo primed 10mM KNO₃ and drought exposed, T₁₂= halo primed (5mM NaCl)and drought exposed, T₁₃= Halo primed (10mM NaCl) and drought exposed, , T₁₄= hydro primed and salt exposed, T₁₅= Osmo primed (5mM KNO₃) and salt exposed, T₁₆= Osmo primed 10mM KNO₃ and salt exposed, T₁₇= halo primed (5mM NaCl)and salt exposed, T₁₈= Halo primed (10mM NaCl) and salt exposed, , T₁₉= hydro primed and heat exposed, T₂₀= Osmo primed (5mM KNO₃) and heat exposed, T₂₁= Osmo primed 10mM KNO₃ and heat exposed, T₂₂= halo primed (5mM NaCl)and heat exposed, T₂₃= Halo primed (10 mM NaCl) and heat exposed. V₁= NARC-2009 and V₂= NARC-2011

89% increase in promptness index followed by 10mM KNO₃ with 85%, hydro priming with 46%, 5mM NaCl with 15% and 10mM

NaCl with 6% increase as compared to stressed seeds. Under heat stress, hydro priming was most effective with 75% increase followed by 5mM KNO₃ with 60%, 10mM KNO₃ with 55%, 5mM NaCl with 12% and 10mM NaCl 2% increase in promptness index as compared to stressed seeds. Variance analysis of promptness index showed significant results ($p \leq 0.05$) in both the varieties.

Seedling Vigor Index:

A very pronounced effect of seed priming was seen on seedling vigor index. Seed priming was effective under well watered conditions as well as stress conditions. 5mM KNO₃ was prominently most effective under well water conditions and caused a 49% increase as compare to control (Fig.4). Under drought stress, hydro priming showed 79% increase in seedling vigor index followed by 5mM KNO₃ with 67%, 10mM KNO₃ with 41%, 5mM NaCl with 11% and 10mM NaCl with 5% increase in seedling vigor index as compared to stressed seeds. Under salt stress, 5mM KNO₃ priming resulted in highest seedling vigor index with 86% increase followed by 10mM KNO₃ with 64%, hydro priming with 41%, 5mM NaCl with 8% and 10mM NaCl with 5% increase in seedling vigor index as compared to stressed seeds. Under heat stress, hydro

priming was most effective with 70% increase followed by 5mM KNO₃ 57%, 10mM KNO₃ 17 %, 5mM NaCl with 4% and 10mM NaCl 2% increase in seedling vigor

index as compared to stressed seeds. Results were significant ($p \leq 0.05$) when subjected to ANOVA. Significant difference was observed among both varieties.

Fig. 4. Effect of Hydro priming, Osmo priming (5mM, 10mM KNO₃) and halo priming (5mM, 10mM NaCl) on Seedling vigor index of two wheat varieties under drought, salt and heat stress.

T0= well watered and un primed, T1= hydro primed and well watered, T2= Osmo primed (5mM KNO₃) and well watered, T3= Osmo primed (10mM KNO₃) and well watered, T4= halo primed (5mM NaCl)and well watered, T5= Halo primed (10mM NaCl) and well watered, T6= drought stressed, T7= salt stressed, T8= heat stressed, T9= hydro primed and drought exposed, T10= Osmo primed (5mM KNO₃) and drought exposed, T11= Osmo primed 10mM KNO₃ and drought exposed, T12= halo primed (5mM NaCl)and drought exposed, T13= Halo primed (10mM NaCl) and drought exposed, T14= hydro primed and salt exposed, T15= Osmo primed (5mM KNO₃) and salt exposed, T16= Osmo primed 10mM KNO₃ and salt exposed, T17= halo primed (5mM NaCl)and salt exposed, T18= Halo primed (10mM NaCl) and salt exposed, T19= hydro primed and heat exposed, T20= Osmo primed (5mM KNO₃) and heat exposed, T21= Osmo primed 10mM KNO₃ and heat exposed, T22= halo primed (5mM NaCl)and heat exposed, T23= Halo primed (10 mM NaCl) and heat exposed. V1= NARC-2009 and V2= NARC-2011

Germination Stress Tolerance Index:

Germination stress tolerance index (fig.5) was reduced under drought, salt and heat stressed seeds. Under water stress, hydro primed seeds showed 55% increase in germination stress tolerance index followed by 5mM KNO₃ with 37%, 10mM KNO₃ with 24%, 5mM NaCl with 10% and 10mM NaCl with 3% increase as compared to stressed seeds. Under salinity stress, 5mM KNO₃ primed showed 51% increase in germination tolerance index followed by 10mM KNO₃ with 42%, hydro priming with 31%, 5mM NaCl with 15% and 10mM NaCl with 5% increase as compared to stressed seeds. Under heat stress, hydro priming was most effective with 45% increase followed by 5mM KNO₃ 39%, 10mM KNO₃ with 30 %, 5mM NaCl with 11% and 10mM NaCl 2% increase in germination stress tolerance index as compared to stressed seeds. Variance analysis of germination stress tolerance index showed significant results ($p \leq 0.05$) in both the varieties NARC-2009 variety showed more tolerance towards stress conditions as compared to NARC-2011 variety.

Fig. 5. Effect of Hydro priming, Osmo priming (5mM, 10mM KNO_3) and halo priming (5mM, 10mM NaCl) on Germination stress tolerance index of two wheat varieties under drought, salt and heat stress.

T6= drought stressed, T7= salt stressed, T8= heat stressed, T9= hydro primed and drought exposed, T10= Osmo primed (5mM KNO_3) and drought exposed, T11= Osmo primed 10mM KNO_3 and drought exposed, T12= halo primed (5mM NaCl)and drought exposed, T13= Halo primed (10mM NaCl) and drought exposed, , T14= hydro primed and salt exposed, T15= Osmo primed (5mM KNO_3) and salt exposed, T16= Osmo primed 10mM KNO_3 and salt exposed, T17= halo primed (5mM NaCl)and salt exposed, T18= Halo primed (10mM NaCl) and salt exposed, , T19= hydro primed and heat exposed, T20= Osmo primed (5mM KNO_3) and heat exposed, T21= Osmo primed 10mM KNO_3 and heat exposed, T22= halo primed (5mM NaCl)and heat exposed, T23= Halo primed (10 mM NaCl) and heat exposed. V1= NARC-2009 and V2= NARC-2011

Discussion

On account of global environmental changes, drought, salinity and high temperature are important stresses that cause a limitation in crop establishment, growth and productivity. According to these situations, present work was planned to improve germination, morphology, physiology and biochemical attributes of crop by use of seed priming technology in relation to abiotic stress tolerance. Different

priming strategies were used in order to screen the most effective seed priming under different stress conditions. Even and fast germination are two important factors required for crop assemblage. Seed soaking technology is used to enhance the seed performance and even germination and improved seedling establishment in many plants [18]. Break down of seed dormancy is possible by priming and thus enhances rate of germination under different ranges of environmental changes [10].

During present work, it was found that germination and seedling growth was greatly influenced under stress conditions. Under drought stress, germination was decreased due to shortage of water required for early processes of germination. Salinity had a toxic effect on germinating seeds and excessive salt hinders seeds from water uptake during germination due to decreased solute potential. High range of temperature caused a reduction in seed germination rate and seedling growth because high temperature effects enzyme functions and initiates drought. These results agree with findings of [19]. In present study, it was found that hydro priming had a profound effect on seed germination, tolerance and seedling vigor index under drought and heat stress. This is due to the fact that hydropriming initiates many mechanisms

such as renovation and production of nucleic acids, protein, membrane repair and many biochemical processes in relation to enzyme activation. Even germination of hydroprimed seeds is probably due to improvement in water uptake, ATP synthesis and cell division. Primed seeds can better imbibe water than stressed seeds thus enhancing tolerance under stress conditions. Metabolism is also stimulated by priming which helps in early emergence of radical and plumule. Same were the findings of [20,21, 22] who previously found that hydro priming increased germination and seedling vigor of cotton and melon. Present study also revealed that Osmo priming was proved to be best treatment under saline conditions improving tolerance, germination properties and seedling vigor. This is due to effect of osmo priming on oxidative metabolism by increasing activity of anti oxidant enzymes such as peroxidase and superoxide dismutase. Osmopriming also stimulates ATPase function, RNA processing and water uptake by acting as a osmoticum. Results agree with the findings of [23, 24, 25]. The reason for increasing salinity tolerance in the plants grown from primed seeds is due to higher capacity of seeds adapted to osmotic conditions. KNO_3 priming was proved to be effective in decreasing salt effects of different varieties like chickpea, sunflower.

Authors' contributions

Conceived and designed the experiments: N Ilyas, Performed the experiments: SR Mirza, Analyzed the data: N Batool & N Ilyas, Contributed reagents/materials/analysis tools: N Ilyas, Wrote the paper: SR Mirza & N Batool.

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