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DOES SEED TREATMENT WITH GLYCINEBETAINE IMPROVE GERMINATION RATE AND SEEDLING GROWTH OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER OSMOTIC STRESS

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Abstract

Sunflower (*Helianthus annuus* L.) cv Suncross and Gulshan-98 achenes were germinated in Petri-plates containing filer papers moistened with solution of polyethylene glycol, PEG-8000 (control and osmotic potential -0.6, -1.2 MPa) with and without glycinebetain (GB) application (0, 25 and 50 mM). PEG treatment severely reduced germination percentage and fresh and dry biomass and mean germination time (days to 50% germination) in both sunflower cultivars. Addition of GB in growth medium was not effective in alleviating the adverse effects of osmotic stress on germination percentage, mean germination time and seedling dry biomass. In contrast, a slight increase in seedling fresh biomass was observed by the application of 25 mM GB under control and osmotic stress.

Keywords: Osmotic stress, polyethylene glycol, glycinebetaine, sunflower, germination rate, and seedling growth

Introduction

Seed sowing generally considered the first critical and most sensitive stage in the live cycle of plants and seeds are frequently exposed to unfavorable environmental conditions that may compromise the establishment of seedling (Figueiredo-e-Albuquerque & Carvalho 2003). Water stress not only affects seed germination but also increases mean germination time in crop plants (Willanborb *et al.*, 2004). Germination is regulated by duration of wetting and the amount of moisture in the growth medium (Schutz & Milberg 1997; Gill *et al.*, 2002). The adverse effects of water shortage on germination and seedling growth have been well reported in different crops sunflower (Lenzi *et al.*, 1995; Mohammad *et al.*, 2002).

The sunflower (*Helianthus annuus* L.), belonging to the family Asteraceae is the world's fourth largest oil seeds crop (Jasso de Rodriguez *et al.*, 2002). Being oil seed crop sunflower achene germination is particularly susceptible to water stress (Sajjan *et al.*, 1999). It was observed that osmotic stress upto -0.4 MPa had no significant effect on germination percentage in sunflower (El-Midaoui *et al.*, 2001). In another study, it was found that germination of sunflower is inhibited in presence of polyethylene glycol (PEG-6000), of osmotic pressure lower than –0.5 MPa (Smok *et al.*, 1993). Although, most of the researchers (Smok *et al.*, 1993; Ashraf & Naqvi, 1995; Hu & Jones, 2004) used PEG-6000 to create water deficit environment in growth chamber studies, the use of small molecular weight compounds does no prevent the risk of uptake of these solutes by plants from the growth medium. Hence, scientists have recently emphasized

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the use of higher molecular weight compounds such as PEG-8000 (Li *et al.*, 2003; Willamborb *et al.*, 2004) instead of lower molecular mass compounds in order to prevent the chances of uptake and translocation of these compounds by plants.

Plants have evolved various biochemical and physiological mechanisms to combat water stress (Sadeghian & Yavari 2004; Sakamoto & Murata 2002). One such mechanism that is ubiquitous in plants is the accumulation of certain organic metabolites of lower molecular weight especially during seed germination and early stages of growth (Bewley & Black, 1994; Bolarin *et al.*, 1995). Exogenous application of some of these compounds such as proline and glycinebetaine to enhance stress tolerance ability of different crop plants has got attention of many researcher since many years (Yancey, 1994, Mansoor, 1998; Rehman *et al.*, 2002) but the information regarding the role of exogenous glycinebetaine on germination and early seedling growth is scarce. Hence, the study was conducted to investigate the role of exogenously applied glycinebetaine in water stress tolerance of sunflower at germination and early seedling growth stages.

Materials and Methods

Two sunflower lines, namely, Gulshan-98 (local) and Sun cross (exotic) were obtained from regional office of Pakistan Seed Council, Faisalabad, Pakistan. The experiment was conducted in growth chamber of Department of Botany, University of Agriculture, Faisalabad, Paksitan. Three osmotic stress levels (control and osmotic potential, -0.6 and -1.2 MPa) and three glycine betaine levels (0, 25 and 50 mM) were applied to both lines of sunflower before seed dispessal. Polyethylene glycol (PEG-8000) 0, 17.5 and 34.5 g was dissolved in 100 cm^3 of half strength Hoagland's nutrient solution separately to prepare solutions of control, -0.6 and -1.2 MPa (osmotic potential was measured by Osmometer, VAPRO vapor pressure osmometer, Model 5520, USA) osmotic potential, respectively. The seeds were surface sterilized with 100 % sodium hypochlorite solution for five minutes and washed three times with distilled water. Twenty-five seeds were sown in each petri dish containing filter papers.

The solutions (5 cm^3) containing different concentrations of PEG-8000 and glycinebetaine were added separately in these petri plates. The experiment was laid out in split plot design with five replication for each experimental unit. Five cm^3 of appropriate treatment solution (PEG) was applied daily in each Petri dish after washing out the previous solution. Number of seeds germinated was counted daily and data were recorded for 14 days. A seed was considered as germinated when both plumule and radicle had emerged more than 5 mm. Total germination was expressed as percent of that in the control treatment for each line and then data were analyzed statistically. Rate of germination was determined on the basis of days to 50% germination. Fresh and dry biomass per seedling were recorded after 14 days of the start of the experiment.

The data collected were analyzed by analysis of variance technique. Duncan's New Multiple Range test at 5% level of probability was used to test the significance of means (Steel & Torrie, 1980).

Results

Germination percentage decreased significantly ($P \geq 0.001$) by increasing the concentration of external osmoticum (-0.6 and –1.2 MPa osmotic potential). Mean germination was maximum under the control (77 %) followed by the osmotic stress

treatment of –0.6 MPa (58 %) and –1.2 MPa (16 %). The two sunflower lines did not differ significantly regarding this variable. Addition of GB (0, 25 and 50 mM respectively) in the growth medium had no effects on germination percentage at all the osmotic stress levels (Fig. 1). Interactions among all the factors were also statistically non-significant with respect to germination percentage (Table 1).

Increasing the concentration of PEG-8000 in the growth medium had a highly significant (P≥0.001) adverse effect on mean germination time in sunflower lines. Under control, mean germination time was minimum (4 days) followed by –0.6 MPa (6 days) and –1.2 Mpa (10 days), respectively. Exogenous application of GB at all concentrations was not effective in alleviating the adverse effects of osmotic stress on this variable (Fig. 2). The two sunflower lines did not differ significantly regarding mean germination time (Table 1).

Fresh biomass per seedling of sunflower lines decreased significantly (P≥0.001) by osmotic stress treatments. Osmotic stress treatments of –1.2 and –0.6 MPa caused a 67 and 21% reduction in fresh biomass per seedling respectively, as compared with control. The two sunflower lines showed non significant differences with respect to seedlings fresh biomass production. Addition of GB in the growth medium had significant (P≥0.05) contribution in preventing the adverse effects of osmotic stress on fresh biomass/seedling (Table 1, Fig. 3). Maximum fresh biomass/seedling were observed under the application of 25 mM GB (272 mg) followed by 50 and 0 mM GB (265 and 256 mg respectively).

A marked adverse effect (P≥0.001) of PEG-8000 treatments on seedling dry biomass production was observed in two sunflower lines. The water stress treatment of –1.2 MPa produced 75% less dry biomass than control, whereas, a 28% reduction in this variable was observed due to water stress treatment of –0.6 MPa in respect of control. All the levels of GB had almost similar effects on dry biomass per seedling (Fig. 4). The two sunflower lines exhibited non-significant differences regarding this variable. Interactions among all factors were also statistically non-significant (Table 1).

Discussion

In general, a crop species or genotype within the species with better germination and seedling growth under water stress will be more stress tolerant at later stages and will

Table 1. Mean Square values from analysis of variance of germination percentage, mean germination time, fresh biomass seedling^{-1}, dry biomass seedling^{-1} of two sunflower lines subjected to water deficit and Glycinebetaine levels at different growth stages.

Source of Variation	d.f.	Germination percentage	Mean germination time	Fresh biomass seedling^{-1}	Dry biomass seedling^{-1}
Water deficit (D)	2	22948***	191.54^{NS}	391045***	20089***
Sunflower lines (S)	1	45^{NS}	0.056^{NS}	59^{NS}	10^{NS}
DxS	2	25^{Ns}	0.264^{NS}	16^{NS}	28^{NS}
GB level (L)	2	2^{NS}	0.042^{NS}	1466*	83^{NS}
DxL	4	5^{NS}	0.146^{NS}	741^{NS}	55^{NS}
SxL	2	20^{NS}	0.014^{NS}	114^{NS}	7^{NS}
DxSxL	4	4^{NS}	1.035^{NS}	170^{NS}	8^{NS}
Error	54	14	0.769	358	29

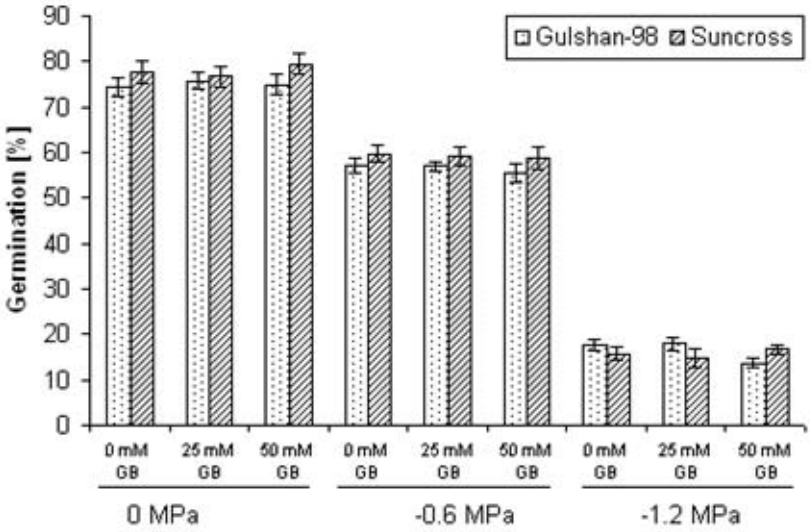

Fig.1: Germination percentage in two sunflower lines subjected to different levels of water stress and glycinebetain.

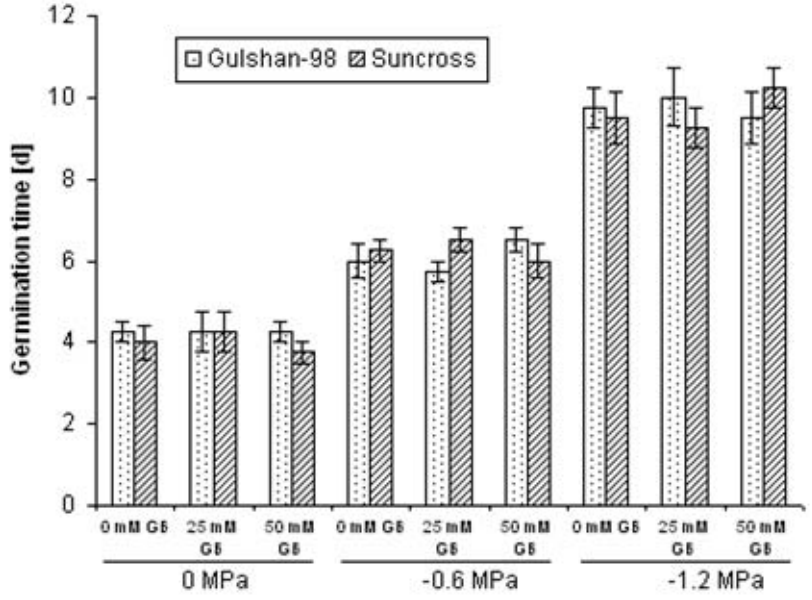

Fig. 2: Mean germination time in two sunflower lines subjected to different levels of water stress and glycinebetain.

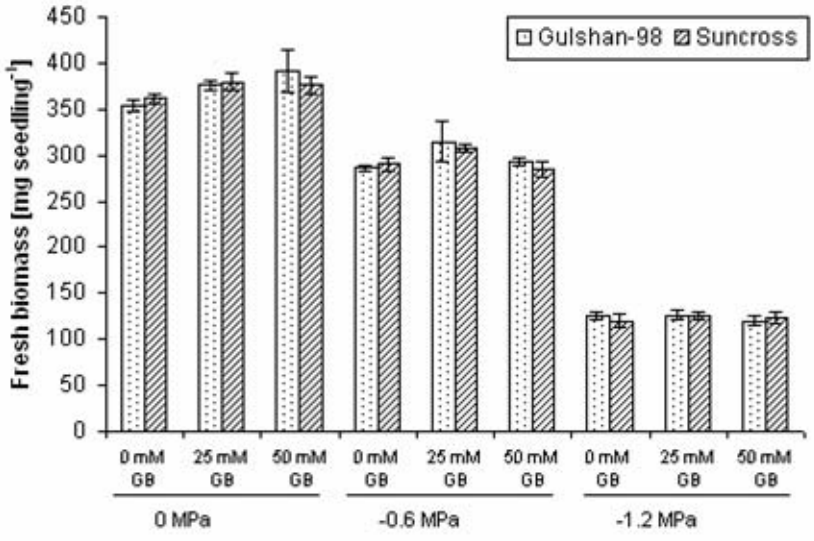

Fig.3: Fresh biomass/seedling in two sunflower lines subjected to different levels of water stress and glycinebetain.

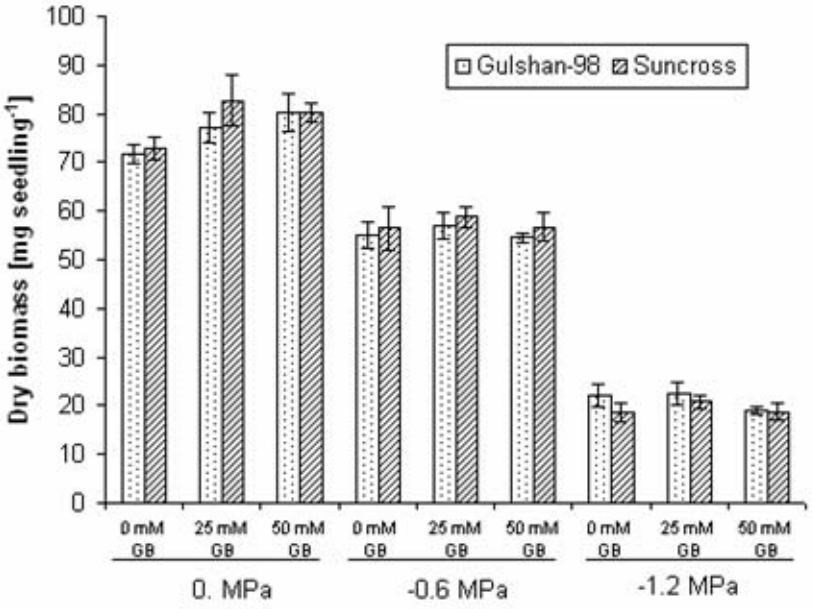

Fig.4: Dry biomass/seedling in two sunflower lines subjected to different levels of water stress and glycinebetain.

produce good crop stand and economic yield (Ashraf & Khan 1990; Ashraf & Naqvi 1995). On the other hand, some scientist were argued that seed germination capacity is not associated with stress tolerance ability but depends upon seed maturity (Shete *et al.,* 1992), storage conditions (Elemer, 1991), seed biochemical composition (Reuzean *et al.,* 1992), genetic variability (Sajjan *et al.,* 1999) and ecological conditions (Smok *et al.,* 1993). The present findings also support the later view, the two sunflower lines did not differ in respect of achene germination, although both these lines showed differential responses to water stress at later growth stages (unpublished data). In the present study since every effort was made to collect the seed of same age and size and provide identical environmental conditions to germinating achenes, it is felt that such precautions eliminated the effect of above mentioned factors on seed germination. El-Midaoui *et al.* (2001) reported that water stress treatments of -0.8, -0.01 and -1.2 MPa had almost similar effects on sunflower achene germination. In contrast, a marked adverse effect of water stress on sunflower achene germination during the present study was observed when PEG-8000 concentration was increased from -0.6 MPa water potential to -1.2 MPa. The differences in the results of the present investigation and as reported by El-Midaoui *et al.* (2001) may be due to differences in genotypes used for the study and the nature of growth conditions. The decrease in the germination percentage due to ascending concentration of PEG-8000 in the growth medium was due to less availability of water during imbibitions, because seed germination mostly depends upon this process (Ashraf & Naqvi 1995). Sajjan et al. (1999) also observed a gradual decrease in germination percentage of sunflower with increase in concentration of external osmotic. Addition of GB in the growth medium did not prevent the adverse effect of water stress on germination ability of sunflower lines. This indicates that exogenous GB had no significant role in water availability for imbibitions of germinating seeds during water limited conditions.

Exogenous application of GB also have no effects on mean germination time under normal and water stress conditions, though mean germination time increased with water stress treatments. The increase in germination time might be due to less availability of water under higher concentration of PEG-8000. The increase in germination duration due to water stress has been well reported in sunflower (El-Midaoui *et al.,* 2001) and in other crops (Ashraf *et al.,* 1990; Ashraf & Naqvi, 1995; Dhanda *et al.,* 2004).

It is now widely accepted that exogenous application of glyceinbetaine counteracts the inhibitory effects of water stress on growth and biomass production in different crops (Agboma *et al.,* 1997abc; Makela *et al.,* 1998a). In the present experiment, a marginal increase in fresh biomass per seedling was observed by the application of 25 mM glycinebetaine under control and under water stress treatment of -0.6 MPa. This means that exogenously applied glycinebetaine was absorbed by the developing seedling due to which it acquired ability to maintain a better water status under water stress and increased the fresh biomass. Addition of 50 mM glycinebetaine in the growth medium was not effective in alleviating the adverse effects of water stress on fresh biomass per seedling. There are some other reports showing a higher plant water content and slower decrease in leaf water potential of water stressed plants by the application of glycinebetaine in other crop plants (WeiBing & Rajashakar 1999). Exogenous application of glycinebetaine, however, did not mitigate the inhibitory effect of water stress on seedling dry matter production. Makela *et al.* (1998b) also

reported non-significant effects of exogenous glycinebetaine in alleviating the adverse effects of water stress in relatively short-term experiments. There are, however, some contrasting reports (Agboma *et al.*, 1997b; Makela *et al.*, 1998a, Luts, 2000) indicating improved growth of stressed plants due to exogenous application of glycinebtaine.

In conclusion, glycinebetaine did not mitigate the adverse effects of water stress on germination and germination time of sunflower achenes. It may be absorbed by developing seedling and increased the fresh biomass production under water stress but have no effects on dry matter production.

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