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ORIGINAL ARTICLE

**The Effect of Salicylic Acid and Gibberellin on Seed Reserve Utilization, Germination and Enzyme Activity of Sorghum (*Sorghum bicolor* L.) Seeds Under Drought Stress**

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Seed priming methods have been used to increase germination characteristics under stress conditions. The study aimed to determine the effect of salicylic acid and gibberellin on seed reserve utilization, germination and enzyme activity of sorghum (*Sorghum bicolor* L.) seeds under drought stress. Factorial experiment was carried out in completely randomized design with three replications. The first factor was the seed treatments (unpriming, salicylic acid and gibberellin) and the second factor was drought stress (0, -4, -8 and -12 bar). The results indicated that for these traits: germination percentage, germination index, weight of utilized (mobilized) seed, seed reserve utilization efficiency, seedling dry weight and seed reserve depletion percentage was a significant treatment × drought interaction. Thus priming improved study traits in Sorghum (*Sorghum bicolor* L.) seeds under drought stress. Also, priming improves enzyme activity as compared to the unprimed seeds.

*Key words:* Stress, Sorghum (*Sorghum bicolor* L.), Seed reserve utilization, Priming.

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In arid regions, cereal production is widely limited by poor stand establishment (Jones and Wanji, 1992). Particularly in drought-prone environments, cereal germination tends to be irregular and can extend over long periods (Bougne

*et al.*, 2000). Sorghum bicolor is the fourth most important world cereal grain, following wheat, rice, and corn.

Drought stress is widespread problem around

the world. Seed germination negatively affected by drought stress in more crops (Davidson and Chevalier, 1987; Ansari and Sharif-Zadeh, 2012). Seed germination is the most sensitive stage to abiotic stress (Patade *et al.*, 2011; Redmann, 1974; Ansari *et al.*, 2012). Soltani *et al.* (2006) and Ansari *et al.* (2012) reported that seed reserve utilization, seedling growth and weight of mobilized seed reserve decreased with increasing drought and salt intensity. Seed priming can be taken to counteract the adverse effects of abiotic stress (Patade *et al.*, 2009. Ashraf and Foolad, 2005). Seed priming techniques have been used to increase germination characteristics and improves germination uniformity in more field crops under stress conditions (Iqbal and Ashraf, 2007; Kaya *et al.*, 2006; Patade *et al.*, 2011; Saglam *et al.*, 2011; Ansari *et al.*, 2012). Seed priming increases seed reserve utilization, seedling dry weight and seed reserve depletion percentage in mountain rye (Ansari *et al.*, 2012) and wheat (Soltani *et al.*, 2006). In monocotyledon plants like wheat (Soltani *et al.*, 2006) and mountain rye (Ansari *et al.*, 2012), gibberellic acid after synthesis in the scutellum migrates in to the aleurone layer. The hetrotraphic seedling growth (mg per seedling) could be quantitatively described as the product of the following two components: the weight of mobilized seed reserve (WMSR; mg per seed), and the conversion efficiency of mobilized seed reserve to seedling tissue (mg mg<sup>-1</sup>) (Soltani *et al.*, 2006; Ansari *et al.*, 2012; Ansari *et al.*, 2013). Also, the priming strategies enhanced activities of free radical scavenging enzymes such as CAT and APX (Ansari and Sharif-Zadeh, 2012; Rouhi *et al.*, 2012).

Although effects of drought stress and seed priming in wheat, mountain rye and perisian silk tree (*Albizia julibrissin* Durazz.) are documented, no

reports are available on sorghum seeds under drought stress. Therefore, in the present study, we investigated seed reserve utilization, seedling growth and enzyme activity of treated seeds of sorghum seeds as affected by drought stress.

## MATERIALS AND METHODS

Drought stress at osmotic potentials of 0 (as control), -4, -8 and -12 bar were adjusted using PEG-6000 (polyethylene glycol 6000 mw) according to Michel and Kaufmann (1973) before the start of the experiment.

Seeds of were pretreated with salicylic acid 50 ppm at 10 °C for 24 h and gibberellin 50 ppm at 10 °C for 24 h. sorghum seeds were exposure in 20 cm glass petri dishes containing 15 ml solution. The imbibed seeds were then washed 4 times with tap water and dried on filter paper at 15±1°C for 24 h (Ansari and Sharif-Zadeh, 2012).

Standard germination test was carried out by place 50 seeds in 9 cm petri dishes at 25 °C. Seeds were observed daily until day 10<sup>th</sup> and germinated seeds were recorded. Three replicates of 50 seeds were weighed (W1), dried at 104°C for 24 h and then reweighed (W2). Seed water content was calculated as [(W1-W2)/W2]. The initial seed dry weight was calculated using the data for seed water content and W1. After test time expiration, some germination indexes correlating to seed vigor were evaluated such as: germination percentage and germination index. Also, after 7 days, oven-dried weight of seedlings was determined. The weight of utilized (mobilized) seed reserve was calculated as the dry weight of the original seed minus the dry weight of the seed remnant. Seed reserve utilization efficiency was estimated by dividing seedling dry weight by the utilized seed reserve. The ratio of utilized seed reserve to initial seed dry

weight was considered as seed reserve depletion percentage (SRDP) (Soltani *et al.*, 2006; Ansari *et al.*, 2012).

All extraction procedures were carried out at 4 °C. The seed samples, weighting about 0.3 gr, were homogenized with 3 ml of tris (pH 7.8), followed by centrifugation of 20000 g for 20 min. The supernatants were used for determination of enzyme activity. The supernatants were used for determination of enzyme activity. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometric ally following H<sub>2</sub>O<sub>2</sub> consumption at 240 nm (Chiu *et al.*, 1995). Ascorbate peroxidase (APX, EC 1.11.1.7) activity was determined according to the procedures of Johnson and Cunningham (1972). The activities of APX and CAT were expressed per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min.

All data were analyzed statistically by analysis of variance using SAS Software. Data for germination and normal germination percentages were subjected to arcsine transformation before analysis of variance was carried out with SAS software. Mean comparisons were performed using an ANOVA protected least significant difference (Duncan) ( $P < 0.01$ ) test.

## RESULTS AND DISCUSSION

Our results have shown that salicylic and gibberellin increased germination percentage and germination index as compared to the unprimed (Fig. 1 and 2). Also, priming improved germination percentage and germination index in sorghum seeds under drought stress (Fig. 1 and 2).

The highest germination percentage (86%) and germination index (31.33) were attained from gibberellin in control conditions (0 bar). The minimum studied traits were obtained from

unprimed in osmotic pressure -12 bar (Fig. 1, 2, 3, 4, 5 and 6). Our results showed that in across drought osmotic pressures, priming with gibberellin had a greater weight of utilized (mobilized) seed reserve, seed reserve depletion percentage and dry weight than unprimed and priming by salicylic acid (Fig. 3, 4 and 5).

The highest seed reserve utilization efficiency obtained from priming by salicylic acid and – 8 bar osmotic pressure (Fig. 6). The highest seedling dry weight was attained from priming by gibberellin and minimum osmotic pressure (Fig. 5), also in higher levels of osmotic pressures the highest seedling dry weight were obtained from priming by gibberellin (Fig. 5). Thus could say that priming by gibberellin lead to improvement in mentioned traits in sorghum seeds under drought stress. The results are in agreement with the earlier study who reported the significant reduction in the germination as well as growth of Pea (Okçu *et al.*, 2005).

The results of our study suggested that priming treatments caused an improvement in germination percentage and germination index. These results agree whit those of Patade *et al.* (2011), Ansari *et al.* (2012) and Ansari and Sharif-Zadeh (2011). Across drought osmotic pressures, gibberellin had a greater weight of utilized (mobilized) seed reserve and seedling dry weight than unprimed and hydro-priming. While seed reserve utilization efficiency was declined with drought in seed treatments. Decline in seed reserve utilization efficiency to drought were also reported by other researchers (Soltani *et al.*, 2006; Sadghi *et al.*, 2011; Ansari *et al.*, 2012). Decline in seedling growth and different indices of seeds under drought stress also reported for wheat (Soltani *et al.*, 2006), tomato (Bhatt and Srinivasa-Rao, 1987), mountain rye (Ansari *et al.*,

2012) and mung bean (De and Kar, 1995).

Also, our results showed that priming by gibberellin and salicylic acid increased catalase (Fig. 7) and ascorbate peroxidase (Fig. 8) activity as compared to the unprimed seeds.

Seed priming highly increased germination indexes, APX and CAT activities in mountain rye

(Ansari and Sharif-Zadeh, 2012). Also, Rouhi *et al.* (2012) showed that germination characteristics and antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) in treated seeds of Berseem clover (*Trifolium alexandrinum* L.) were significantly increased compared to those in control group.

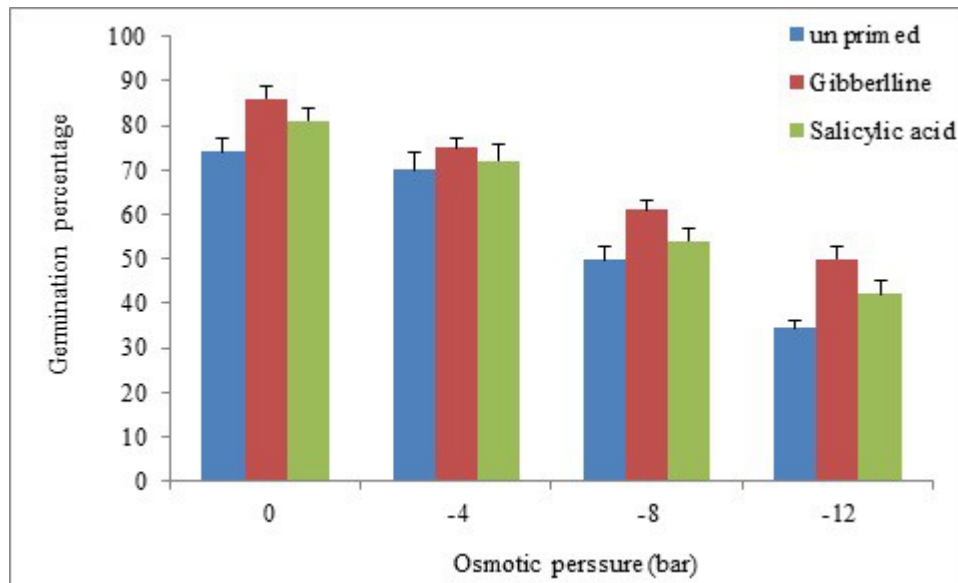


Figure 1. Effect of priming on germination percentage in sorghum seeds under drought stress.

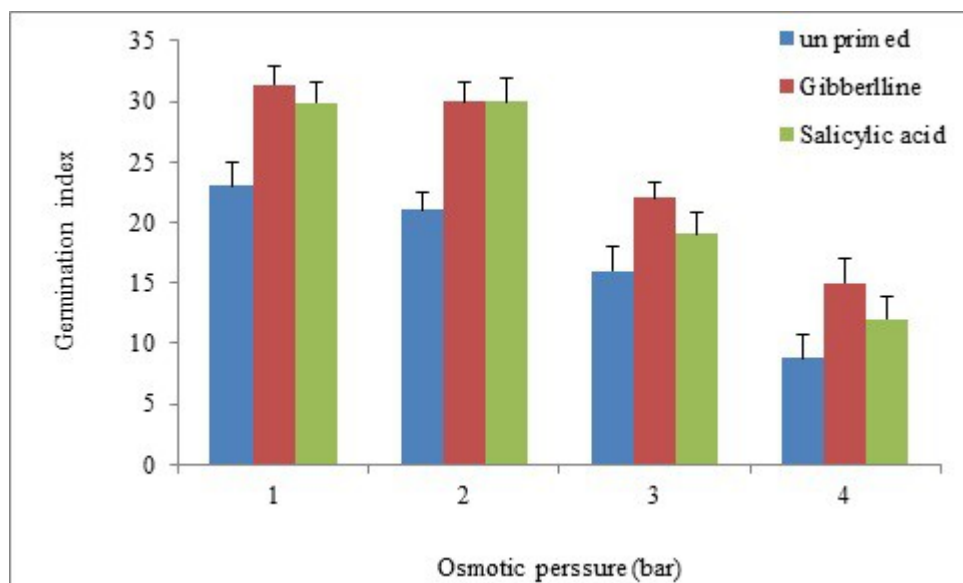


Figure 2. Effect of priming on germination index in sorghum seeds under drought stress.

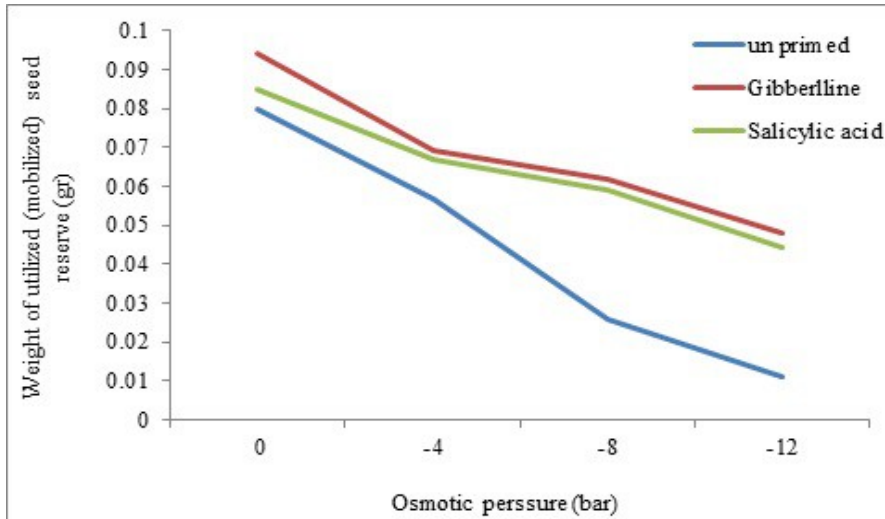


Figure 3. Effect of priming on weight of utilized (mobilized) seed reserve in sorghum under drought stress.

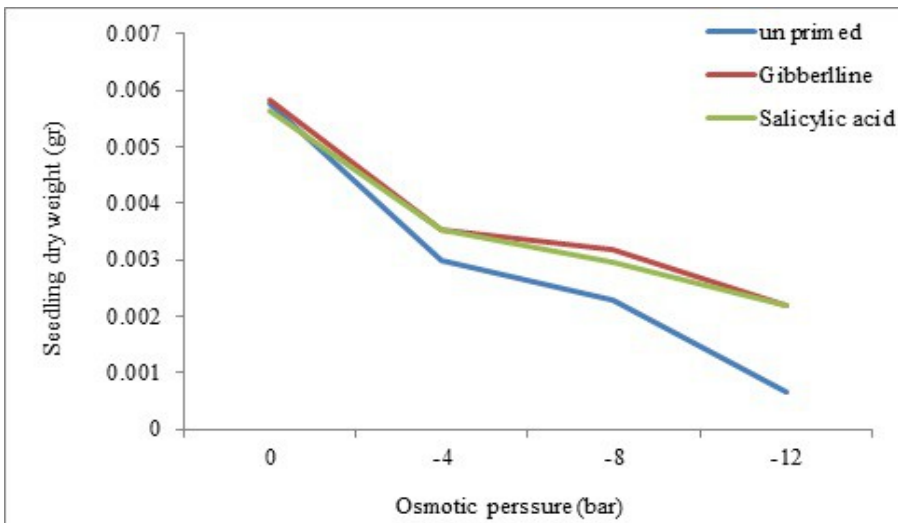


Figure 4. Effect of priming on seed reserve depletion percentage in sorghum under drought stress.

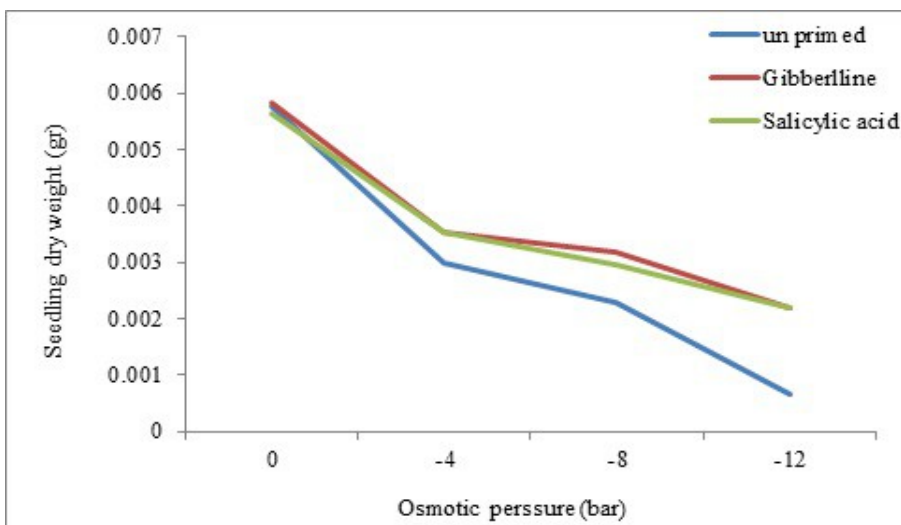


Figure 5. Effect of priming on seedling dry weight in sorghum under drought stress.

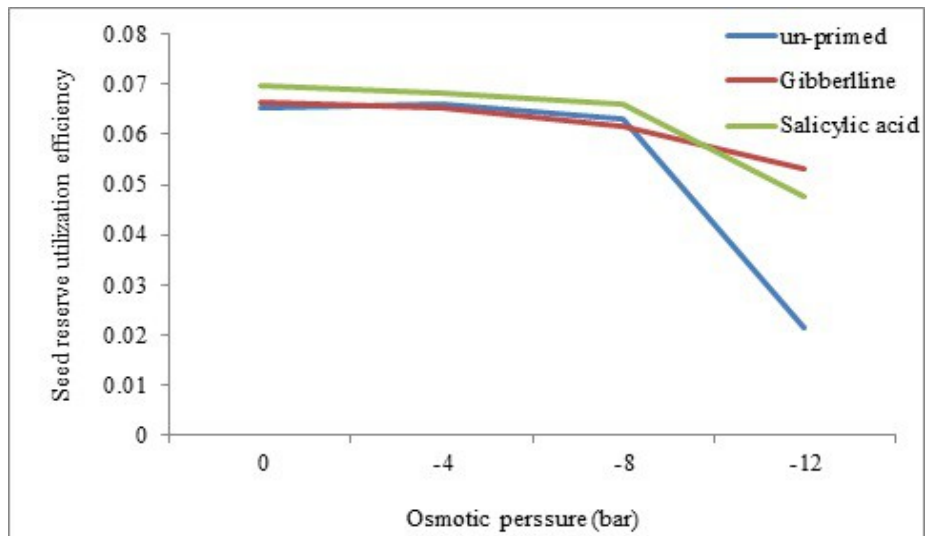


Figure 6. Effect of priming on seed reserve utilization efficiency in sorghum under drought stress.

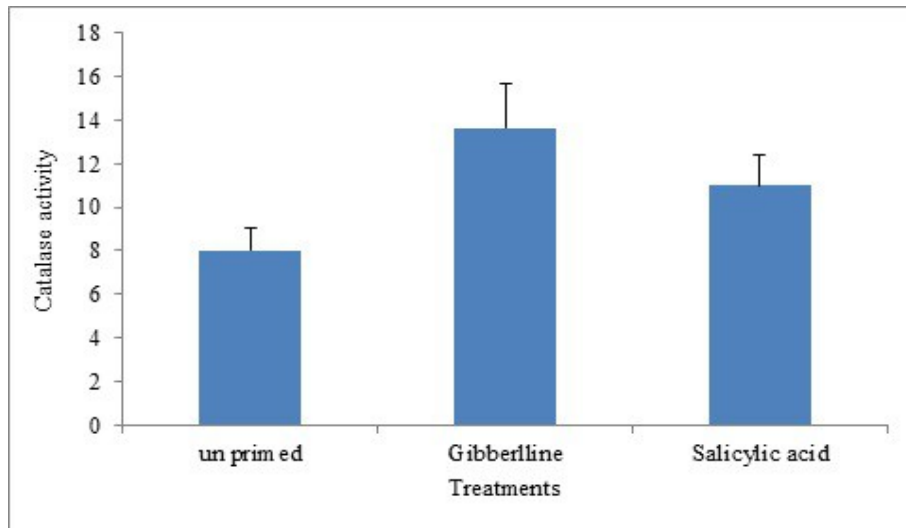


Figure 7. Effect of priming on catalase activity in sorghum seeds.

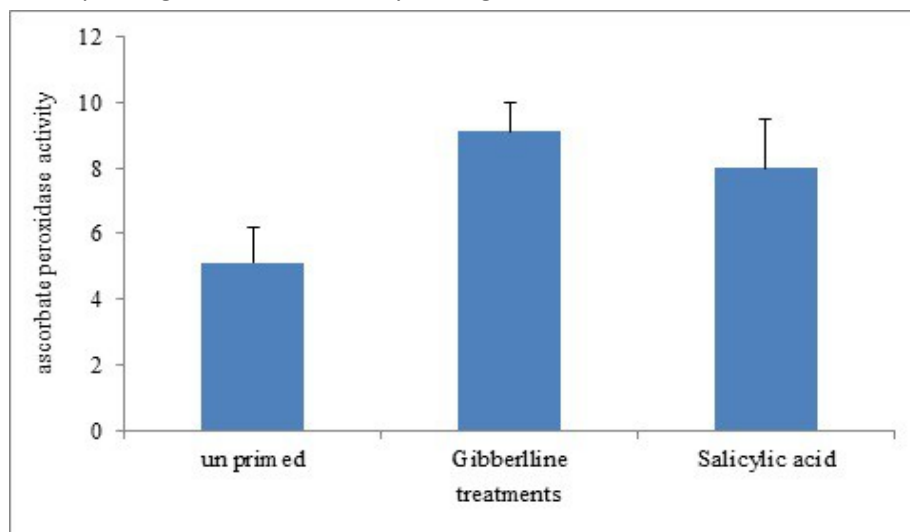


Figure 8. Effect of priming on ascorbate peroxidase activity in sorghum seeds.

## CONCLUSION

Priming increased characteristics as compared to the unprimed. The highest germination percentage and germination index were obtained from priming by gibberellin in control conditions. Priming improved seed reserve utilization and seedling growth in sorghum seeds under drought stress. Also priming improves enzyme activity as compared to the unprimed seeds.

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