

## GIBBERELIC ACID PRIMING ENHANCES MAIZE SEED GERMINATION UNDER LOW WATER POTENTIAL

### *Priming Asam Giberelat Meningkatkan Perkecambahan Benih Jagung pada Tekanan Potensial Air Rendah*

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#### ABSTRACT

Germination is a portentous yield determining factor that is a challenge in the low water potential environment due to disrupted imbibition. Hormonal seed priming can markedly increase the germination on maize even in such a stressful environment. Therefore, research was aimed to analyze the application of gibberellic acid ( $GA_3$ ) priming to maize seeds to minimize the deleterious effects of reduced water potential.  $GA_3$  priming was done at 0, 50, 100, 150, and 200 ppm for 12 hours and subjected to drought levels of 0, 0.15, 0.50, 1.05, and 1.75 MPa by using NaCl solution. Different germination parameters, i.e., germination percentage, mean germination time, germination index, relative water content, seedling vigor index, root length, and shoot length, were determined on the final day of the experiment. The results showed that all germination parameters were adversely influenced by low water potential. Every level of  $GA_3$  priming has hastened all parameters.  $GA_3$  priming at 100 ppm decreased the mean germination time by 35 hours under 1.75 MPa compared to non-primed seeds.  $GA_3$  priming increased the shoot length of maize seedlings with a shoot length of 2.9 cm in non-primed seedlings compared to 6.4 cm in 200 ppm  $GA_3$  priming under 1.05 MPa.  $GA_3$  priming is the best method for the early establishment of maize seedlings in low water potential conditions. As a result, it may be utilized as a low-cost and straightforward approach for establishing maize crops under saline and drought conditions.

[**Keywords:** drought stress, germination, NaCl, seed priming]

#### ABSTRAK

Perkecambahan merupakan faktor penentu hasil yang menjadi tantangan di lingkungan pada tekanan potensial air rendah karena akan mengganggu proses imbibisi. Penggunaan hormon tumbuh pada benih jagung diharapkan dapat secara nyata meningkatkan perkecambahan benih dalam lingkungan yang tertekan. Oleh karena itu, penelitian ini bertujuan untuk menganalisis pengaruh perlakuan priming asam giberelat ( $GA_3$ ) pada benih jagung untuk meminimalkan efek merugikan akibat rendahnya tekanan potensial air. Priming  $GA_3$  dilakukan pada 0, 50, 100, 150, dan 200 ppm selama 12 jam dan pada kondisi tingkat kekeringan 0, 0.15, 0.50, 1.05, dan 1.75 MPa dengan menggunakan larutan NaCl. Pengukuran parameter perkecambahan,

yaitu persentase perkecambahan, waktu perkecambahan rata-rata, indeks perkecambahan, kadar air relatif, indeks vigor semai, panjang akar, dan panjang tunas, dilakukan pada hari terakhir percobaan. Hasil penelitian menunjukkan bahwa semua parameter perkecambahan dipengaruhi secara negatif oleh tekanan potensial air yang rendah. Setiap konsentrasi  $GA_3$  yang diuji dapat mempercepat semua parameter perkecambahan biji. Perlakuan priming  $GA_3$  100 ppm menurunkan waktu perkecambahan rata-rata 35 jam pada kondisi kekeringan di bawah tekanan 1.75 MPa dibandingkan dengan kontrol tanpa priming. Priming  $GA_3$  meningkatkan panjang tunas 2,9 cm pada benih non-priming dibandingkan 6,4 cm pada benih dengan  $GA_3$  200 ppm di bawah tekanan 1,05 MPa. Priming  $GA_3$  merupakan metode terbaik untuk perlakuan benih jagung pada kondisi tekanan potensial air rendah. Oleh karena itu, priming dengan asam giberelat merupakan cara yang murah dan efektif untuk meningkatkan perkecambahan benih jagung pada lahan salin dan lahan kering.

[**Kata kunci:** cekaman kekeringan, NaCl, perkecambahan, priming benih]

#### INTRODUCTION

Seed germination is crucial and the most vulnerable first stage in the life cycle of plants (Rajjou et al. 2012), and if the seeds exposed to low water potential may have to compromise the establishment of the seedling and adversely affect the crop yield (Fetri et al. 2014; Ma et al. 2018). Though maize (*Zea mays* L.) is one of the major cereal crops, its productivity has declined in the saline and drought region due to low water potential (Farooq et al. 2015). In such a low water potential, the viable maize seed germination is reduced and delayed due to constraints on water absorption and lack of metabolism (Khajeh-Hosseini et al. 2003). Therefore, various strategies are employed in improving seed germination, seedling growth, and productivity in water stress conditions.

Seed priming comprises a pre-sowing treatment of soaking seeds in a specified solution, allowing partial hydration and some metabolic activities to proceed

before germination and drying back to original moisture (Chunthaburee et al. 2014; Farooq et al. 2009). Previous studies suggested that priming raises the germination percentage, shortens germination time, and enhances seedling establishment (Ibrahim 2016; Tsegay and Andargie 2018). Seed priming commonly can be divided into four categories based on the priming agents: hydro priming (with water), halo priming (with salt solutions), osmopriming (with osmotic solutions), and hormonal priming (with hormones) (Chatterjee et al. 2018; Wojtyla et al. 2016).

In hormonal priming, plant growth regulators like gibberellic acid ( $GA_3$ ), abscisic acid (ABA), or salicylic acid (SA) have been used more commonly.  $GA_3$  plays a significant role in plant growth and development, such as seed germination, stem elongation, leaf expansion, flower and fruit development, and floral transition (Cornea-Cipcigan et al. 2020). They are frequently used to overcome seed dormancy and improve seed germination in many species by activating embryo growth, mobilizing reserves, and weakening the endosperm layer (Pallaoro et al. 2016).

At present, the priming technique is the need to increase germination and establishment in maize to allow full use of the available soil moisture. Numerous results published in the literature suggested an improvement in germination rate and uniformity. They demonstrated a significant improvement in the behavior of the seedlings obtained in terms of plant growth and stress resistance (Iqbal and Ashraf 2013). Under low water potential conditions, presoaking seeds with an optimum phytohormone concentration is beneficial to the growth and yield of certain crop species because it raises nutrient reserves through increased physiological activity and root proliferation (Anosheh et al. 2011). Seed priming stimulates the biochemical changes in seeds, thereby initiating the germination process upon sowing, higher imbibition rate, and restoring seed metabolism, resulting in increased germination rate and better seedling establishment (Shrestha et al. 2019; Tian et al. 2014). The probable reason for the early emergence of the primed seed may be the completion of pre-germination metabolic activities, making the maize seed ready for radical protrusion and the primed seed germinated soon after planting compared with the untreated, dry seed (Arif 2005). Another probable reason is that priming may also leach germination inhibitors from seeds (Heydecker and Coolbear 1977).

Drought damages plants during germination, reducing their qualitative and quantitative performance and creating weakness and heterogeneity in plant development in farmers' fields (Fetri et al. 2014). Hormonal seed priming, a low-cost and low-risk

strategy, is widely regarded as the most efficient method for improving agricultural seed germination and seedling establishment under adverse conditions (Shrestha et al. 2019; Benincasa et al. 2016). Farmers may implement this simple, low-cost, low-risk approach and positively impact the more comprehensive farming system and livelihoods (Hoseini et al. 2013). To tackle low seedling establishment under low water potential conditions, farmers want readily available priming chemicals that are economical and user pleasant. Although several studies on seed priming have been conducted, the actual content of  $GA_3$  at various levels of drought remains unknown. Thus, the study aimed to determine the optimal concentration of  $GA_3$  solution for priming maize seeds to mitigate the negative effects of drought and low water potential during seedling establishment.

## MATERIALS AND METHODS

### Seeds and Priming

Seeds of maize cultivar 'new 940' were collected from certified seed supplier, Amit Farm (Pvt.) Ltd. Sundarbazar, Nepal. All growth chambers (plant growth chamber PRC 1200 WL) and Petri dishes (12 mm diameter) were sterilized with 70% ethanol. The treatments were drought stress and  $GA_3$  priming. For priming, seeds were soaked in  $GA_3$  solution of concentration 0, 50, 100, 150, and 200 ppm for 12 hours at room temperature without light. After priming, seeds were removed and left to dry between papers for 24 hours in the shade to return their initial dry weight.

### Drought Stress

Drought stress was created by using the NaCl solution. As proposed by Braccini et al. (1996), different sodium chloride (NaCl) solutions were used for irrigation for drought stress. NaCl of 1.76 g was dissolved in 1 liter of water to prepare a salt (NaCl) solution with an osmotic potential of 0.15 MPa. Similarly, 5.74, 12.07, and 20.88 g NaCl were dissolved in 1 liter of water to prepare a solution of NaCl with the osmotic potential of 0.50, 1.05, and 1.75 MPa, respectively. Normal distilled water was used for control (0 Mpa) (Mohammadkhani and Heidari 2008).

### Experimental Design

The study was carried out in the Agronomy Laboratory at Lamjung Campus, Tribhuvan University, Nepal. A completely random design (CRD) was used to study

the effect of GA<sub>3</sub> priming on germination and seedling establishment of maize seeds under low water potential. The experiment was in two factorial designs with two factors (drought stress and GA<sub>3</sub> priming) replicated three times. Seed germination was recorded every 12 hours. A seed was considered as germinated when the radicle emerged by 5 mm or above in length. Twenty seeds of maize were planted in each Petri dish (12 mm diameter). Each Petri dish was rinsed with their respective osmotic solution to wet the filter paper at the base to be neither dry nor get excess water on the Petri dish. Germination tests were performed in the germination chamber with  $24 \pm 1^\circ\text{C}$  and 16 hours light and 8 hours dark lighting conditions. A ruler was used to measure all the shoots and root lengths of the seedlings. Shoot and fresh root weight was measured using a sensitive balance. The separated shoots and roots were dipped in tap water for 24 hours to take turgor weights and then dried at  $70^\circ\text{C}$  until constant weight is achieved for the dry weight (Kaydan and Yagmur 2008).

### Measurements of Traits

Emergence was recorded every 12 hours after the seed water imbibition for 7 days. Root length, shoot length, fresh weight, turgor weight, and dry weight were recorded on the seventh day. Based on these data, all the final germination percentage, mean germination time, germination index, relative water content, and seedling vigor index were calculated using the following relationship:

#### Final Germination Percentage (FGP)

FGP only represents the final percentage of germination achieved and offers no image of germination speed or uniformity. It was calculated using the formula in equation (1) (Scott et al. 1984).

$$\text{FGP} = \frac{\text{final number of seeds germinated}}{\text{total seed}} \times 100\% \quad (1)$$

#### Mean Germination Time (MGT)

MGT is a quantitative indicator of the time taken for a lot to germinate but does not equate with the time distribution or germination uniformity. Instead, it reflects on the day most germination activities took place. MGT was determined using Equation 2, according to Orchard (1977).

$$\text{MGT} = \frac{\sum n_i \cdot t_i}{\sum t_i} \quad (2)$$

Where,  $t$  = time from the beginning of germination test in terms of the hour or day

$n$  = number of newly germinated seeds in time  $t$

#### Germination Index (GI)

The GI tends to be the most detailed measuring parameter integrating percentage and speed of germination (spread, length, and 'high/low' events). In the GI, the seeds germinated on the first day are given maximum weight, and those germinated after that are given less. For seeds germinated on the last day, the lowest weight will be. Therefore, the GI examines both germination percentage and speed. A higher GI value corresponds to a more significant percentage and rate of germination. GI was determined using Equation 3 according to Benech Arnold et al. (1991),

$$\text{GI} = (7 \times N_1) + (6 \times N_2) + \dots + (1 \times N_7) \quad (3)$$

$N_1, N_2, \dots, N_7$  is the number of germinated seeds on the first, second, and subsequent days until the 7th day and the multipliers (e.g., 7, 6 ...etc.) are weights given to the days of the germination.

#### Relative Water Content (RWC)

RWC was determined using Equation 4 according to Turner (1981),

$$\text{MGT} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100\% \quad (4)$$

Where FW = fresh weight  
DW = dry weight  
TW = turgor weight

#### Seedling Vigor Index (SVI)

SVI was determined using Equation 5 according to Abdul-Baki and Anderson (1970),

$$\text{SVI} = (\text{SL} + \text{RL}) \times \text{GP} \quad (5)$$

Where SL = average shoot length (cm)  
RL = average root length (cm)  
GP = germination percentage

### Data Analysis

Data obtained from the experiments were subjected to variance analysis (Two-way ANOVA) using the R program (version 4.0. 2). The difference between means

was determined by Fisher's Post Hoc Least Significant Difference Test. The graphs were also created from the same program using different packages.

## RESULTS

### Final Germination Percentage

Both drought levels and concentration of GA<sub>3</sub> priming affected the germination of maize seeds. The germination of maize was unaltered by drought except when it increased to 1.75 MPa, while all the concentrations of GA<sub>3</sub> (50–200 ppm) increased the germination percentage compared to control. The interaction between the factors showed declining germination percentage with increasing drought levels to 1.75 MPa in almost all primed treatments. In contrast, in control, the germination percentage declined even at drought levels of 1.05 MPa. Though there was no significant difference in final germination percentage by priming at all drought levels, when the drought reached 1.75 MPa, final germination was significantly improved compared to the GA<sub>3</sub> priming control group (Figure 1).

### Mean Germination Time

Drought levels and different concentrations of GA<sub>3</sub> priming had a significant effect on mean germination time. The results showed that mean germination time increased with increasing drought stress, and all GA<sub>3</sub> priming decreased mean germination time. The maximum mean germination time was found at 1.75 MPa with 98.75 hours, while that of control was 51.67 hours. Priming had reduced the mean germination time significantly compared to control, while there was no significant difference among different concentrations of GA<sub>3</sub> priming (50–200 ppm) (Figure 2). GA<sub>3</sub> priming at 100 ppm decreased the mean germination time by 35 hours under 1.75 MPa compared to non-primed seeds. Moreover, 100 ppm GA<sub>3</sub> priming has increased the final germination number of maize seeds and reduced the time of emergence in every drought level (Figure 3).

### Germination Index

Analysis of variance showed that drought and GA<sub>3</sub> priming significantly affected the germination index at  $p \leq 0.05$ . Comparison of means showed that the use of GA<sub>3</sub> priming increased the germination index of maize under low water potential conditions compared to control. The maximum effect of priming on germination index was observed in 100 ppm with 103.00. The result showed a maximum germination index of 0.15 MPa moisture stress with 110.93, which is statistically at par with the control

condition (0 MPa). The minimum germination index was on extreme moisture stress (1.75 MPa) with 68.73. Moreover, in the 0.50 MPa moisture stress condition, the maximum germination index was 100 ppm GA<sub>3</sub> priming with 112.00, while in the control condition, it was 96 (Figure 4).

### Relative Water Content

The result showed a highly significant effect of drought level and GA<sub>3</sub> priming on the relative water content of maize seedlings. The results revealed that relative water content was decreased with an increase in drought stress level, while every concentration of GA<sub>3</sub> priming increased relative water content. The highest relative water content was found at control drought stress (0 MPa) with 82.88%. In contrast, the lowest relative water content was found in 1.75 MPa drought stress with 51.78%. In GA<sub>3</sub> priming, the highest was shown at 200 ppm with 74.29% and lowest at 0 ppm with 63.50%. In extreme drought conditions, i.e., 1.75 MPa, relative water content was only 39.03% in control, which increased to 61.70% while priming with 200 ppm GA<sub>3</sub> (Figure 5).

### Seedling Vigor Index

The analysis results showed a significant effect of the interaction between drought levels and different concentrations of GA<sub>3</sub> priming on seedling vigor index at  $p \leq 0.01$ . The results showed that GA<sub>3</sub> priming increased the seedling vigor index. The maximum and minimum seedling vigor indexes were observed in 50 ppm priming and control (0 ppm) with 1422.05 and 1167.52, respectively. The result showed that the drought stress had a maximum effect on decreasing the seedling vigor index. The maximum seedling vigor index was found in control (0 MPa) moisture stress with 2275.52, and the minimum was seen on higher moisture stress (1.75 MPa) with 257.01 (Figure 6).

### Shoot Length

Drought levels and different concentrations of GA<sub>3</sub> priming had a significant effect on shoot length. The results showed that shoot length decreased with increasing drought and all GA<sub>3</sub> priming increased the shoot length. The maximum shoot length was recorded in 200 ppm GA<sub>3</sub> priming with 6.43 cm while that of control was only 4.6 cm. There was no significant difference between the different concentrations of GA<sub>3</sub> priming (50–200 ppm). Furthermore, the results

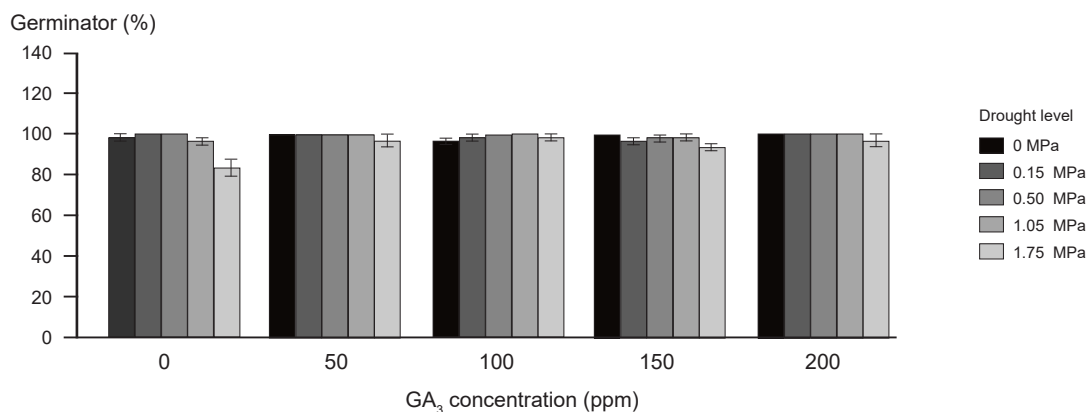


Figure 1. The effect of GA<sub>3</sub> priming on final germination percentage of maize under different levels of drought stress. The bars represent the standard error.

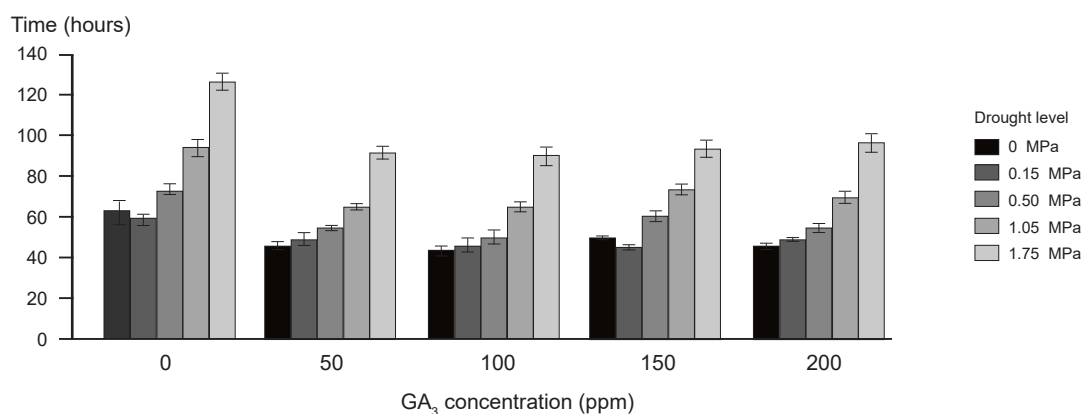


Figure 2. The effect of GA<sub>3</sub> priming on mean germination time of maize under different levels of drought stress. The bars represent the standard error.

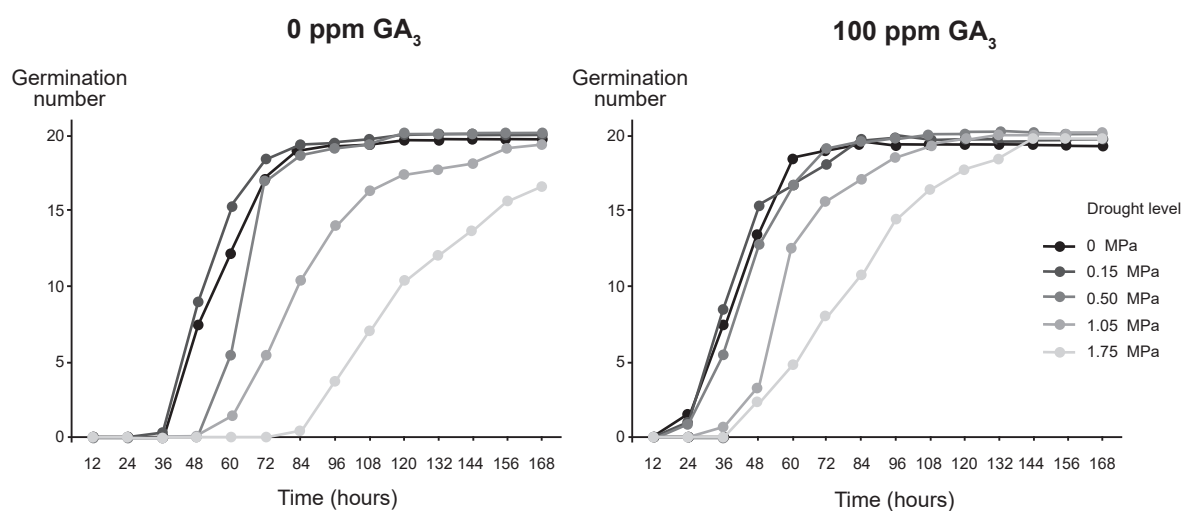


Figure 3. The effect of GA<sub>3</sub> priming on germination number and germination time of maize seed under different levels of drought stress.

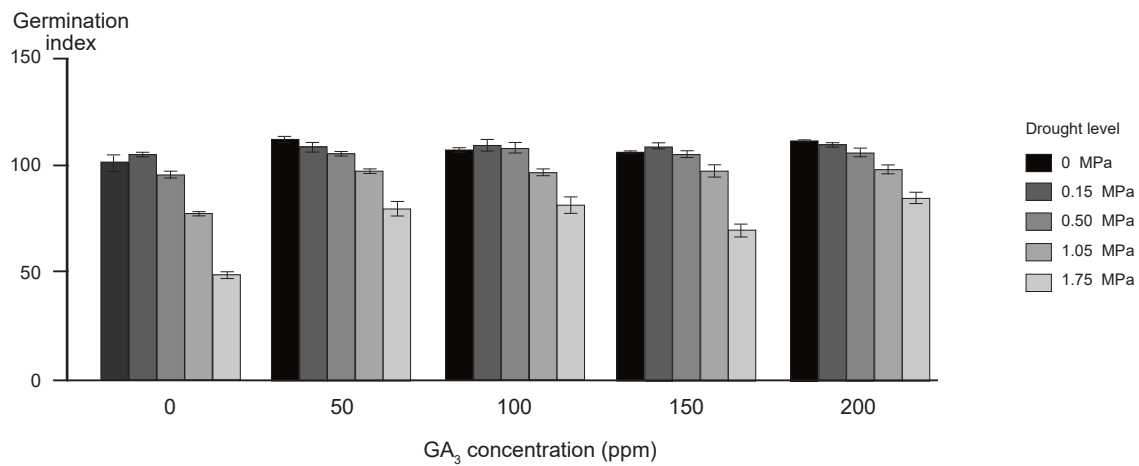


Figure 4. The effect of GA<sub>3</sub> priming on germination index of maize under different levels of drought stress. The bars represent the standard error.

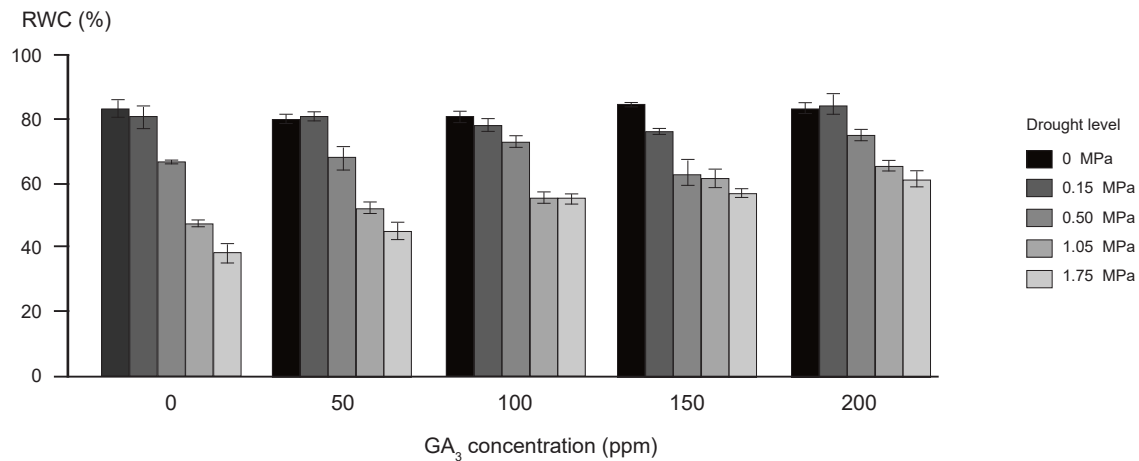


Figure 5. The effect of GA<sub>3</sub> priming on relative water content (RWC) of maize under different levels of drought stress. The bars represent the standard error.

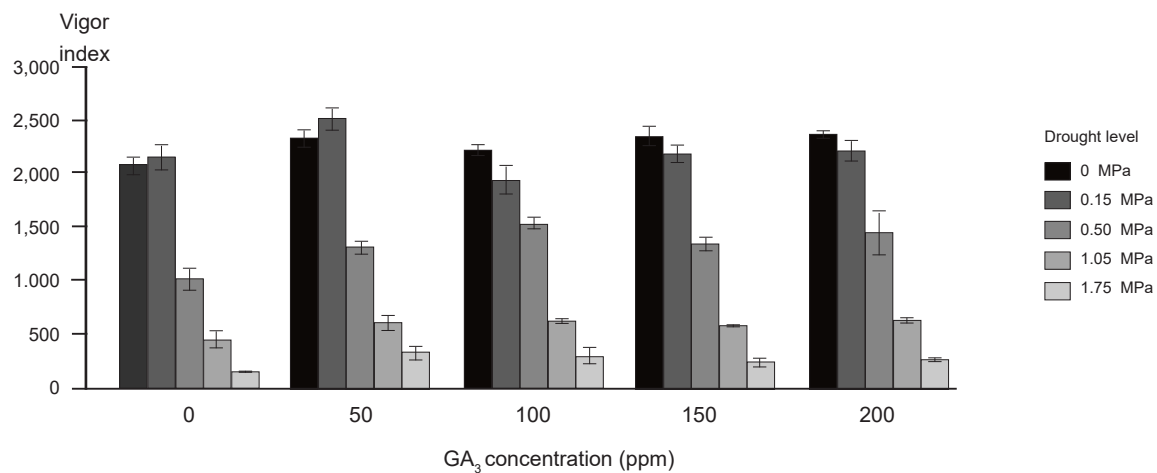


Figure 6. The effect of GA<sub>3</sub> priming on seedling vigor index of maize under different levels of drought stress. The bars represent the standard error.



showed that GA<sub>3</sub> priming had counteracted the effect of drought on the shoot length of maize seedlings. GA<sub>3</sub> priming (200 ppm) increased shoot length from 2.9 cm to 5.04 cm under a 0.5 MPa drought level (Figure 7).

### Root Length

The analysis results showed that the interaction between drought levels and different concentrations of GA<sub>3</sub> priming had a significant effect on root length at  $p \leq 0.05$ . Root length was reduced with increasing drought levels, but all the GA<sub>3</sub> priming increased the root length. The maximum root length was recorded in control drought (0 MPa) with 12.09 cm, and minimum root length was observed in extreme drought (1.74 MPa) with 1.66 cm. GA<sub>3</sub> priming with 50 ppm concentration showed maximum root length of 7.92 cm which was statistically at par with all other priming concentrations (Figure 8).

### DISCUSSION

Seed germination is adversely affected by moisture stress (Rad and Rad 2013). In this research, low water potential decreased germination percentage, mean germination time, shoot length, and root length. When NaCl is dissolved in water, it reduces water absorption by seeds, and subsequent seed germination is delayed or stopped in extreme conditions (Luan et al. 2014). A reduction in water imbibition is due to a decrease in water potential with the addition of salt. Water moves from higher potential to lower potential (Achakzai 2009). Due to the reduction in osmotic pressure under low water potential, the imbibition process is disturbed, and the alpha-amylase enzyme activity is inhibited (Soualem et al. 2018). This study finds that GA<sub>3</sub> priming enhances the germination of maize seeds under both low water potential and normal conditions. Similar results

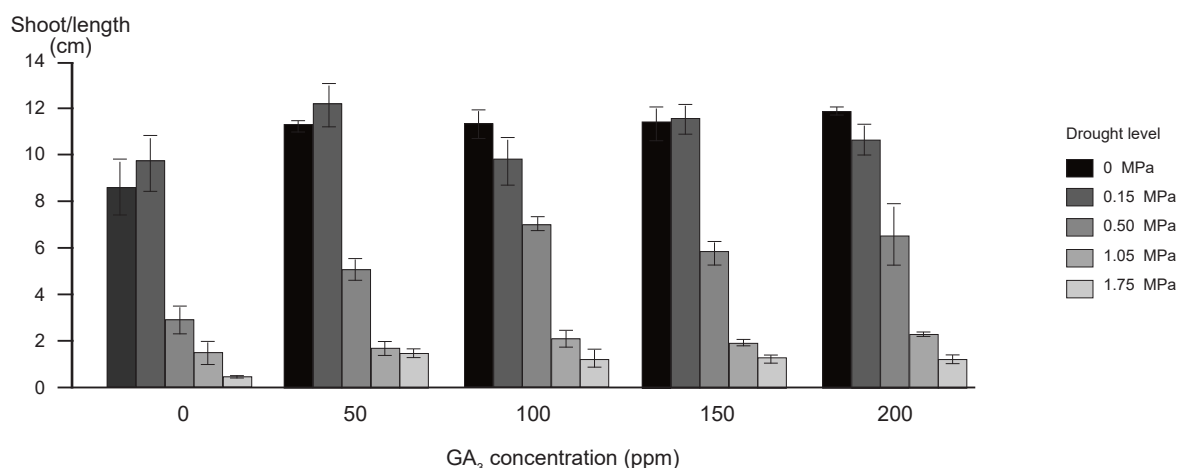


Figure 7. The effect of GA<sub>3</sub> priming on shoot length of maize under different levels of drought stress. The bars represent the standard error.

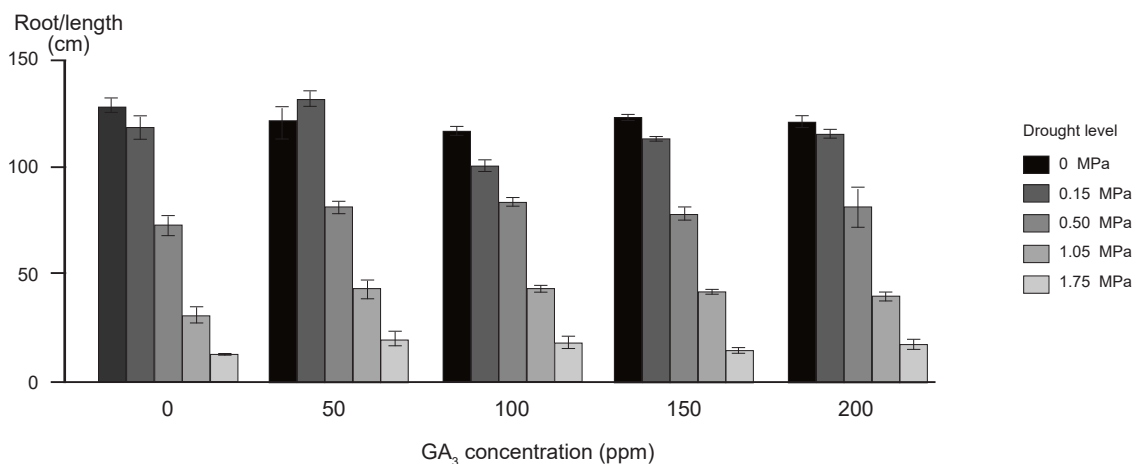


Figure 8. The effect of GA<sub>3</sub> priming on root length of maize under different levels of drought stress. The bars represent the standard error.

were also found by Ghodrat and Rousta (2012), Yuan et al. (2014) on their studies. GA<sub>3</sub> priming reduces germination time and speeds up the seed germination process (Hamza and Ali 2017). Therefore, this is the reason primed seeds are better compared to non-primed maize seeds. Furthermore, it increases antioxidant enzymes such as glutathione in primed seeds, and this enzyme may reduce lipid peroxidation activity during germination, leading to increased germination (İşeri et al. 2014).

With increasing drought stress, root and shoot length decreased (Bayoumi et al. 2008). The decrease in shoot length of maize seedling with an increase in drought level may be due to the unavailability of water for seedling growth. Shoot cell growth in seedlings depends on water availability to seedlings, so when the seedling is exposed to water shortage, it shortens shoot growth (Batool et al. 2014). Moreover, seeds' reduction in water absorption causes reduced stress hormones secretion and enzyme activity in the maize seeds, which harms seedling growth (Kafe et al. 2005). Additionally, with increased drought stress, the seedling vigor index decreased. Similar findings were recorded in the Chinese cabbage germination analysis by Yan (2015).

Our findings also found that the priming of gibberellic acid had a beneficial effect on all the germination traits tested compared with the control. One of the effective and positive reasons for gibberellic acid on seed germination is probably due to hormonal balance and decreased proportion of growth-inhibiting substances such as abscisic acid (ABA). These enzymes also mobilize the storage reserves of endosperms that fuel germination and growth (Pallaoro et al. 2016). Priming with GA<sub>3</sub> will speed up metabolic reactions before germination and make seed germination possible under salinity stress conditions with low water potential (Iqbal and Ashraf 2013).

Among the different traits we studied, relative water content is very responsive to drought stress and has been shown to correlate well with drought tolerance (Colom and Vazzana 2003). The leaves' relative water content has been considered a better indicator of water stress than other growth traits of the plant (Sinclair and Ludlow 1985). Relative water content is usually higher in plants adapted to dry conditions, and Carter and Paterson (1985) had earlier recorded similar observations in soybean. In our experiment, relative water content of seedling shoots has reduced with an increase in drought levels due to reduced external water potential. At the same level of water potential, GA<sub>3</sub> priming has increased relative water content, an indicator of drought tolerance through osmoregulation with higher relative water content under different drought stresses. It is the only adaptive and positive response beneficial to the plant under water stress conditions (Turner 1986). Osmoregulation enables

the plant to maintain high turgor pressure and survive under stress conditions (Datta et al. 2011).

Drought and salinity are a global problem that affects around 7% of the world's total land area, 20% of which is total cultivated land and 33% irrigated land, with a global loss estimated at 20% (Jamil et al. 2011). In addition, 10 million hectares of agricultural land are projected annually ruined by salinized soil (Pimentel et al. 2004). Salt impacted regions may rise to over 50% of the entire arable land globally in 2050, without appropriate and sustainable control (Jamil et al. 2011). In soil with such low water potential, salinity-sensitive plants such as maize cannot germinate and are established. The average yield in such circumstances similarly decreased by 50–80% (Panta et al. 2014). GA<sub>3</sub> priming, under this scenario, can be a conveniently accessible, easy-to-use approach that enhances the tolerance of agricultural crop to abiotic stress and may be employed in a sustainable agricultural production environment in drought-, saline- and flood-prone regions across the world. GA<sub>3</sub> priming enhances the tolerance for abiotic stress and assures a harmonized germination by improving viability.

## CONCLUSION

In general, it can be concluded that low levels of water moisture harm maize seed germination and development. GA<sub>3</sub> priming plays a significant part in mitigating the deleterious impact that low water potential produces. Therefore, this research demonstrated the effectiveness of applying seed priming techniques in drought-stressed habitats to reduce the adverse effects of low water potential on germination and early growth of seedlings under laboratory conditions. But to see the output of the maize crop (primed with GA<sub>3</sub>) on vegetative growth and yield at field level, further research is needed.

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## AUTHOR CONTRIBUTIONS

AG initiated and designed the research; AG performed experiments; RS supervised the project; AG and RS



conducted data analysis and interpretation; AG drafted, revised, and approved the final version of the manuscript for publication.

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