Original Article | Open Access

Nasir et al., 2019. 21: 14-22. J Environ Agric Sci (ISSN: 2313-8629)

Seed Priming to Alleviate Drought Stress in Cotton

Muhammad Waqar Nasir[1,2,*], Azra Yasmeen[2], Muhammad Imran[2], Tóth Zoltan[1]

Edited by:
Wajid Nasim,
The Islamia University, Bahawalpur, Pakistan

Reviewed by:
Fahd Rasul,
University of Agriculture, Faisalabad, Pakistan
Javaid Iqbal,
Ghazi University, Dera Ghazi Khan, Pakistan

Received
July 10, 2019
Accepted
September 12, 2019
Published Online
December 28, 2019

Abstract: Water scarcity being a major issue threatens the exposure of crops to drought stress. Cotton is one of the most important cash crops and its yield is also affected by water shortage. Therefore, a pot trial was established to study the effect of various priming agents to mitigate drought stress in cotton. Two water levels i.e. 100% field capacity (control) and 70% field capacity (drought stress) were maintained. Sowing materials (seeds) were primed with water, benzyl amino purine (BAP), moringa leaf extract (MLE), calcium chloride ($CaCl_2$) and their performance was compared with non primed seeds (control). Bi factorial randomized complete block design was used. Data regarding emergence index, mean germination time, number of bolls per plant, boll weight per plant, lint weight, seed weight, plant height and ginning out turn was obtained and analyzed statistically. The results revealed that all parameters under observation were significantly higher in well watered pots than in water stressed pots. All priming agents produced better results than control, however, BAP priming proved to be the most promising. Under drought condition; highest emergence index (0.85) was computed for MLE primed seeds. CaCl2 primed seeds took least time to germinate (MGT) (13 days). Maximum no. of bolls per plant under control (8.50) and drought condition (4), highest bolls weight per plant under control (22.1 g), lint weight (9.56 g) and seed weight (11.21 g) were observed for BAP priming. On the basis of results it can be concluded that seed priming can be used to get better yield results under water shortage; particularly use of BAP priming is recommended to obtain better growth and yield under drought conditions.

Keywords: Priming agents, Moringa leaf extract, benzyl amino purine, calcium chloride, tolerance, water stress.

***Corresponding author:** Muhammad Waqar Nasir: nasir.muhammad.waqar@2018.georgikon.hu

Cite this article as: Nasir, M.W., A. Yasmeen, M. Imran and T. Zoltan. 2019. S**eed priming to alleviate drought stress in cotton**. Journal of Environmental& Agricultural Sciences. 21:14-22.

1. Introduction

Cotton is one of most important seed and fiber crop of Pakistan (Ali and Rahut, 2018; Government of Pakistan, 2015; Nawaz et al., 2019; Nawaz et al., 2016). Its yield is affected by various factors including but not limited to sowing time, nutrient deficiency (Coker et al., 2009), weed management, water availability (Loka et al., 2011), biotic stress (Sabir et al., 2011) etc. Global warming; an ever increasing threat causing climate change induced anomalies in the amount, distribution, frequency and duration of precipitation (Darand et al., 2017; Nawaz et al., 2016; Kijne et al., 2003). Global warming is the major reason of drought stress leading to reduced agricultural production (Kim et al., 2019; Leng and Hall, 2019; Zhang et al., 2019). Drought is usually accompanied by other abiotic stresses (heat stress, nutrient stress) as plants are unable to uptake nutrients or to stabilize their temperature effectively under water stress condition (Constable and Bange, 2015; Nachimuthu and Webb, 2017; Yan et al., 2010).

Resistance to drought stress depends upon several factors i.e. plant genotype, developmental stage of plant, extent and duration of drought stress etc. (Hussain et al., 2014; Pabuayon et al., 2019; Saleem et al., 2016;). Under the ever increasing water shortage threat, plants may have to grow under water stress since very early stage of their life (germination). Germination is the first most water sensitive stage that can affect the final yield of cotton (Chen et al., 2018; Ghodrat et al., 2017; Manonmani et al., 2019).

[1]Festetics Doctoral School, Department of Crop Production, Georgikon Faculty, University of Pannonia, Keszthely, Hungary
[2]Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan.

Forecasting the water threat; a number of agronomic and breeding strategies have been practiced to help plant grow under water stress conditions (Elliot et al., 2018; Lesk et al., 2016; Stagge et al., 2015; Tardieu et al., 2018). Adjusting the planting time, foliar application of plant growth regulators (PGRs), use of short season hybrid varieties, use of more drought resistant varities and genetic engineering to produce more resistant varities are some of techniques utilized (Fan et al., 2018; Hatfield and Dold, 2019; Saleem et al., 2016).

Seed priming is also an effective strategy that gives an early boost to plants facing water shortage and helps in attaining better yield under stress condition (Ahmad et al., 2015; Sharif et al., 2019). Seed priming is a quick and economical treatment that significantly affects the germination particularly speeds of germination leading to a better uniform stand promising a better yield (Ashraf et al., 2018). Seed priming with plant growth hormones, natural plant extracts, osmoprotectants etc have been reported an effective water stress tolerant treatment by several researchers (Nawaz et al., 2017; Yasmeen et al., 2013; Ullah et al., 2019).

Cytokinins are growth regulators that effect chlorophyll synthesis in plants. Cytokinins are commercially available in the form of trans-zeatin, benzyl amino purine (BAP) etc. They are costly but the use of BAP has been reported to improve yield of crops under water stress condition (Shang, 2000; Taiz and Zeiger, 2006). Usefulness of osmoprotectants such as KCl and $CaCl_2$ has also been reported for increasing drought resistance in rice (Farooq et al., 2009) and tomato (Afzal et al., 2006). Seed priming of rice with $CaCl_2$ improved germination providing a better start to crop under stress condition that resulted in higher 1000 kernel weight. Afzal et al., (2006) also reported an increase in defense mechanism and total soluble proteins in tomatoes by $CaCl_2$ priming.Use of plant extracts for priming and foliar application is trending and economical now a days. Moring leaf extracts (MLE) is a rich source of zeatin, vitamin c, calcium and potassium. MLE priming has been reported to improve metabolism and positively affect seed emergence (Foidle et al., 2001; Ashraf et al., 2008).

Therefore this research was planned to evaluate the effect of natural (MLE), synthetic (BAP) and nutrient ($CaCl_2$) priming agents on growth and water stress resistance of cotton.

2. Materials and Methods

2.1. Experimental material and site

In the wire net house of Faculty of Agricultural Sciences, Bahauddin Zakariya University, Multan, Pakistan; a pot trial was conducted to evaluate the effect of different priming agents on drought resistance in *Gossypium hirsutum* L. Clay loan soil and farm yard manure (3:1) were used as growing media filled @ 10 kg per pot. Bi factorial randomized complete block design was used with three replications. *Gossypium hirsutum* L. *cv*. FH 114 were obtained from Ayoub Agriculture Research Institute, Faislabad, Pakistan. 15 seeds per pot were sown and thinned to 2 plants per pot after emergence.

2.2. Treatments

The main factor was two water levels i.e. well watered (100% FC) and drought stressed (60% FC) and sub factors included hydro priming, priming with Benzyl amino purine (BAP), priming with Moringa leaf extract (MLE) @30:1 (Yasmeen et al., 2013), priming with calcium chloride ($CaCl_2$) (Farooq et al., 2006).

Field capacity was measured by gravimetric method described by Nachabe, (1998) and drought stress was imposed from the sowing and maintained till harvesting. Priming was done in the aerated solution of each priming agent separately.

2.3. Observations

To assess the effect of priming agents on drought resistance in cotton; various emergence, growth, yield and biochemical parameters were observed. In the start of trial mean emergence time (MET) and emergence index (EI) were calculated by formula (eq 1) and methodology described by Ellis and Robert, (1981) and Association of Official seed Analyst, (1990) respectively.

$$MET = \sum \mathrm{Dn} / \sum \mathrm{n} \qquad [1]$$

Where, n is total number of seeds emerged, D is total number of days taken for complete emergence (eq 2).

$$EI = \left(\frac{E_{DAE1}}{DAE}\right) + \left(\frac{E_{DAE2}}{DAE}\right) + \cdots + \left(\frac{E_{DAEn}}{DAE}\right) \qquad [2]$$

Where, DAE = days after first seeds emerged, E_{DAE1} = seeds emerged on 1 DAE, E_{DAE2} = seeds emerged on 2 DAE, E_{DAEn} = seeds emerged on final DAE.

Table 1. Mean square and level of significance of treatments applied on germination and yield of cotton

Source of variation	DF	EI	MGT	Bolls weight (g)	Lint (g)	GOT (%)	Plant height (cm)	Seed weight (g)	Total number of bolls
Priming (P)	04	0.34**	2.57**	24.24**	4.41**	442.69**	29.18**	3.60**	2.97**
Water level (W)	01	0.26**	2.97**	740.23**	114.41**	23.82**	110.21**	175.26**	93.63**
P × W	04	0.03**	17.68**	18.63**	4.61**	528.13**	15.73**	3.19**	2.63**
Min. value		0.44	10.48	5.53	2.20	59.00	41.5	3.75	2.50
Max. value		1.16	17.33	22.10	9.56	94.89	54.0	11.21	8.50

EI, emergence Index; GOT, ginning out turn; MGT, mean germination time; ** $P < 0.01$.

After complete emergence of seeds, thinning was performed to reduce the number of plants to 2 plants per pot. At the time of harvesting plant height (cm), number of bolls per plant, bolls weight per plant, seeds cotton weight (g), lint weight (g) and ginning out turn (GOT percentage was measured by the formula (eq 3) described by Singh, (2004).

$$GOT(\%) = \frac{Lintweight\ (g)}{Seedcottonweight\ (g)} \times 100 \qquad [3]$$

To quantify chlorophyll (Chl), and total protein (TP) in plants; procedure described by Yasmeen, (2011) was adopted. For enzymatic antioxidants determination of leaf sample, extraction was done in 5 ml of 50 mM phosphate buffer (pH 7.8), after centrifugation at 15000 × *g* for 20 min, the supernatant was used in further assay for superoxide dismutase (SOD) activity (Giannopolitis and Ries, 1977), peroxidase activity (Chance and Maehly, 1955) by recoding absorbance at 560 and 240 nm respectively.

The collected data was recorded on MS Excel 2007 to calculate standard deviation to make graphical presentation of data. Statistix 8.1 software was used to statistically analyze the data. Analysis of variance and mean comparison was performed at 5% and 1% significance level.

3. Results

The analysis of variance indicated that seed priming, water levels and there interaction significantly affected all phenological parameters under study in cotton. All parameters under observation very significantly higher in well watered pots than in water stressed pots. All priming agents produced better results than control, however, BAP priming proved to be most promising. Highest emergence index (1.16) was computed for MLE primed seeds (Fig 1a).

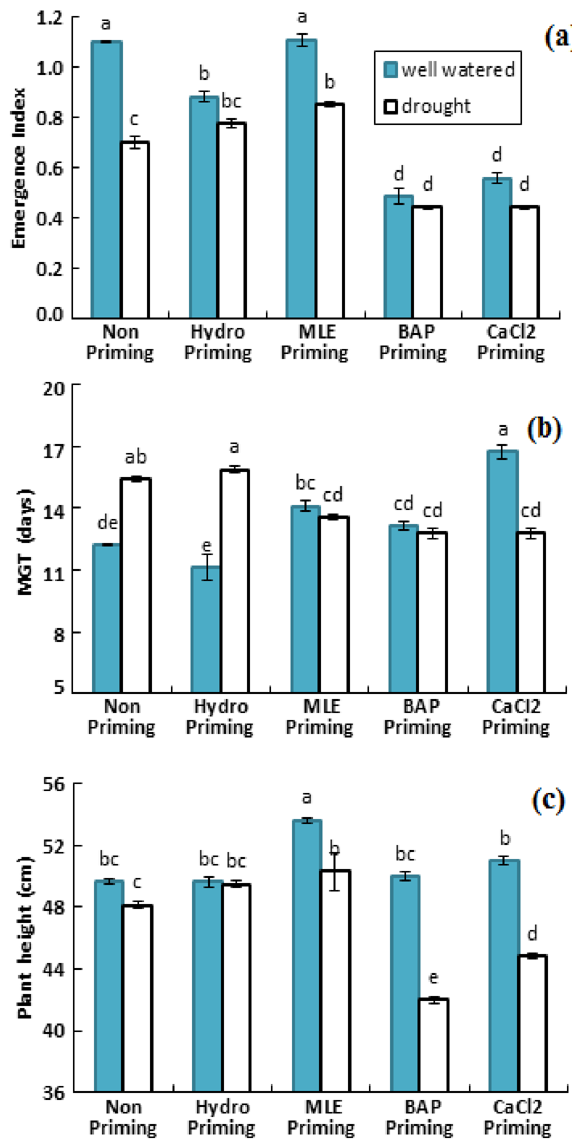

Fig. 1. Impact of different priming agents and varying levels of water stress on emergence Index (a), mean germination time (MGT) (b) and plant height (c) of cotton.

Table 2. Mean comparisons of different priming agents on emergence and yield contributing parameters of cotton under different water levels

Factors	Level	EI	MGT	Bolls weight	Lint weight	GOT	Plant height	Seed weight	Total number of balls
Water level	W1	0.83 a	13.46 b	17.49 a	7.07 a	75.69 a	50.80 a	9.03 a	6.86 a
	W2	0.65 b	14.09 a	7.55 b	3.17 b	73.91 b	46.96 b	4.19 b	3.33 b
Priming	P1	0.90 b	13.81 b	11.44 d	4.29 c	86.55 a	48.91 bc	6.51 b	4.66 b
	P2	0.83 b	13.50 bc	12.33 c	5.40 b	72.20 c	49.58 b	6.66 b	4.83 b
	P3	0.98 a	13.85 b	9.88 e	4.38 c	70.09 d	52.00 a	5.62 c	4.33 b
	P4	0.47 c	12.96 c	14.91 a	6.40 a	64.89 e	46.00 d	7.79 a	6.00 a
	P5	0.50 c	14.76 a	14.02 b	5.14 b	80.26 b	47.91 c	6.48 b	5.66 a
Water level × Priming	W1 × P1	1.10 a	12.20 de	17.22 c	6.19 cd	78.56 d	49.66 bc	9.24 b	6.33 b
	W1 × P2	0.88 b	11.12 e	16.31 d	6.64 c	81.35 c	49.66 bc	8.72 b	7.00 ab
	W1 × P3	1.11 a	14.11 bc	12.42 e	5.38 d	78.49 d	53.66 a	7.09 c	5.00 c
	W1 × P4	0.49 d	13.15 cd	22.05 a	9.52 a	70.24 e	50.00 bc	11.21 a	8.00 a
	W1 × P5	0.56 d	16.74 a	19.42 b	7.64 b	69.81 e	51.00 b	8.89 b	8.00 a
	W2 × P1	0.70 c	15.42 ab	5.65 i	2.40 g	94.54 a	48.16 c	3.78 d	3.00 de
	W2 × P2	0.78 bc	15.88 a	8.35 fg	4.15 e	63.05 f	49.50 bc	4.60 d	2.66 e
	W2 × P3	0.85 b	13.59 cd	7.34 h	3.38 ef	61.69 f	50.33 b	4.15 d	3.66 de
	W2 × P4	0.45 d	12.78 cd	7.78 gh	3.28 efg	59.53 g	42.00 e	4.37 d	4.00 cd
	W2 × P5	0.45 d	12.78cd	8.63 f	2.64 fg	90.72 b	44.83 d	4.08 d	3.33 de

Where, EI, emergence Index; GOT, ginning out turn; MGT, mean germination time; W1= well watered, W2 = water stressed, P1 = control, P2 = Hydro priming, P3 = MLE priming, P4 = BAP priming, P5 = $CaCl_2$ priming.

$CaCl_2$ primed seeds took most time to germinate (MGT) (17.33 days) (Fig 1b). Maximum no. of bolls per plant (8.50) (Fig 2a), highest bolls weight per plant (22.1 g) (Fig 2b), lint weight (9.56 g) (Fig 2c) and seed weight (11.21 g) were observed for BAP priming (Fig 2d).

Table 1 showed that interaction of water levels and priming agents have significant effect on all physiological traits. Emergence index was highest for MLE primed seeds under well water condition that was at par with control followed by MLE primed seeds in drought condition. Minimum mean germination time was observed for hydro primed seeds under non-stress condition. While $CaCl_2$ primed seeds under well water condition took maximum mean germination time that was at par with hydro primed seeds under water stress condition.

Highest bolls weight per plant and lint weight were observed for BAP primed seeds in well watered condition followed by $CaCl_2$ primed seeds in non stressed environment. Under stressed condition $CaCl_2$ produced highest boll weight. Similar fashion was observed for total number of balls per plant and seed weight where BAP primed seeds under well watered pots produced maximum seed weight followed by $CaCl_2$ under well watered pots while the two treatments were at par for highest number of balls produced per plant.

Surprisingly, GOT (%) was highest in non primed plants under stressed condition followed by $CaCl_2$ in water stressed condition. Tallest plants were observed for MLE primed seeds in well water condition followed by $CaCl_2$ in well watered pots that was at par with MLE primed seeds under water stressed condition.

The mean table for effect of water level and different priming on SOD, POD, chlorophyll and total protein showed significant effect on all physiochemical parameters. POD and total protein were higher and well watered conditions while SOD and chlorophyll were produced in significantly larger amount under stressed condition (Table 3). All priming treatments were significantly higher than control. SOD calculated was highest in $CaCl_2$ primed seeds under drought condition followed by MLE primed seeds under well watered conditions. POD was highest in hydro primed seeds in well watered condition followed by $CaCl_2$ under well watered conditions. Maximum chlorophyll content were observed in hydro primed seeds under stress condition that was statistically at par with $CaCl_2$ primed seeds under well watered conditions. However, maximum TP were observed in well watered plants primed with $CaCl_2$ and water respectively

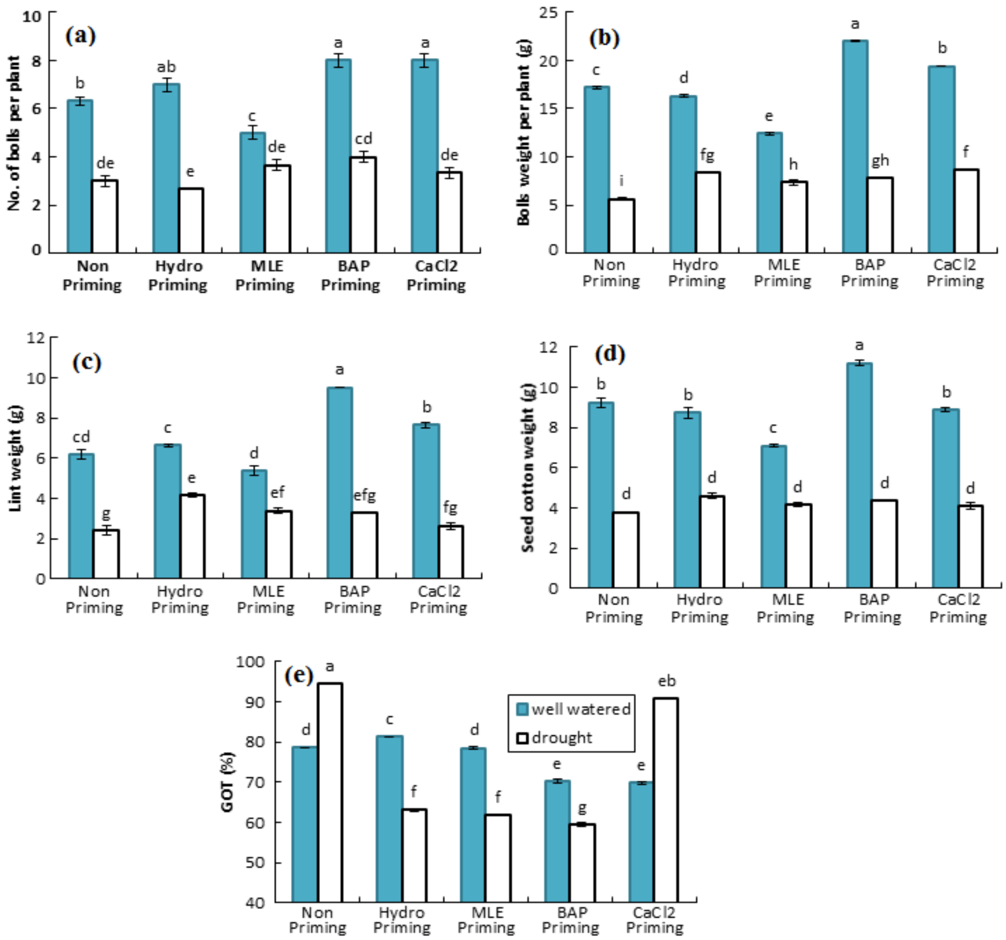

Fig. 1. Impact of different priming agents and varying water stress levels on yield components of cotton.

Table 3 Mean comparison of different priming agents on antioxidants, chlorophyll and total protein concentration of cotton under different water levels

Factors	Level	SOD	POD	CHL	TP
Water level	**W1**	60.65 b	16.75 a	13.67 b	24.04 a
	W2	163.90 a	8.13 b	15.35 a	13.28 b
Priming	**P1**	45.73 d	6.58 c	13.93 c	8.76 e
	P2	107.67 c	33.03 a	15.32 a	20.64 b
	P3	120.94 b	5.94 c	14.37 bc	12.70 c
	P4	0.49 e	1.69 d	14.08 bc	39.79 a
	P5	286.55 a	14.98 b	14.83 ab	11.42 d
Water level × Priming	**W1 × P1**	45.47 d	5.24 f	11.62 d	11.69 f
	W1 × P2	18.02 e	46.34 a	14.05 bc	35.72 b
	W1 × P3	230.37 b	0.57 h	12.83 cd	23.01 d
	W1 × P4	0.35 h	3.07 g	13.75 bc	47.58 a
	W1 × P5	9.06 g	28.56 b	16.08 a	2.23 h
	W2 × P1	46.00 d	7.93 e	16.25 a	5.84 g
	W2 × P2	197.33 c	19.72 c	16.58 a	5.56 g
	W2 × P3	11.52 f	11.32 d	15.91 a	2.39 h
	W2 × P4	0.64 h	0.31 h	14.41 b	32.00 c
	W2 × P5	564.03 a	1.4 h	13.58 bc	20.61 e

Where, CHL, Chlorophyll; SOD, Superoxide dismutase; POD, Peroxide dismutase; TP, total protein; W1= well watered, W2 = water stressed, P1 = control, P2 = Hydro priming, P3 = MLE priming, P4 = BAP priming, P5 = $CaCl_2$ priming.

4. Discussion

More than half of Agricultural production comes from drought facing countries (Revenga et al., 2000). Cotton is the most important fiber crop. All developing stages of cotton are affected by water stress (Loka et al., 2011) leading to morphological (Alishah and Ahmadikhah, 2009), physiological and biochemical changes (Loka and Oosterhuis, 2011). Kaya et al. (2006) reported that drought at early stage effects germination and seedling stand in crops that in turn causes the reduced yield. Therefore, this experiment was established to tackle the drought stress at very early stage of cotton.

The results revealed a significant effect of drought on all germination and yield contributing factors. Seeds sown under well watered condition germinated faster and uniformly, were taller than stressed plants, produced more and heavier bolls per plant, lint and fiber weight as well as GOT. Water stress condition effects germination of cotton and protein and antioxidant status is reduced making it difficult for plant to resist stress eventually leading to lower yield. Several researchers also reported similar results (Abd-El-Malak, and Radwan, 1998; Enciso et al., 2003; Pedroza and Flores, 1998; Sahit et al., 2015)

Moringa leaf extract is rich source of Cytokinins and potassium (Foidel et al., 2001). In present study, MLE improved emergence and plant height of cotton. Farooq et al., (2010) reported that MLE being the source of nutrients and vitamins might transfer them to seeds during priming process and improve the germination and growth of primed seeds. MLE also increases the vegetative life span and antioxidant status of plants making it more resistant to unfavorable condition (Yasmeen et al., 2013).

Priming with $CaCl_2$ improved yield and antioxidant status of cotton under well watered as well as stressed conditions. $CaCl_2$ improves the root growth that helped in better uptake of water and nutrients (Khan et al., 2015). Harris et al. (1999) and Harris et al., (2001) also reported an increase in economic yield of plant (bolls in case of cotton) by $CaCl_2$ priming due to better assimilate portioning. Results also revealed that $CaCl_2$ triggered the antioxidant production that scavenges on reactive oxygen species leading to resistance against stress. Kaczmarek et al., (2017) also reported similar results.

BAP significantly affected yield contributing factors.BAP is a commercial form of cytokinin that improves chlorophyll and protein content of plant under stressed and normal conditions (Taiz and Zeiger, 2006). Extra number of bolls produced by BAP primed seeds lead to higher boll weight per pot and eventually higher lint and fiber weight. Brathe et al. (2002) and Iqbal et al. (2006) also reported BAP is a growth regulator that can improve plant stress tolerance.

5. Conclusion

It is evident from the results that under drought condition cotton growth is affected. Moreover, results also suggest that seed priming can be used to improve cotton growth under control as well as drought conditions. Seed priming improves germination of cotton, leading towards a better crop stand that can produce good yield even under water shortage. Therefore, use of seed priming agents particularly BAP can be advised for better growth and yield of cotton.

List of Abbreviations: BAP: Benzyl amino purine; MLE: Moringa leaf extract; PGR: plant growth regulator; MET: Mean emergence time; EI: Emergence Index; GOT: Ginning out turn; SOD: Superoxide dismutase; POD: Peroxide dismutase; CHL: Chlorophyll content; TP: Total protein.

Competing Interest Statement: All the authors declare that they have no competing interest.

Author's Contribution: M.W. Nasir and A. Yasmeen designed the study. M.W. Nasir and M. Imran conducted the experiment under the supervision of A. Yasmeen. T. Zoltán performed statistical analysis while M.W. Nasir wrote the manuscript. Finally, A. Yasmeen approved the current version of manuscript. All the authors read and approved the final manuscript.

Acknowledgments: The work/publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

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