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Effect of Soaking Seed with Growth Regulators, Antioxidants and Natural Extracts on Maize Germination and Seedlings Characters

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Abstract: A laboratory experiment was conducted to study the effect of soaking seed treatments for 18 hours with different concentrations of some growth regulators (indol acetic acid and indole butyric acid at the rates of 200, 400 and 600 mg l^{-1} and gibberellic acid at the rates of 500, 1000 and 1500 mg l^{-1}), antioxidants (ascorbic acid and oxalic acid at the rates of 200, 400 and 600 mg l^{-1}) and natural extracts as biostimulants (black seed extract and yeast extract at the rates of 50, 100 and 150 ml l^{-1}) on germination and seedlings parameters of maize hybrid Single Cross-10 (SC-10). The soaking maize seeds in yeast extract at 150 ml l^{-1} for 18 hours as a bio-stimulant before starting germination improvement of germination parameters. This was followed by soaking maize seeds in antioxidant substances such as ascorbic acid at 600 mg l^{-1} for 18 hours and then soaking maize seeds in yeast extract at 100 ml l^{-1}. In order to maximize germination and seedlings parameters of maize seeds hybrid SC-10, soaking seeds in yeast extract at 150 ml l^{-1} as a bio-stimulant or ascorbic acid at 600 mg l^{-1} as antioxidant for 18 hours could be recommended.

Keywords: *Zea mays*, GA_3, IAA, IBI, Ascorbic acid, Oxalic acid, Black seed extract

Maize (*Zea mays* L.) is the most important cereal grain in the world and for higher production and productivity of crop use of good quality seeds and proper management practices play significant role. Good quality seeds imply vigour, uniformity and structural soundness besides its genetic and physical purity. To provide higher quality seeds, many researchers have developed new technologies called Seed Enhancement Techniques to improve quality of seeds. Seed soaking could improve early seedling growth under stress conditions compared to plants grown from untreated seed (Sharifi and Khavazi 2011). Indole acetic acid (IAA) is the common natural auxin that shows all auxin doing actions and extensively affects plant's physiology and seed germination (Kelen et al 2004). Indole butyric acid (IBA) shows paradoxical behavior relative to other auxins, such as IAA, NAA or 2,4-dichlorophenoxyacetic acid (2,4-D). It displays only weak auxin activity in root growth inhibition assays, but its ability to induce lateral and adventitious roots is comparable with that of other auxins (Ludwig-Muller et al 2005). The. Gibberellic acid is the most important growth regulator, which breaks seed dormancy, promotes germination, intermodal length, hypocotyls growth and cell division in cambial zone and increases the size of leaves. GA_3 stimulates hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and thus speeds germination by promoting seedling elongation growth of cereal seeds. Anosheh et al (2014) observed that seed priming with optimal concentrations of plant growth hormones, such as auxin, gibberellins, abscisic acid, and ethylene, improve the germination parameters. Yuan et al (2014) concluded that seed soaking with 6-BA or 10% PEG improved maize seed germination parameters under drought stress, including seedlings dry weight, seed vigor and germination rate. Hamza and Ali (2017) showed that soaking seeds in GA_3 improved emergence and seedling growth of corn under salt stress. Ghodrat et al (2019) observed that soaking in IBA improved germination traits and increased the resistance of wheat in germination stage under saline conditions.

Pre-sowing treatment with ascorbic acid is widely used and improves performance and stand establishment at different external factors such as high salinity (Afzal et al 2005). Oxalic acid is considered antioxidant substance, which acting a significant role in regulating a number of physiological processes i.e. ions uptake and transport, transpiration, photosynthesis, growth and plant metabolism (Singh et al 2010). Kandil et al (2015 a and b) revealed that soaking maize grains in ascorbic acid at the rate of 100 ppm for 18 hours significantly increased germination. Yeast is natural source of cytokinins and has stimulatory effects on plants (Amer 2004). Furthermore, yeast extract was recommended to participate in a beneficial role on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation (Wanas 2002). Seadh et al (2017) showed that seed soaking for about 18 hours with yeast extract (100 ml l^{-1}) resulted in high growth, yield and its

components of maize as compared with soaking in water, gibberellic acid (100 ppm) and oxalic acid (400 ppm). The objective of this investigation was to study the effect of soaking seed treatments with different concentrations of some growth regulators (indole acetic acid, indole butyric acid and gibberellic acid, antioxidants (ascorbic acid "AA" and oxalic acid "OA") and natural extracts as biostimulants (black seed extract and yeast extract) on germination and seedlings parameters of maize hybrid Single Cross-10 (SC-10).

MATERIAL AND METHODS

This investigation was conducted at Mansoura University, Egypt, during June 2018. The experiment was carried out in completely randomized design with four replications. The soaking seed treatments with different concentrations of some growth regulators, antioxidants and natural extracts of maize (23 treatments) were shown in Table 1.

Maize seeds were soaked for 18 hours in all soaking treatments and then air dried before start germination test. Indol acetic acid (IAA), indol butyric acid (IBA) and gibberellic acid (GA_3) as growth regulators and ascorbic acid (AA) and oxalic acid (OA) as antioxidants were produced by El-Nasr Pharmaceutical Chemicals Co., Egypt, and obtained from El-Gomhouria Company for Trading Pharmaceutical Chemical & Medical. Black seed extract (BSE) and yeast extract (YE) as natural biostimulants were prepared by using a technique modified by Spencer et al (1983).

Germination test: Random sample of 400 seeds per each treatment were sown on top filter paper in sterilized Petri-dishes (14 cm diameter). Each Petri-dish contain 25 seeds, and four Petri-dishes kept close together and assessed as though they were one 100-seeds replication at 8th June 2018 as the rules of International Seed Testing Association (ISTA 1996). Dishes were inspected daily and distilled water added as required. Seeds are considered physiologically germinated when the radical pierced the coleorhiza and reach approximately 2 to 3 mm long. The germinated seeds were counted and first count defined as the number of germinated seeds at the fourth day. Then, every 24 hours the number of germinated seeds were counted until end of germination test (7 days). Seeds were categorized as germinated (radical 2 mm long), hard (no imbibitions or swelling) or nonviable (abnormal, dead or infected seeds) as described by ISTA (1996).

Germination parameters

1- Final germination percentage (FG %) ISTA (1996):

$$FG\% = \frac{\text{Number of normal seedlings}}{\text{Number of total grains}} \times 100$$

2- Speed germination index (SGI) (ISTA 1996):

$$SGI = \frac{\text{No. of germinated grains}}{\text{Days to first count}} + \frac{\text{No. of germinated grains}}{\text{Days to final count}}$$

3- Germination index (GI) as (Karim et al 1992):

$$GI = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in control treatment}}$$

4- Co-efficient of germination (CG) (Copeland 1976):

$$CG = \frac{100\ (A_1 + A_2 + \ldots\ldots + A_n)}{A_1T_1 + A_2T_2 + \ldots\ldots\ A_nT_n)}$$

Where;

A = Number of seed germinated, T = Time (days) corresponding to A.

n = No. of days to final count.

5- Mean germination time (MGT) (Ellis and Roberts 1981)

$$MGT = \frac{\Sigma\ Dn}{\Sigma\ n}$$

Where (n) is the number of seeds, which were germinated on day, D is number of days counted from the beginning of germination.

6- Energy of germination (EG) It was the percentage of germinating seeds at the first count (4 days after sowing) relative to the total number of tested seeds (Ruan et al 2002).

7- Abnormal and hard seedlings percentage counted and expressed by the percentage of abnormal seedlings after 7 days according to ISTA (1996).

9- Root and shoot length was conted from ten seedlings selected at random in each replicate The root length was estimated from the grain to the tip of the radical and 10- Shoot length (was calculated from seed? to the tip of the leaf blade

11- Seedling vigor index (SVI). as follow (Abdul Baki and Anderson 1973):

$$SVI = \frac{(\text{Root length} + \text{Shoot length}) \times \text{Germination percentage}}{100}$$

12- Total chlorophyll content (TCC by SPAD unit): was assessed in wide leaf of shoot by SPAD-502 (Minolta Co. Ltd., Osaka, Japan).

13- Root dry weight (RDW) and Shoot dry weight (SDW) of ten seedling roots and shoot at random per replicate were recorded and expressed in milligram (mg) after oven drying at 70 º C until constant weight (Agrawal 1986).

Data were subjected to the statistical analysis using “MSTAT-C” computer software package. Means of treatments were compared using Duncan's multiple range tests at 5 % level of probability (Duncan 1955).

RESULTS AND DISCUSSION

There were significant differences in germination parameters i.e. final germination percentage (FG %), speed germination index (SGI), germination index (GI), co-efficient of germination (CG), mean germination time (MGT), energy of germination (EG), abnormal seedlings percentage (AS %) and hard seeds percentage (HS %) and seedling parameters i.e. root length (RL), shoot length (SL), seedling vigor index (SVI), total chlorophyll content (TCC), root dry weight (RDW) and shoot dry weight (SDW) among soaking maize seeds treatments in different concentrations of some growth regulators indol acetic acid and indol butyric acid, gibberellic acid and antioxidants ascorbic acid and oxalic acid and natural extracts as biostimulants (black seed extract and yeast extract as compared to besides untreated seeds (control treatment) and soaking maize seeds in distilled water.

Soaking maize seeds in yeast extract (YE) at 150 ml L^{-1} for 18 hours as a biostimulant significantly increased final germination percentage (FG %), speed germination index (SGI), germination index (GI), co-efficient of germination (CG), mean germination time (MGT), energy of germination (EG), abnormal seedlings percentage (AS%), hard seeds percentage (HS%), root length (RL), shoot length (SL), seedling vigor index (SVI), total chlorophyll content (TCC), root dry weight (RDW) and shoot dry weight (SDW) and resulted in best results of them, in addition increased these characters by 20.48 , 21.8), 20.41, 9.01), 20.51, 26.58, 100.00, 100.00, 262.26, 133.47, 262.79, 300.58, 149.59 and 187.13% as compared with the control treatment, respectively. This was followed by soaking maize seeds in antioxidant substances ascorbic acid (AA) at 600 mg l^{-1} for 18 hours and yeast extract at 150 ml l^{-1} and at 100 ml l^{-1}

The favourable effect of soaking maize seeds in yeast extract may be attributed to its stimulatory effect on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation (Amer 2004). Besides, the desirable effect of ascorbic acid (AA) before starting germination test may be ascribed to ascorbic acid is consider as an important metabolite involved in many cellular processes, including cell division. In addition, the advantageous effect of oxalic acid which acting a significant role in regulating a number of physiological processes *i.e.* ions uptake and transport, transpiration, photosynthesis,

Table 1. Final germination (FG) percentage, speed germination index (SGI), germination index (GI), co-efficient of germination (CG) and mean germination time (MGT) as affected by soaking seed treatments

Soaking seed treatments (mg/ml l^{-1})	Characters									
	FG (%)		SGI		GI		CG		MGT	
Untreated (control)	81.34	h	5.03	i	98	h	24.2	c	20.33	g
Distilled water	84.28	g	5.24	h	101.5	g	24.35	b	21.07	f
IAA at 200	86.24	fg	5.39	gh	103.9	fg	24.5	b	21.56	ef
IAA at 400	90.16	de	5.68	de	108.6	de	24.43	b	22.78	cd
IAA at 600	94.08	c	5.88	bc	113.3	c	24.5	b	23.52	bc
IBA at 200	88.2	ef	5.5	efg	106.2	ef	24.43	b	22.05	de
IBA at 400	90.16	de	5.64	def	108.6	de	24.5	b	22.54	d
IBA at 600	94.08	c	5.88	bc	113.3	c	24.5	b	23.52	bc
GA_3 at 500	91.14	d	5.68	de	109.8	d	24.44	b	22.78	cd
GA_3 at 1000	94.08	c	5.88	bc	113.3	c	24.5	b	23.52	bc
GA_3 at 1500	97.02	ab	6.06	ab	116.8	ab	24.5	b	24.25	ab
AA at 200	91.14	d	5.7	cd	109.8	d	24.5	b	22.78	cd
AA at 400	95.06	bc	5.94	ab	114.5	bc	24.5	b	23.76	ab
AA at 600	98	a	6.13	a	118	a	24.5	b	24.5	a
OA at 200	89.18	de	5.62	def	107.4	de	24.43	b	22.54	d
OA at 400	91.14	d	5.7	cd	109.8	d	24.5	b	22.78	cd
OA at 600	95.06	bc	5.94	ab	114.5	bc	24.5	b	23.76	ab
BSE at 50	88.66	de	5.48	fg	106.8	de	24.5	b	22.16	de
BSE at 100	89.18	de	5.57	def	107.4	de	24.5	b	22.29	de
BSE at 150	94.08	c	5.88	bc	113.3	c	24.5	b	23.52	bc
YE at 50	97.02	ab	6.06	ab	116.8	ab	24.5	b	24.25	ab
YE at 100	97.02	ab	6.06	ab	116.8	ab	24.5	b	24.25	ab
YE at 150	98	a	6.13	a	118	a	26.38	a	24.5	a

IAA, IBA, GA3, AA, OA, BSE and YE are Indol acetic acid, Indol butyric acid, Gibberellic acid, Ascorbic acid, Oxalic acid, Black seed extract and Yeast extract

Table 2. Energy of germination (EG), abnormal seedlings (AS) percentage, hard seeds percentage (HS %), root length and shoot length as affected by soaking seed treatments

Soaking seed treatments (mg l^{-1})	Characters									
	EG		AS (%)		HS (%)		Root length (cm)		Shoot length (cm)	
Untreated (control)	77.42	i	9.8	a	8.54	a	5.22	k	4.72	l
Distilled water	82.32	h	9.61	ab	7.84	ab	5.68	k	5.15	kl
IAA at 200	86.24	fg	8.82	ab	2.94	cd	8.86	i	5.93	ij
IAA at 400	90.16	de	5.88	bcd	1.96	cd	9.21	hi	6.24	hi
IAA at 600	94.08	bc	2.94	def	0.98	cd	10.12	h	6.81	gh
IBA at 200	87.22	efg	7.84	abc	3.92	cd	9.87	hi	6.49	ghi
IBA at 400	90.16	de	4.9	cde	2.94	cd	12.22	g	6.93	fg
IBA at 600	94.08	bc	2.94	def	0.98	cd	12.78	fg	7.42	ef
GA_3 at 500	90.16	de	4.9	cde	1.96	cd	12.83	fg	7.64	de
GA_3 at 1000	94.08	bc	2.94	def	0.98	cd	13.65	ef	8.08	cd
GA_3 at 1500	97.02	ab	0	f	0.98	cd	14.35	cde	9.33	b
AA at 200	91.14	cd	4.9	cde	1.96	cd	14.4	cde	8.5	c
AA at 400	95.06	ab	0.98	ef	1.96	cd	16.98	b	9.87	b
AA at 600	98	a	0	f	0	d	13.05	fg	7.08	efg
OA at 200	89.18	def	3.92	cdef	4.9	bc	12.91	fg	8.08	cd
OA at 400	91.14	cd	4.9	cde	1.96	cd	13.91	def	7.47	ef
OA at 600	95.06	ab	1.96	def	0.98	cd	14.97	cd	8.4	c
BSE at 50	84.39	gh	8.82	ab	3.92	cd	5.88	jk	5.61	jk
BSE at 100	89.18	def	3.92	cdef	4.9	bc	6.22	jk	5.93	ij
BSE at 150	94.08	bc	3.92	cdef	0.98	cd	7	j	6.56	gh
YE at 50	97.02	ab	0.98	ef	0	d	15.14	c	9.4	b
YE at 100	97.02	ab	0	f	0	d	16.71	b	9.82	b
YE at 150	98	a	0	f	0	d	18.91	a	11.02	a

See Table 1 for details

Table 3. Seedling vigor index (SVI), total chlorophyll content in shoots, root dry weight and shoot dry weight as affected by soaking seed treatments

Soaking seed treatments	Characters							
	SVI		Total chlorophyll content (SPAD)		Root dry weight (mg)		Shoot dry weight (mg)	
Soaking seed treatments (mg/ml l^{-1})	8.25	n	3.43	n	147.4	q	20.82	o
	9.00	mn	3.74	n	160.7	p	22.69	n
Untreated (control)	13.02	k	4.78	m	168.5	n	28.17	l
Distilled water	14.22	jk	6.00	kl	183.7	k	29.40	l
IAA at 200	16.25	i	6.86	jk	193.0	i	31.85	k
IAA at 400	14.74	j	6.76	jk	173.4	m	29.40	l
IAA at 600	17.62	h	6.88	jk	184.4	k	31.85	k
IBA at 200	19.40	fg	8.13	hi	193.7	i	34.54	j
IBA at 400	19.05	fg	8.50	gh	198.2	h	39.20	h
IBA at 600	20.86	e	10.07	def	239.6	f	44.10	g
GA_3 at 500	23.45	cd	10.97	cd	261.6	d	46.30	f
GA_3 at 1000	19.53	fg	10.41	cde	241.8	f	47.77	e
GA_3 at 1500	22.22	d	10.09	def	281.9	c	49.00	e
AA at 200	26.85	b	11.88	b	288.6	b	55.12	b
AA at 400	18.31	gh	7.49	ij	178.1	l	31.36	k
AA at 600	19.90	ef	8.77	gh	189.6	j	32.34	k
OA at 200	24.28	c	9.18	fg	205.8	g	36.01	i
OA at 400	22.68	d	9.67	ef	161.9	p	22.78	n
OA at 600	9.87	lm	4.31	mn	165.3	o	26.21	m
BSE at 50	11.05	l	5.17	lm	178.1	l	28.42	l
BSE at 100	13.03	k	6.37	k	256.7	e	50.96	d
BSE at 150	26.24	b	11.24	bc	282.2	c	53.65	c
YE at 50	29.93	a	13.74	a	367.9	a	59.78	a

See table 1 for details

growth and plant metabolism (Singh et al 2010). The effective role of gibberellic acid (GA_3) in enhancing final germination percentage comparing with the control treatment may be due to GA_3 is the most important growth regulator, which breaks seed dormancy, promotes germination, intermodal length, hypocotyls growth and cell division in cambial zone. The positive effects of indole acetic acid (IAA) in enhancing final germination percentage comparing with the control treatment may be due the common natural auxin that extensively affects plant's physiology and seed germination (Kelen et al 2004). These results are in accordance with those reported by Hamama and Murniati (2010), Anosheh et al (2014), Kandil et al (2015 a and b), Hamza and Ali (2017) and Seadh et al (2017).

CONCLUSION

For maximizing germination and seedlings parameters of seeds maize hybrid SC-10, soaking in yeast extract at 150 or 100 ml L^{-1} as a biostimulant or ascorbic acid at 600 mg L^{-1} as antioxidant for 18 hours were very effective is very effective recommended.

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