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Enhancement Wheat Germination and Seedlings Vigor By Using Some Growth Regulators, Antioxidants and Macro And Micronutrients

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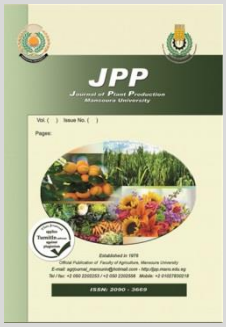

ABSTRACT

To enhancing seedlings parameters of bread wheat Misr 1 cultivar by soaking in ascorbic acid (AsA) as a source of antioxidant, indole-3-acetic acid (IAA) as a source of growth regulator, Truefert powder as a source of macronutrients (NPK) and Folifert powder as a source of micronutrients (Zn, Fe, Mn, Cu, B and Mo), a laboratory experiment was conducted at Agronomy Department Laboratory of Seed Testing, Faculty of Agriculture, Mansoura University, Egypt, during November 2018. The experiment was carried out in completely randomized design (CRD) with four replications. Results showed that treating wheat seeds with solution of ascorbic acid (AsA) + micronutrients at the rates of 0.1 g/l + 3.75 g/l, respectively for 12 hours was the best treatment which produced the maximum values of most seedlings characters, followed by soaking wheat seeds in indole-3-acetic acid (IAA) + micronutrients at the rates of 0.05 g/l+ 3.75g/l, respectively. It could be recommended that soaking wheat seeds Misr 1 cultivar in combination of ascorbic acid (AsA) + Micro (Zn, Fe, Mn, Cu, Mo and B) at the rates of 0.1 g/l+ 3.75g/l, respectively for 12 hours or indole-3-acetic acid (IAA) + micro (Zn, Fe, Mn, Cu, Mo and B) at the rates of 0.05 g/l+ 3.75g/l, respectively for 12 hours to improving germination and seedlings parameters.

Keywords: Wheat, soaking, Ascorbic acid (AsA), Indole-3-Acetic Acid (IAA), macronutrients, micronutrients, germination characters.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the largest source of food security in the world especially the Arab countries, where wheat is found as an essential ingredient in daily diets. It can be simply changed into several species of food like bread, macaroni, biscuit, pizza and sweets. All parts of the plant have a significant economic return, and it has many purposes as straw which used as coarse fodder for animals, as bedding for poultry farms, or as a source of organic matter. So, efforts must be intensified to enhance wheat productivity to face the increasing demand for it. So, rising domestic production is necessary to face population growth of about 2.5% per annum in Egypt (El-Sayed *et al.*, 2018). The government is fighting to make up the gap among the Egyptian's production and consumption by rising the cultivated area, production per unit area by using suitable agronomic practices and promising cultivars. An important attention has been given to soak seeds before drilling, which can be used to apply only small quantities of indole-3-acetic acid, ascorbic acid macro and micro-nutrients (Seadh *et al.*, 2017). The importance of ascorbic is due to its role in plants, which serves as a co-factor for various enzymes and help to the detoxification of ROS. The antioxidant activity of ascorbic is led to longevity in plants and fight the oxidative stress. Hence, the endogenous level of ascorbic is recommended to be important to the organizing of developmental senescence (Gadalla, 2009).

Indole-3-acetic acid (IAA) is one of the most important plant hormone, furthermore. It is helpful in vascular tissue development, cell elongation, and apical dominance. IAA helps the cell to elongation by changing bad conditions as, increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure (Hanaa and Safaa, 2019). These important macronutrients may be lost from the soil by several ways as volatilization and leaching. Therefore, macronutrients must be added by any method. Because its deficiency influences on yield and growth. Nitrogen (N) helps in creation of protein and building substances from which the living material or protoplasm of every cell is made. Also, N is useful in created in chlorophyll the green coloring matter of leaves. Chlorophyll enables the plant to carry energy from sunlight by photosynthesis (Wagan *et al.*, 2003). Phosphorus (P) is considered as an important element for plant. P contributes in plants is the metabolic reactions, make nucleic acid (DNA, RNA), nucleotides, phospholipids and phosphoproteins were difficult without phosphorus (Islam *et al.*, 2017). Potassium (K) has a key role in the carrying prepared food from the leaves to other parts of plants, quality of seeds and fruits, supports the roots, stem and branches of plants and reduce lodging (Brhane *et al.*, 2017).

Soil does not have available quantity of microelements as boron (B) copper (Cu) iron (Fe) manganese (Mn) molybdenum (Mo) zinc (Zn). So, micro must be added exogenous. Zinc (Zn) subscribe in important metabolic roles in plant and help as regulatory cofactor for different enzymes (Mahmoud, Nadia, *et al.*, 2014). Iron (Fe) acts a central role in nucleic acid metabolism and it is vital to respiration and photosynthesis

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processes. Manganese (Mn) plays a chief role in the biosynthesis of ATP, acyl lipids, proteins, fatty acids and play a part in RuBP carboxylase reactions (Tripathi, *et al*., 2015). Boron (B) is one of the most main micronutrients which play a substantial function in the RNA metabolism, respiration, (IAA) metabolism, membranes, root growth, pollination and may benefit in the translocation of sugar. Molybdenum (Mo) is an essential microelement for plant growth, especially for more needs species in nitrogen or activate the process of biological nitrogen fixation (BNF) (Zoz *et al*., 2012). Copper (Cu) has an essential role in the metabolic processes of plant like photosynthesis and lower respiration in pollen capability and its deficiency rises infertility of spikelet in lot of unfilled grains (Ali *et al*., 2016).

Hence, this investigation was established to determine the influence of antioxidant, growth regulator, macro and micronutrient in addition to their mixture on germination and seedlings vigour of bread wheat Misr 1.

MATERIALS AND METHODS

A laboratory experiment was conducted at Agronomy Department Laboratory of Seed Testing, Faculty of Agriculture, Mansoura University, Egypt, during November 2018 to improve germination and seedlings vigour of wheat Misr 1 cultivar by soaking seeds in some antioxidant, growth regulator, macro and micronutrient in addition to their mixture .

This s investigation was carried out in completely randomized design (CRD) with four replications. The studied soaking wheat seeds treatments for 12 hours in ascorbic acid (AsA), indole-3-acetic acid (IAA), macronutrients (NPK) and micronutrients in addition to their mixture were as follows:

1- Without (control).
2- distilled water.
3- Ascorbic acid (AsA) at the rate of 0.1 g/L.
4- Indole-3-acetic Acid (IAA) at the rate of 0.05 g/L.
5- Truefert powder as a source of macronutrients (NPK) at the rate of 45 g/L.
6- Folifert powder as a source of micronutrients (Zn, Fe, Mn, Cu, B and Mo) at the rate of 3.75 g/L.
7- The combination of AsA and IAA at the aforementioned rates.
8- The combination of AsA and macronutrients at the same rates.
9- The combination of IAA and macronutrients at the same rates.
10- The combination of AsA and micronutrients at the same rates.
11- The combination of IAA and micronutrients at the rate of 0.05 g/L + at the same rates.
12- The combination of macronutrients and micronutrients at the same rates.
13- The combination of AsA, IAA, macronutrients and micronutrients at the aforesaid rates.

Standard germination test:

Random sample of 400 seeds per each treatment were allowed to germinate during November 2018, as the rules of International Seed Testing Association (ISTA, 2013) on top filter paper in sterilized Petri-dishes (14 cm diameter). Each Petri-dish contains 25 seeds, and four Petri-dishes kept close together and assessed as though they were one 100-seed replication under the environmental conditions of Laboratory for Seed Testing in Agronomy Department, Faculty of Agriculture, Mansoura University, Egypt.

Dishes were inspected daily, and distilled water added as required. Grains are considered physiologically germinated when the radical pierced the coleorhiza and reach approximately 2 to 3 mm long.

A. GERMINATION PARAMETERS:

1. Final germination percentage (FGP%). It was counted after 8 days from planting according to the following equation as described by ISTA (1996):

$$FGP = \frac{\text{Number of germination seeds}}{\text{Total Number of tested seeds}} \times 100$$

2. Abnormal seedlings percentage (AS%). It was counted and expressed by the percentage of abnormal seedlings after 8 days according to ISTA (1996).

3.Sold grains(%). It was counted and expressed by the percentage of sold grains at the end of germination test according to ISTA (1996).

4.Rotten grains (%). It was counted and expressed by the percentage of rotten grains at the end of germination test according to ISTA (1996).

5.speed germination index (SGI). It was calculated by the following formula (ISTA, 1996):

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

6. Germination index (GI %). It was calculated according to the following equation Karim et al. (1992) as the following equation:

$$GI = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in the control}} \times 100$$

7. Co-efficient of germination (CG). It was calculated using the following formula according to Copeland (1976):

$$CG = \frac{100\,(A_1 + A_2 + \dots + A_n)}{A_1 T_1 + A_2 T_2 + \dots A_n T_n}$$

Where;
A = Number of seed germinated.
T = Time (days) corresponding to A.
n = No. of days to final count.

8. Mean germination time (day). It was measured due to the following equation as showed by 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.

9. Germination energy percentage (GE %). It was determined from the percentage of germinating grains at the first count (4 days after sowing) relative to the total number of tested seeds as described by Ruan et al. (2002)

$$EG = \frac{\text{Number of germinated seeds after four days}}{\text{Number of seeds tested}} \times 100$$

B. SEEDLING PARAMETERS:

After 8 days from planting, ten seedlings were randomly selected from each replication and each treatment for evaluating the followed parameters:

1. Shoot length (cm). Ten of seedlings from the seed to the tip of the bade were recorded in centimeters (cm).

2. Root length (cm). Ten of seedlings from the seed to the tip of the root were recorded in centimeters (cm).

3. Seedling vigor index (SVI). It was calculated according to the formula of AbdulBaki and Anderson (1973):

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

4. Shoot fresh weight (g). The weight of ten seedling shoots were weighted in gram (g).

5. Root fresh weight (g). The weight of ten seedling roots were weighted in gram (g).

6. Shoot dry weight (g). The weight of ten seedling shoots were recorded after oven drying at 70 ℃ for 24h according to Agrawal (1986).

7. Root dry weight (g). Weight of ten seedling roots were recorded after oven drying at 70 ℃ for 24 h according to Agrawal (1986).

8. Photosynthetic pigments. Its were extracted for 24 hours from the 1st leaf in methanol with traces of sodium carbonate, and after that determined spectrophotometrically (T60 UV-visible spectrophotometer, PG Instruments Limited, Uk) as described by Lichtenthaler and Wellburn (1985).

Chlorophyll-a = 15.65 A666 – 7.340 A653

Chlorophyll-b = 27.05 A653 – 11.21 A666

Total chlorophylls = Chlorophyll-a + Chlorophyll-b

9. To determine macro-elements (NPK) in wheat shoot, 0.2 g of crude dried pea shoot was kept powder from each previous sample and wet digested with a mixture of concentrated sulphuric and perchloric acid (Peterburgski, 1968). Nitrogen content (N %) was determined in dried pea shoot using Kjeldahl method described by Jackson (1967). Phosphorus content (P %) was determined coloremitricaly using the chlorostannus reduce molybdo phosphoric blue colours method in sulphoric system (Jackson, 1967). Potassium content (K %) was determined in the digested pea shoot using a flame photometer according to Black (1965).

10-The electrical conductivity (EC). of solution was measured after the removal of samples from incubator with a pipette-type conductivity cell to a bulk conductivity meter (WPA linton CAMBRIDGE CMD 830). Between readings the dip cell rinsed in distilled water according to Matthews and Alison (1987) method. The mean of the four samples conductivity reading for each replicate was calculated and expressed per gram of seed and reported as μ mhos/g.

Rendering to the system of analysis of variance (ANOVA) was used for the Completely Randomized Design (CRD) as published by Gomez and Gomez (1984) of the subjected data was statistically analyzed. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5 % level of probability as described Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Regarding to soak wheat seeds treatments in Ascorbic Acid (AsA), indole-3-acetic acid (IAA), macronutrients (NPK) and micronutrients and some mixture of them for 12 h meaningfully influence on most of germination characteres. Results showed that soaking wheat seeds in ascorbic acid (AsA) + micro (Zn,Fe,Mn,Cu,Mo,B) at the rates of 0.1 g/l+ 3.75g/l, respectively for 12 hours resulted the maximum value of final germination percentage (FGP%), speed germination index (SGI), germination index (GI), co-efficient of germination (CG), mean germination time (MGT), germination energy percentage (GE %), shoot length, root length , seedling vigor index (SVI), shoot fresh weight ,root fresh weight, shoot dry weight, root dry weight, chlorophyll -a, chlorophyll -b, total chlorophyll, nitrogen percentages, phosphorus percentages and potassium percentages, as shown in Tables (1,2,3 and 4).

Also, soaking wheat seeds in indole-3-acetic acid (IAA) + Micro (Zn,Fe,Mn,Cu,Mo,B) at the rates of 0.05 g/l+ 3.75g/l , respectively for 12 hours presented the second rank after aforementioned treatment ,as shown in Tables (1,2,3and 4).

By contrast, Soaking wheat seeds in Ascorbic acid (AsA) + Micro (Zn,Fe,Mn,Cu,Mo,B) at the rates of 0.1 g/l+ 3.75g/l , respectively for 12 hours before starting germination test reduced and presented the minimum value of abnormal seedlings percentage (AS %), sold grains percentage (SG%), rotten grains (RG%) and the electrical conductivity (EC) as compared with control treatment as compared with control treatment, as shown in Tables 1 and 4 .

The favorable effect of soaking wheat grains in ascorbic acid (AsA) on seedlings parameters may be back to the role of ascorbic acid (AsA) is consider as a vital metabolite which was used in many cellular processes, as cell division. Furthermore, AsA is utilized during the early phases of germination by both zygotic in addition somatic embryos (Kandil *et al.* 2015b) and the central role of microelements as an enzymatic activator responsible for increased the plant, hence development early growth, increased dry matter accumulation and energizing the building of metabolic products (Badawi *et al.*, 2014). Also, soaking seeds with IAA has an important effect on germination because IAA support the germination of seeds and buds, it helps to elongate the stem and growth of leaves, flowering activates and assistances the enhancement of fruits (Al-Shaheen *et al.,* 2020). soaking seeds in macronutrients will support develop the process of water imbibition to the seed. By the water and oxygen get into the seed, it might speed launching the process of oxygen imbibition to the seed to break down the endosperm that will offering energy for the seed germination (Sinay, 2019). Our result is similar with the research of Rehman *et al.* (2015), Kandil *et al.*(2015a) and Badawi *et al.* (2014).

Table 1. Means of final germination, abnormal seedlings, sold, rotten grains percentages, speed germination index (SGI) and germination index (GI %), as affected by soaking wheat seeds treatments in some antioxidant, growth regulator, macro and micro-nutrients as well as their mixture at the end of germination test.

Characters Treatments	Final germination (%)	Abnormal seedlings (%)	Sold grains (%)	Rotten grains (%)	SGI	GI %
Without (control)	33.0	13.5	27.0	26.5	3.55	1.000
Distilled water	42.5	6.0	22.5	29.0	4.67	1.485
Ascorbic acid (AsA)	87.5	3.0	6.0	3.5	10.76	3.080
Indole-3-Acetic Acid (IAA)	83.0	3.0	10.5	3.5	10.19	3.015
Macro-nutrients	60.5	4.5	28.5	6.5	5.25	1.758
Micro-nutrients	87.0	2.5	7.5	3.0	10.70	3.070
AsA + IAA	88.0	2.5	6.5	3.0	10.80	3.132
AsA + Macro-nutrients	71.5	3.5	20.5	4.5	7.37	2.598
IAA+ Macro-nutrients	71.0	4.0	20.0	5.0	8.10	2.520
AsA + Micro-nutrients	92.0	2.0	4.5	1.5	10.98	3.200
IAA+ Micro-nutrients	90.0	2.0	6.0	2.0	10.82	3.195
Macro + Micro nutrients	47.0	4.5	40.5	8.0	5.42	1.582
Macro + Micro + AsA + IAA	43.5	5.0	42.5	9.0	5.19	1.502
LSD at 5 %	11.4	3.71	10.64	12.53	1.37	0.825

Table 2. Means of co-efficient of germination (CG), mean germination time (MGT), germination energy percentage (GE %), shoot length, root length and seedling vigor index (SVI) as affected by soaking wheat seeds treatments in some antioxidant, growth regulator, macro and micro-nutrients as well as their mixture at the end of germination test.

Characters Treatments	CG	MGT (day)	GE %	Shoot length (cm)	Root length (cm)	SVI
Without (control)	19.28	16.50	3.00	3.06	2.54	1.84
Distilled water	20.40	21.25	4.00	3.41	2.72	2.57
Ascorbic acid (AsA)	24.23	43.75	16.00	4.95	4.37	8.11
Indole-3-Acetic Acid (IAA)	23.48	41.50	10.00	4.18	3.83	6.70
Macro-nutrients	22.30	23.50	7.00	3.79	3.22	3.42
Micro-nutrients	24.20	43.50	13.00	4.31	4.18	7.35
AsA + IAA	24.34	44.00	18.00	5.16	4.46	8.46
AsA + Macro-nutrients	23.23	35.75	9.00	4.07	3.72	5.54
IAA+ Macro-nutrients	23.20	35.50	7.00	3.96	3.61	5.39
AsA + Micro-nutrients	24.59	46.00	58.00	6.64	5.37	11.05
IAA+ Micro-nutrients	24.46	45.00	53.00	5.96	4.48	9.39
Macro + Micro nutrients	21.83	22.75	6.00	3.72	3.17	3.23
Macro + Micro + AsA + IAA	21.06	21.75	6.00	3.44	2.87	2.71
LSD at 5 %	19.28	5.71	25.06	1.14	1.10	1.30

Table 3. Means of shoot and root fresh and dry weights, chlorophyll a and chlorophyll b of wheat seedlings affected by soaking wheat seeds treatments in some antioxidant, growth regulator, macro and micro-nutrients as well as their mixture at the end of germination test.

Characters Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)
Without (control)	0.038	0.030	0.016	0.016	0.097	0.030
Distilled water	0.057	0.032	0.019	0.016	0.114	0.053
Ascorbic acid (AsA)	0.132	0.077	0.032	0.025	0.387	0.260
Indole-3-Acetic Acid (IAA)	0.105	0.058	0.030	0.025	0.365	0.183
Macro-nutrients	0.092	0.053	0.024	0.024	0.244	0.137
Micro-nutrients	0.115	0.065	0.032	0.025	0.381	0.230
AsA + IAA	0.157	0.102	0.032	0.026	0.408	0.263
AsA + Macro-nutrients	0.098	0.055	0.028	0.025	0.360	0.169
IAA+ Macro-nutrients	0.098	0.055	0.026	0.025	0.304	0.146
AsA + Micro-nutrients	0.170	0.143	0.035	0.031	0.588	0.326
IAA+ Micro-nutrients	0.165	0.118	0.034	0.028	0.484	0.298
Macro + Micro -nutrients	0.078	0.045	0.023	0.019	0.154	0.117
Macro + Micro + AsA + IAA	0.060	0.045	0.021	0.017	0.138	0.089
LSD at 5 %	0.031	0.028	6.859	6.859	0.202	0.103

Table 4. Means of total chlorophyll,macro-nutrients (NPK) percentages in wheat shoot and electrical conductivity (EC) as affected by soaking wheat seeds treatments in some antioxidant, growth regulator, macro and micro-nutrients as well as their mixture at the end of germination test.

Characters Treatments	Total chlorophyll (mg g^{-1} FW)	N (%)	P (%)	K (%)	EC (μ mhos/g)
Without(control)	1.040	3.280	0.320	0.630	0.178
Distilled water	1.050	3.760	0.320	0.820	0.165
Ascorbic acid (AsA)	1.200	4.420	0.680	1.490	0.085
Indole-3-Acetic Acid (IAA)	1.120	4.080	0.500	1.300	0.112
Macro-nutrients	1.105	3.860	0.390	1.150	0.140
Micro-nutrients	1.125	4.320	0.550	1.330	0.093
AsA + IAA	1.205	4.680	0.700	1.600	0.077
AsA + Macro-nutrients	1.115	4.040	0.450	1.280	0.123
IAA+ Macro-nutrients	1.115	3.950	0.400	1.230	0.125
AsA + Micro-nutrients	1.260	5.040	0.830	1.890	0.057
IAA+ Micro-nutrients	1.260	4.700	0.720	1.770	0.072
Macro + Micro-nutrients	1.085	3.830	0.340	1.030	0.155
Macro + Micro + AsA + IAA	1.085	3.780	0.330	0.980	0.155
LSD at 5 %	0.112	0.052	0.233	0.170	0.0883

CONCLUSION

In conclusion, soaking wheat seeds in combination of ASA + micronutrients or IAA + micronutrients improved germination and seedlings vigor of wheat.

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زيادة إنبات القمح و وقوة البادرات باستخدام بعض منظمات النمو ومضادات الأكسدة والعناصر الغذائية

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لتعزيز صفات بادرات قمح الخبز صنف مصر 1عن طريق النقع في حمض الأسكوربيك كمضادات الأكسدة ، و أندول حمض الخليك كمنظم النمو ، ومسحوق Truefert كمصدر للمغذيات الكبرى (NPK) ومسحوق Folifert كمصدر للمغذيات الدقيقة (الزنك ، الحديد ، المنجنيز ، النحاس ، الموليبدينوم ، البورون) تم إجراء تجربة معملية في مختبر قسم المحاصيل ، كلية الزراعة ، جامعة المنصورة ، مصر خلال شهر نوفمبر 2018. نفذت الدراسة بتصميم تام العشوائية في أربع مكررات. أظهرت النتائج المتحصل عليها أن نقع تقاوى القمح في حمض الأسكوربيك (AsA) + مغذيات دقيقة بمعدل (0,1 جم / لتر + 3,75 جم / لتر) على التوالي لمدة 12 ساعة كانت أفضل معاملة حيث أنتجت القيم القصوى لمعظم صفات البادرات ، متبوعة بـ نقع تقاوى القمح في أندول حمض الخليك (IAA)+ مغذيات دقيقة بمعدل (0,05 جم / لتر + 3,75 جم / لتر) على التوالي لمدة 12 ساعة ، ويوصى بنقع تقاوى القمح صنف مصر 1 ; حيث أثبتت النتائج المتحصل عليها من الدراسة أن معاملة تقاوى القمح بمزيج من حمض الأسكوربيك (AsA) + المغذيات الدقيقة أو بمزيج من أندول حمض الخليك (IAA) + المغذيات الدقيقة لمدة 12 ساعة أدي إلى تحسين إنبات وقوة بادرات القمح.