International Journal of Advanced Research in Biological Sciences

ISSN : 2348-8069

www.ijarbs.com

Research Article

Maize seedlings characters as affected by soaking in some natural and artificial substances

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Abstract

To enhancing seedlings parameters of maize hybrid TWC 352 of both old and new harvested grains by soaking in different times in some natural and artificial substances a laboratory experiment was conducted at Agronomy Department Laboratory of Seed Testing, Faculty of Agriculture, Mansoura University, Egypt, during May 2014. Each type of maize grain of both old or new harvested grains was performed in separate experiment. Every experiment of grain types was carried out in factorial experiment in completely randomized design (CRD). The results revealed that new harvested maize grains significantly exceeded old harvested maize grains in radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content, which recorded highest values of these characters. Soaking maize grains in ascorbic acid (AA) at the rate of 100 ppm significantly increased all studied seedlings characters. Generally, increasing times of soaking significantly increased all studied seedlings characters. Highest percentages of these characters were produced from soaking maize grains in different substances for 18 hours. It could be concluded that for maximizing seedlings parameters of both old or new harvested grains maize hybrid TWC 352, soaking in antioxidant substances such as ascorbic acid or citric acid or salicylic acid or natural substances like yeast extract at the rate of 100 ppm of each one for 18 hours.

Keywords: Maize, Soaking treatments, GA_3, IAA, Salicylic acid, Ascorbic acid, Times of soaking, Seedlings parameter.

Introduction

Maize (*Zea mays* L.) is an important crop in the world, which ranks the third in world production following wheat and rice for the area and production. In Egypt, maize is considered also as one of the main cereal crops, comes the third after wheat and rice. Therefore, a great attention should be paid to raise maize productivity either by increasing the cultivated area or maximizing yield per unit area in order to reduce the gap between its production and consumption.

Higher production and productivity of crop is possible only through use of good quality seeds and proper management practices. 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”. The main objective of this technique is to optimize application of seed treatment products by improving technical quality of seeds. Seed soaking could improve early seedling growth under stress conditions compared to plants grown from untreated seed (**Sharifi and Khavazi, 2011**).

Growth regulators such as gibberellins (GA_3) or Indole acetic acid (IAA) which produced naturally by plants, moreover its applied to plants as a spray to foliage or as a liquid drench to seeds to encouragement the growth of seeds, increase the economic yield and to enable the plant to adapt the adverse conditions. **Anosheh et al. (2014)** found that seed priming with optimal concentrations of plant growth hormones, such as auxin (IAA), gibberellins (GA_3), abscisic acid, and ethylene, has proven that germination performance as well as growth and yield of many crop species under both normal and stress conditions could be improved effectively.

Salicylic acid (SA) is considered as a hormone like substance, which acting an important role in regulating a number of physiological processes such as ion uptake and transport, inhibition of ethylene biosynthesis, transpiration, photosynthesis and growth, nitrate metabolism and stress tolerance (**Khan et al., 2010**). **Tonel et al. (2013)** found that the salicylic acid (SA) application has minimized damages induced by salt stress to maize seed germination by favoring their germination percent. However, without SA was not able on maintaining seed viability.

Ascorbic acid (AsA) is one of the most important antioxidant at cellular processes including cell division and expansion, and at metabolism activity when germination started (**Arrigoni et al. 1992**), cell detoxification, protecting cell from reactive oxygen species and preventing death cell (**Conklin and Barth 2004**). 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**). **Conklin and Barth (2004)** pointed out that ascorbic acid (AsA) and its associated enzyme, ascorbate peroxidase, play diverse roles in several physiological processes in plants.

Citric acid is an organic compound belonging to the family of carboxylic acids. It presents in practically all plants. It is one of a series of compounds involved in the physiological oxidation of fats, proteins and carbohydrates to CO_2 and water (**Abd-Allah et al., 2007**). **Ananou et al. (2007) and Nielsen and Arneborg (2007)** reported that citric acid (CA) is effective in controlling food-borne yeasts and bacterial pathogens, its antimicrobial properties resting primarily in the chelation of divalent cations.

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**).

Therefore, this investigation was established to enhance grain maize hybrid TWC 352 of both old and new harvested grains seedlings parameters by soaking in different times in some natural and artificial substances.

Materials and Methods

This research was conducted at Agronomy Department Laboratory of Seed Testing, Faculty of Agriculture, Mansoura University, Egypt, during May 2014. The objective of this investigation was aimed to enhance grain maize hybrid TWC 352 of both old and new harvested grains seedlings parameters by soaking in different times in some natural and artificial substances. Grain maize hybrid TWC 352 of both old or new harvested grains were produced and obtained from Experimental Farm of Gemmeiza Agriculture Research Station, Agricultural Research Center (ARC), Egypt.

Each type of maize grain of both old or new harvested grains was performed in separate experiment. Old harvested maize grains were resulted from 2012 growing season, whereas new harvested maize grains were resulted from 2013 growing season and both were examined for germination in May 2014.

Every experiment of grain types was carried out in factorial experiment in completely randomized design (CRD). The first factor included seven soaking grain treatments in some natural and artificial substances beside control treatment (untreated "without soaking") as follows; 1) Soaking maize grains in distilled water. 2) Soaking maize grains in gibberellic acid (GA_3) at the rate of 100 ppm. 3) Soaking maize grains in indol acetic acid (IAA) at the rate of 100 ppm. 4) Soaking maize grains in salicylic acid (SA) at the rate of 100 ppm. 5) Soaking maize grains in ascorbic acid (AA) at the rate of 100 ppm. 6) Soaking maize grains in citric acid (CA) at the rate of 100 ppm. 7) Soaking maize grains in yeast extract (YE) at the rate of 100 ppm.

Gibberellic acid (GA) and indol acetic acid (IAA) as artificial growth regulators and salicylic acid (SA), ascorbic acid (AA) and citric acid (CA) as antioxidants were produced by El-Nasr Pharmaceutical Chemicals Co., Egypt, and obtained from El-Gomhouria Company for Trading

Pharmaceutical Chemical & Medical. Yeast extract (YE) as natural biostimulants was prepared by using a technique allowed yeast cells (pure dry yeast) to be grown and multiplied efficiently during conducive aerobic and nutritional conditions. Thus method allowed to produce denovo beneficial bioconstituent, (carbohydrates, sugars, proteins, amino acids, fatty acids, hormones, etc.), then these constituents could release out of yeast cells in readily form. Active dry yeast were dissolved in water at rate 1 g/L followed by adding sugar at ratio 1:1 and kept overnight for activation and reproduction of yeast and two cycles of freezing and thawing for disruption of yeast cells and releasing their content. Such technique for yeast preparation was modified by **Spencer *et al.* (1983)**.

The second factor included three times of soaking treatments at 6, 12 and 18 hours.

Standard germination test:

Random sample of 400 grains per each treatment were sown on top filter paper in sterilized Petri-dishes (14 cm diameter). Each Petri-dish contain 25 grain, and four Petri-dishes kept close together and assessed as though they were one 100 – grains replication under the environmental conditions of Laboratory for Grain Testing in Agronomy Department, Faculty of Agriculture, Mansoura University, Egypt at 14th June 2014 as the rules of International Seed Testing Association (**ISTA, 1986**).

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.

The germinated grains were counted and first count defined as the number of germinated grains at the fourth day. Then, every 24 hours the number of germinated grains were counted until end of germination test (7 days). Grains were categorized as germinated (radical 2 mm long), hard (no imbibitions or swelling) or nonviable (abnormal, dead or infected seeds) as described **ISTA (1996**).

SEEDLING PARAMETERS:

1- Radical length: The radical length of ten seedlings taken by random per each replicate from the grain to the tip of the radical and recorded and expressed in centimeters (cm) as the radical length at the end of standard germination test.

2- Plumel length: The length of the ten seedlings taken by random per each replicate from the grain to the tip of the leaf blade were recorded and expressed in centimeters as the plumel length at the end of standard germination test.

3- Seedling vigor index (SVI). It was calculated according the formula as suggested by **AbdulBaki and Anderson (1973)**:

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

4- Radical dry weight: The weight of ten seedling roots at random per replicate were recorded and expressed in milligram (mg) after oven drying at 70 º C until constant weight (**Agrawal, 1986**).

5- Plumel dry weight: The weight of ten seedling plumel at random per replicate were recorded and expressed in milligram (mg) after oven drying at 70 º C until constant weight (**Agrawal, 1986**).

6- Total chlorophyll (SPAD): Total chlorophyll content was assessed in wide leaf of plumel by SPAD-502 (Minolta Co. Ltd., Osaka, Japan).

Data were subjected to the statistical analysis according to the technique of analysis of variance (ANOVA) for the factorial completely randomized design to each experiment (grain types), then combined analysis was done between grain types experiments as published by **Gomez and Gomez (1984)** by using “MSTAT-C ” computer software package. Least significant of difference (LSD) method was used to test the differences between treatment means at 5 % level of probability as described by **Snedecor and Cochran (1980)**.

Results and Discussion

1- Effect of types of maize grains of both old or new harvested grains:

Results presented in Tables 1 and 2 show that there were significant differences in seedling parameters *i.e.* radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content in maize plumel by SPAD unit between types of maize grains of both old or new harvested grains.

Table 1: Radical length, plumel length and seedling vigor index (SVI) as affected by types of maize grain, soaking grain treatments and times of soaking as well as their interactions.

Character / Treatments	**Radical length (cm)**			**Plumel length (cm)**			**Seedling vigor index (SVI)**		
	Old grains	New grains	Combined	Old grains	New grains	Combined	Old grains	New grains	Combined
Soaking grain treatments:									
Untreated (control)	4.30	5.32	4.81	4.25	4.82	4.53	6.76	8.42	7.59
Distilled water	5.50	6.50	6.00	5.40	6.15	5.77	9.68	11.55	10.62
Gibberellic acid (GA_3)	10.22	11.86	11.04	6.69	7.09	6.89	15.06	17.61	16.33
Indol acetic acid (IAA)	8.24	9.59	8.91	6.17	6.45	6.31	12.90	14.79	13.85
Salicylic acid (SA)	12.31	13.89	13.10	7.98	8.52	8.25	18.85	21.55	20.20
Ascorbic acid (AA)	15.36	17.26	16.31	9.96	10.29	10.12	24.76	27.36	26.06
Citric acid (CA)	14.43	15.06	14.75	8.75	9.00	8.87	22.16	23.33	22.75
Yeast extract (YE)	12.20	14.26	13.23	7.39	7.80	7.60	17.88	20.71	19.30
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	0.99	0.83	0.64	0.51	0.39	0.32	1.14	0.95	0.73
Times of soaking:									
6 hours	9.98	11.45	10.72	6.79	7.32	7.06	14.96	17.32	16.14
12 hours	11.26	12.58	11.92	7.37	7.72	7.55	17.32	19.26	18.29
18 hours	12.30	13.86	13.08	8.26	8.66	8.46	19.71	22.10	20.90
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	0.64	0.54	0.42	0.33	0.26	0.21	0.74	0.62	0.48
Means of grain types	11.18	12.63		7.48	7.90		17.33	19.56	
F. test	*			*			*		
C- Interactions (F. test):									
Grain types × Soaking treatments			NS			NS			NS
Grain types × Soaking times			NS			NS			NS
Soaking treatments × Soaking times			*			NS			*
Grain types ×Soaking treatments ×Soaking times			NS			NS			NS

Table 2: Radical dry weight, plumel dry weight and total chlorophyll content in shoots as affected by types of maize grain, soaking grain treatments and times of soaking as well as their interactions.

Character / Treatments	Radical dry weight (mg)			Plumel dry weight (mg)			Total chlorophyll content (SPAD)		
	Old grains	New grains	Combined	Old grains	New grains	Combined	Old grains	New grains	Combined
Soaking grain treatments:									
Untreated (control)	140.5	150.5	145.5	14.50	21.25	17.87	1.95	3.50	2.72
Distilled water	162.7	171.9	167.3	20.58	26.33	23.45	3.85	5.39	4.62
Gibberellic acid (GA_3)	177.5	187.6	182.6	26.91	32.58	29.75	5.78	7.40	6.59
Indol acetic acid (IAA)	173.0	185.5	179.2	25.00	30.41	27.70	4.35	6.00	5.17
Salicylic acid (SA)	195.1	237.9	216.5	30.83	44.08	37.45	8.36	10.05	9.20
Ascorbic acid (AA)	260.5	308.5	284.5	37.25	57.33	47.29	10.68	11.93	11.30
Citric acid (CA)	233.7	276.3	255.0	32.50	50.25	41.37	9.10	10.70	9.90
Yeast extract (YE)	185.1	195.0	190.1	27.33	33.91	30.62	7.22	8.82	8.02
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	1.6	1.8	1.2	0.88	0.72	0.56	1.00	1.05	0.72
Times of soaking:									
6 hours	176.7	201.0	188.89	23.92	36.78	30.35	5.91	7.45	6.68
12 hours	197.8	222.6	210.25	28.60	39.07	33.83	6.92	8.53	7.73
18 hours	220.1	246.2	233.17	33.35	41.96	37.66	8.31	9.86	9.08
F. test	*	*	*	*	*	*	*	*	*
LSD at 5 %	1.0	1.2	0.7	0.57	0.47	0.37	0.65	0.68	0.47
Means of grain types	198.2	223.2		28.63	39.27		7.05	8.61	
F. test	*			*			*		
C- Interactions (F. test):									
Grain types × Soaking treatments			*			*			NS
Grain types × Soaking times			*			*			NS
Soaking treatments × Soaking times			*			*			NS
Grain types ×Soaking treatments ×Soaking times			*			*			NS

New harvested maize grains significantly exceeded old harvested maize grains in radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content, which recorded the highest values of these characters and increased its by 12.96, 5.61, 12.86, 12.61, 37.16 and 22.12%, respectively as compared with old harvested maize grains. This reduction in seedlings characters of aged grains of both old harvested grains might be due to loss of seed viability which associated with Maillard reactions (**Murthy et al., 2003**), in addition remarkable reduction in seedlings growth which might due to decline in weight of aged grains **(Mohammadi et al., 2011**).

2. Effect of soaking grains treatments:

Regarding soaking maize grain treatments in some natural and artificial substances *i.e.* soaking maize grains in distilled water, gibberellic acid (GA_3), indol acetic acid (IAA), salicylic acid (SA), ascorbic acid (AA), citric acid (CA) and yeast extract (YE) at the rate of 100 ppm of each one, obtained results clearly show that there was a significant differences in seedling parameters *i.e.* radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content in maize plumel by SPAD unit among all studied treatments and control treatment (untreated grains) for both old or new grains and combined data (Tables 1 and 2).

Soaking maize grains in antioxidant substances such as ascorbic acid (AA) at the rate of 100 ppm before starting germination test significantly increased radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content and resulted in the highest values, in addition increased these characters by (257.21, 224.44and 239.09%), (134.35, 113.49 and 123.40%), (266.27, 224.94 and 243.35%), (85.41, 104.98 and 95.53%), (156.9, 169.79 and 164.63%) and (447.69, 240.86 and 315.44%) as compared with control treatment for both old or new grains and combined data, respectively.

However, soaking maize grains in antioxidant substances such as citric acid (CA) at the rate of 100 ppm ranked after aforementioned treatment and followed by soaking maize grains in salicylic acid (SA) at the rate of 100 ppm. The other soaking grain treatments could be arranged as follows; soaking maize grains in natural substances such as yeast extract (YE), indol acetic acid (IAA), gibberellic acid (GA_3), distilled water and untreated grains (control treatment) concerning its effect on radical length, plumel length, SVI, radical dry weight, plumel dry weight and total chlorophyll content for both old or new grains and combined data.

The favourable effect of soaking maize grains in ascorbic acid (AA) on seedlings characters may be ascribed to ascorbic acid is consider as an important metabolite involved in many cellular processes, including cell division. In addition, ascorbic acid is utilized during the initial stages of germination by both zygotic and somatic embryos (**Arrigoni et al., 1997)**. Besides, the desirable effect of citric acid on radical length of maize seedlings may be owing its effective role in controlling seed-borne diseases and bacterial pathogens, also its antimicrobial properties resting primarily in the chelation of divalent cations (**Nielsen and Arneborg, 2007**). In addition, the advantageous effect of salicylic acid on radical length of maize seedlings may be due to salicylic acid significantly stimulated the activities of enzymes involved in germination (**Eastmond and Graham, 2001**). The positive effects of applying yeast extract on radical length of maize seedlings may be attributed to its stimulatory effect on cell division and enlargement, protein and nucleic acid synthesis (**Wanas, 2002)**. The effective role of gibberellic acid (GA_3) in enhancing radical length of maize seedlings 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. Moreover, 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 (**Rood et al., 1990)**. Finally, the efficient role of indole acetic acid (IAA) in enhancing radical length of maize seedlings comparing with control treatment may be payable to indole acetic acid (IAA) is the common natural auxin that shows all auxin doing actions and extensively affects plant's physiology and seedlings characters (**Kelen et al., 2004**). These results in good accordance with those reported by **Dezfuli et al. (2008)** and **Ghalichechi and Azar (2013)**.

3. Effect of times of soaking:

Times of maize grain soaking treatments in some natural and artificial substances at 6, 12 and 18 hours significantly affected all studied characters which were seedling parameters *i.e.* radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content in maize plumel by SPAD unit for both old or new grains and combined data (Tables 1 and 2).

Generally, increasing times of soaking significantly increased radical length, plumel length, seedling vigor index (SVI), radical dry weight, plumel dry weight and total chlorophyll content final germination percentage. Thus, the highest percentages of these characters were produced from soaking maize grains in different substances for 18 hours of old, new grains and combined between them. On the other hand, the lowest percentages of these characters were obtained when soaking maize grains in different substances for 6 hours of both old or new grains and combined data. The favourable effect of increasing times of soaking maize grain treatments on radical length of maize seedlings may be due to increase the quantity of soaking substances obtained by grains, consequently shows best impact of these substances.

4. Effect of interactions:

The results indicated that there was significant effect as a result of the interaction between grain types X soaking grains treatments on radical dry weight (Fig. 1) and plumel dry weight (Fig. 2). Highest values of these characters were resulted from soaking new harvested maize grains in ascorbic acid (AA) at the rate of 100 ppm. Soaking new harvested maize grains in citric acid (CA) at the rate of 100 ppm ranked after previously mention interaction treatment, and followed by soaking new harvested maize grains in salicylic ascorbic acid (SA) at the rate of 100 ppm and then soaking old harvested maize grains in ascorbic acid (AA) at the rate of 100 ppm.

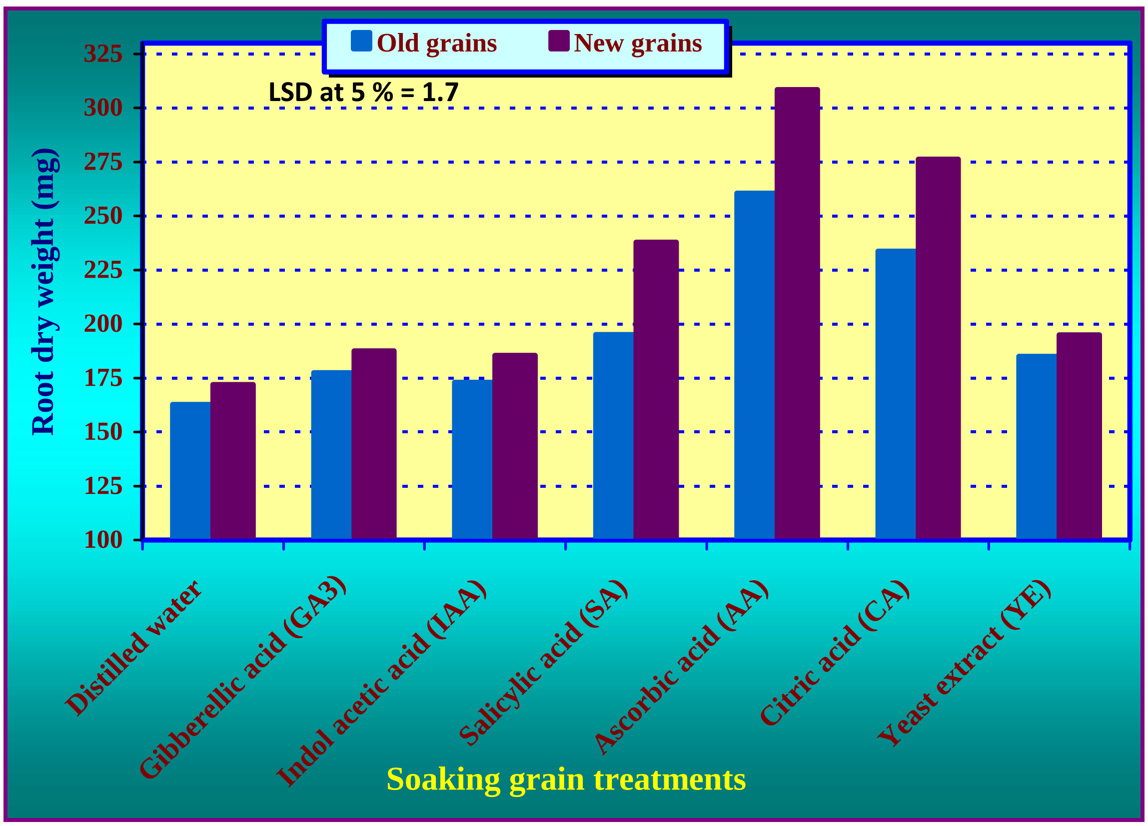

Fig. 1: Radical dry weight (mg) as affected by the interaction between types of maize grains and soaking treatments as combined over times of soaking.

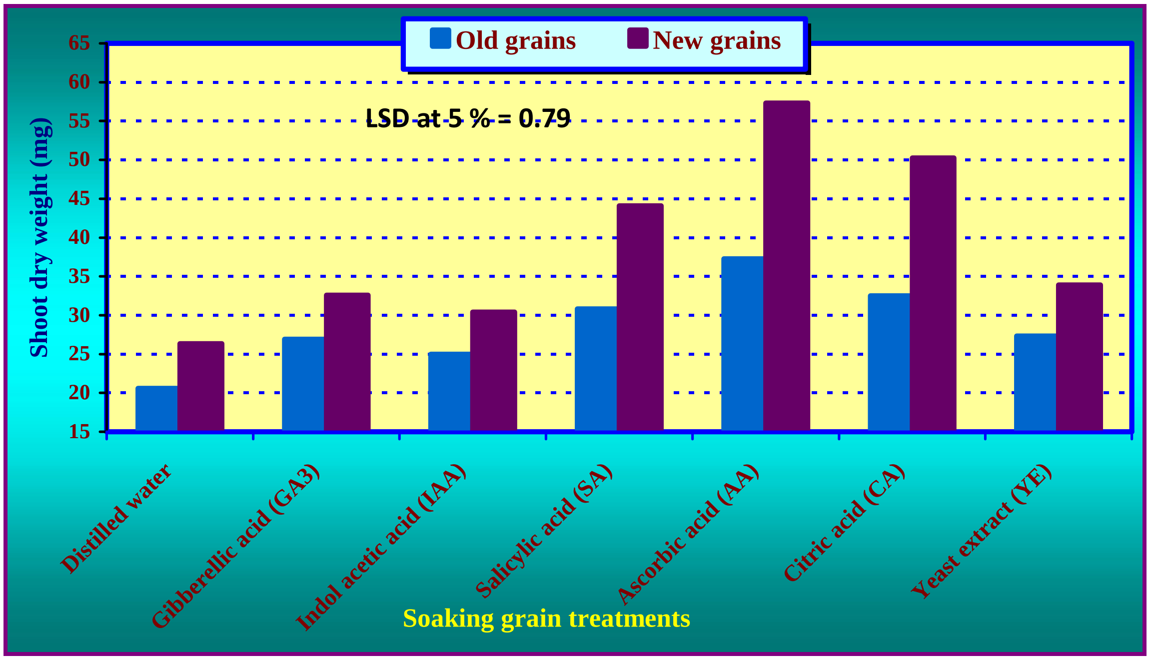

Fig. 2: Plumel dry weight (mg) as affected by the interaction between types of maize grains and soaking treatments as combined over times of soaking.

The results showed that radical dry weight (Fig. 3) and plumel dry weight (Fig. 4) were significantly affected due to the interaction between grain types X soaking times. Highest values of these characters were resulted from soaking new harvested maize grains for 18 hours. Soaking new harvested maize grains for 12 hours ranked after previously mention interaction treatment, and followed by soaking new harvested maize grains for 6 hours and soaking old harvested maize grains for 18 hours.

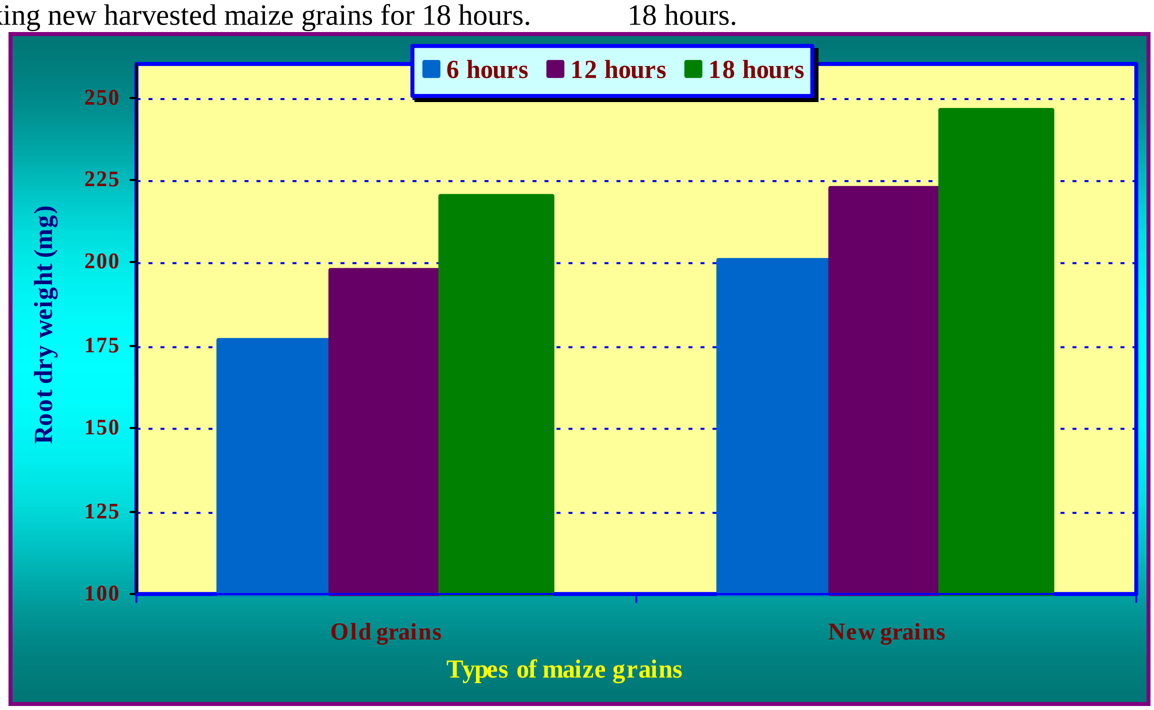

Fig. 3: Radical dry weight (mg) as affected by the interaction between types of maize grains and times of soaking as combined over soaking treatments.

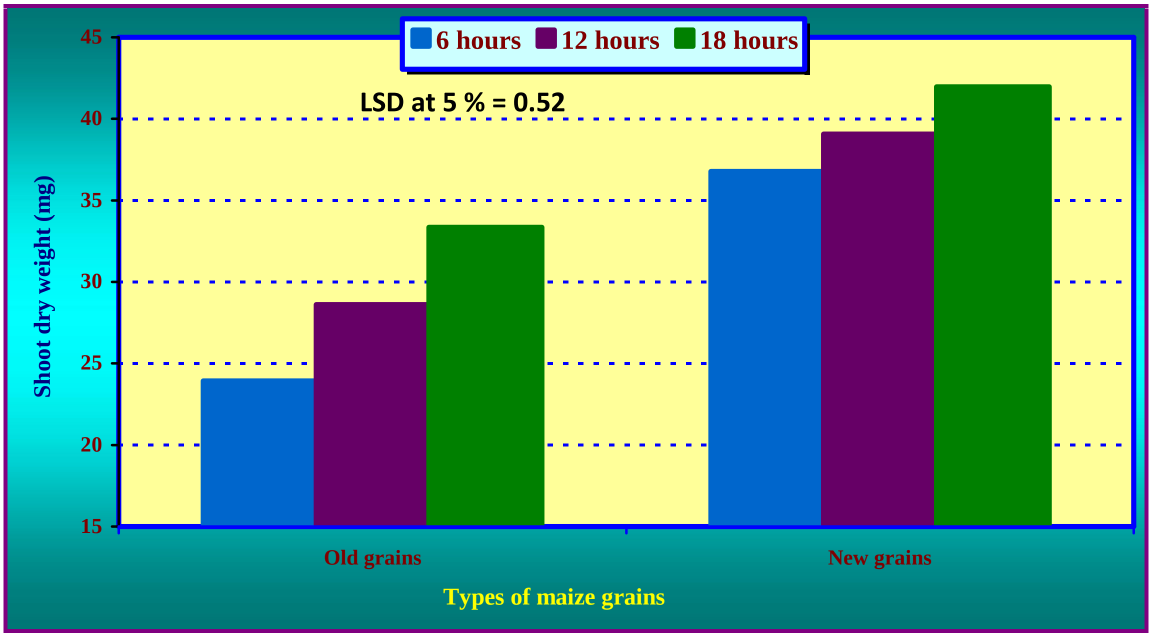

Fig. 4: Plumel dry weight (mg) as affected by the interaction between types of maize grains and times of soaking as combined over soaking treatments.

With respect to the effect of the interaction between soaking grains treatments soaking times, it was significant on radical length (Fig. 5), seedling vigor index (Fig. 6), radical dry weight (Fig. 7) and plumel dry weight (Fig. 8). Soaking maize grains in ascorbic acid (AA) at the rate of 100 ppm for 18 hours resulted highest values of these characters. Soaking maize grains in citric acid (CA) at the rate of 100 ppm from 18 hours ranked after previously mention interaction treatment, and followed by soaking maize grains in ascorbic acid (AA) at the rate of 100 ppm from 12 hours.

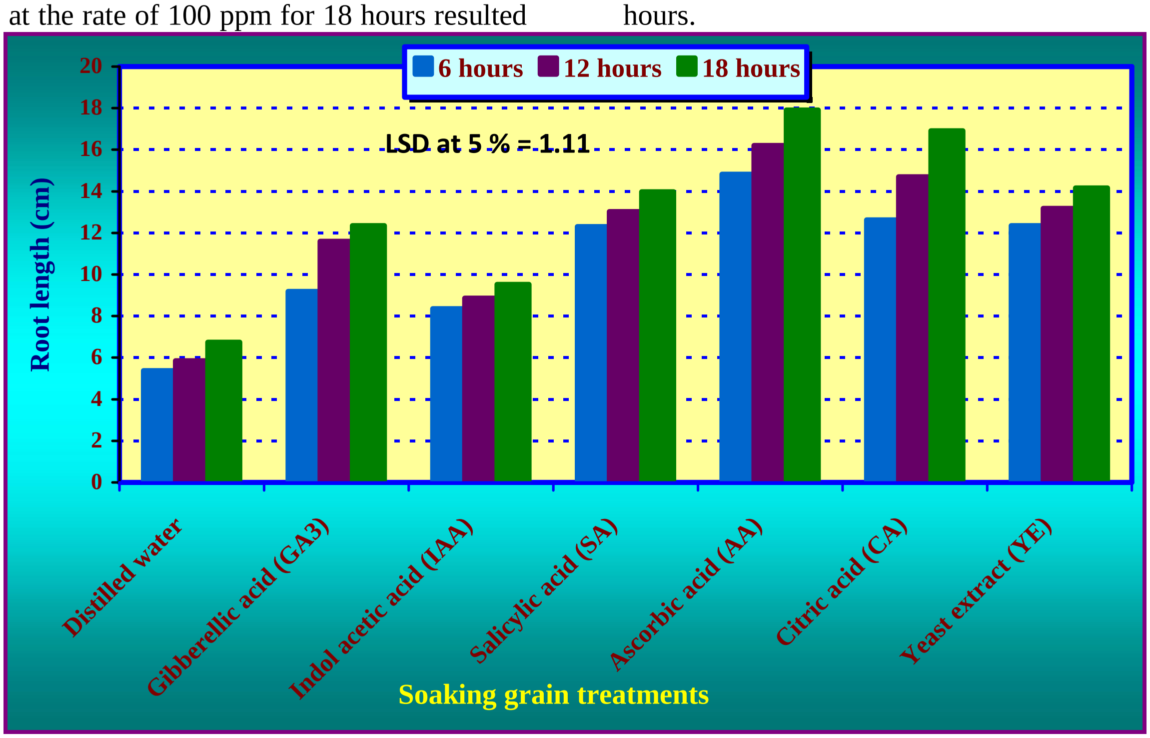

Fig. 5: Radical length (cm) as affected by the interaction between soaking treatments and times of soaking as combined over types of maize grains.

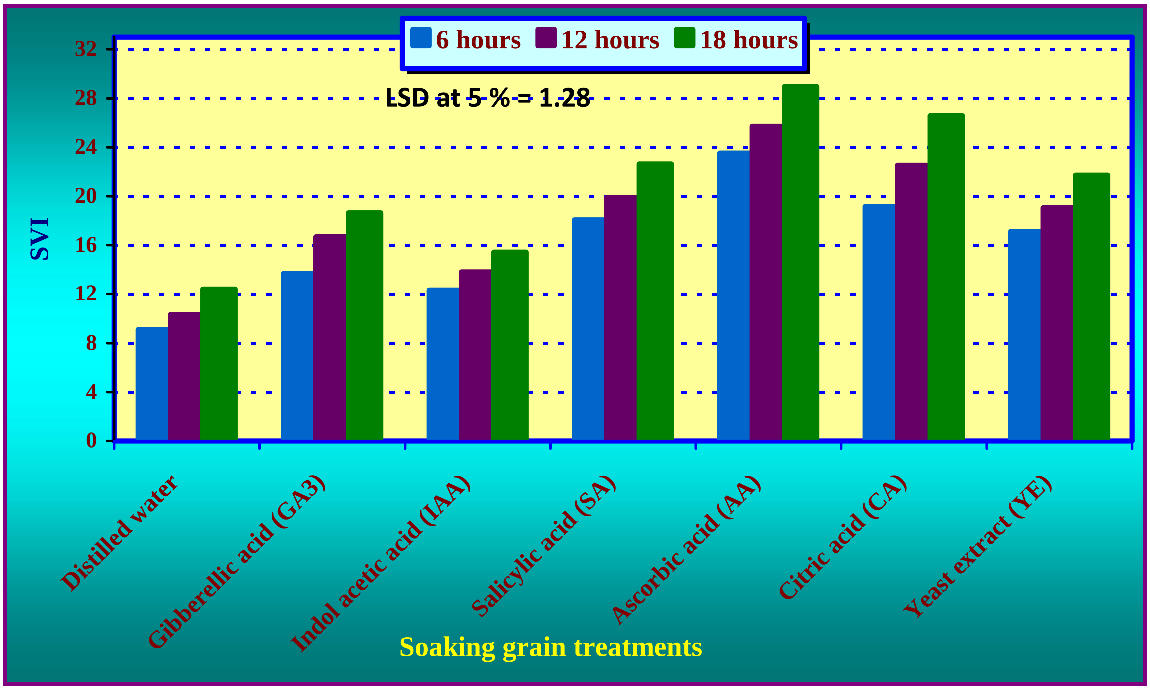

Fig. 6: Seedling vigor index (SVI) as affected by the interaction between soaking treatments and times of soaking as combined over types of maize grains.

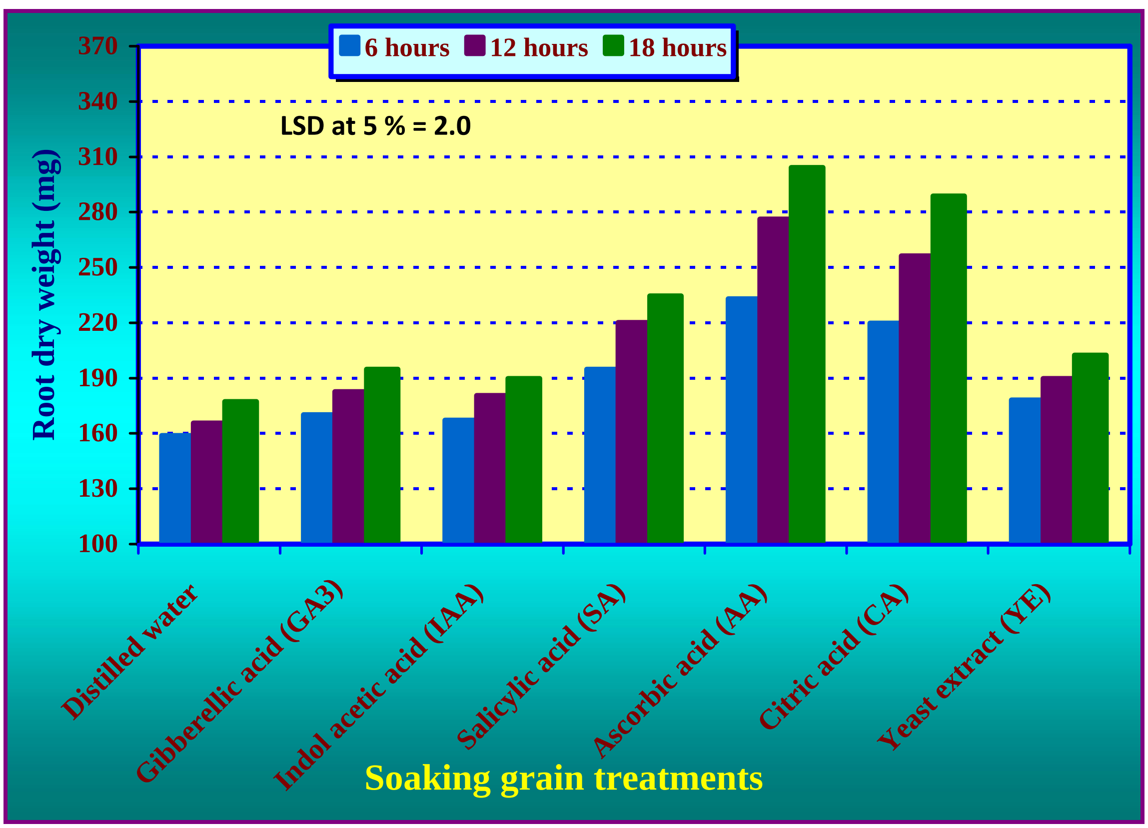

Fig. 7: Radical dry weight (mg) as affected by the interaction between soaking treatments and times of soaking as combined over types of maize grains.

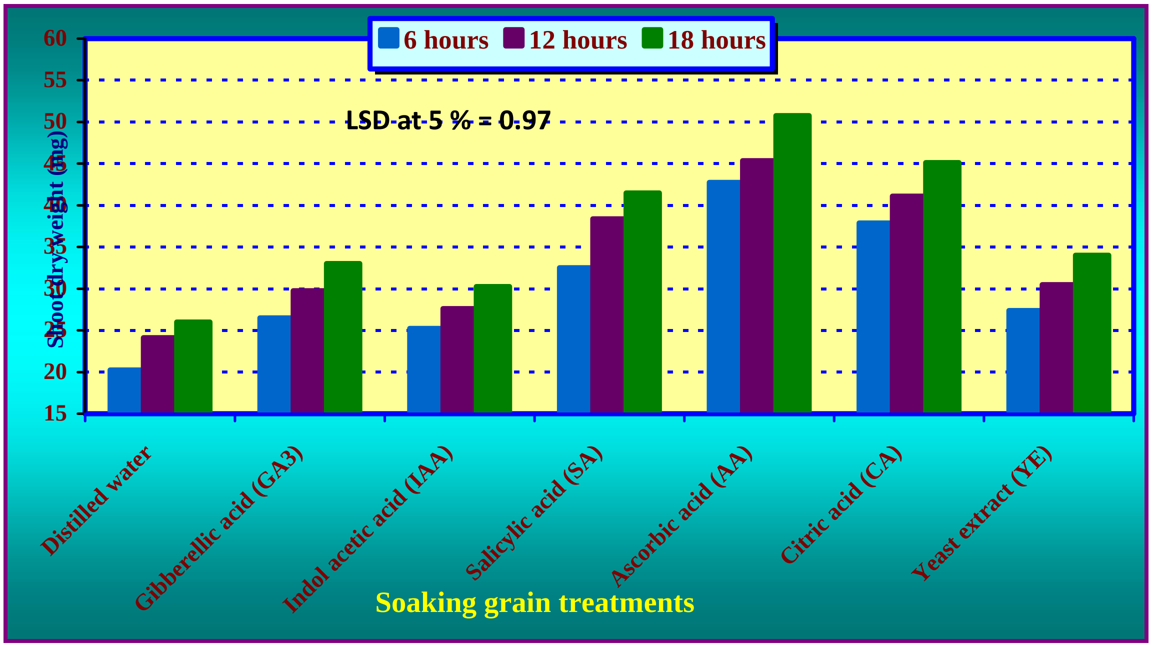

Fig. 8: Plumel dry weight (mg) as affected by the interaction between soaking treatments and times of soaking as combined over types of maize grains.

Concerning the third interaction among studied factors *i.e.* grain types X soaking grains treatments X soaking times, it exhibited significant effect on radical dry weight (Fig. 9) and plumel dry weight (Fig. 10). The highest values of these characters were obtained from soaking new maize grains in ascorbic acid (AA) at the rate of 100 ppm for 18 hours. The second best interaction treatment was soaking new maize grains in ascorbic acid (AA) at the rate of 100 ppm for 12 hours. The third best interaction treatments was soaking new maize grains in CA at the rate of 100 ppm for 18 hours.

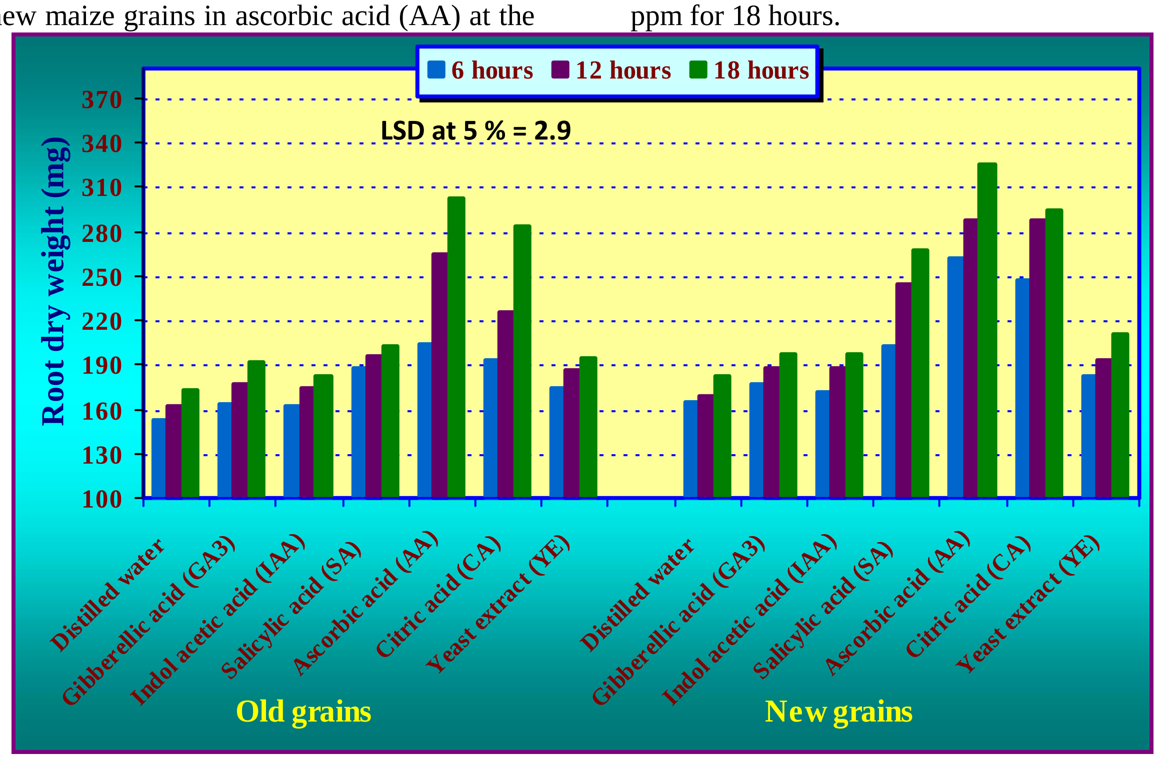

Fig. 9: Radical dry weight (mg) as affected by the interaction among types of maize grains, soaking treatments and times of soaking.

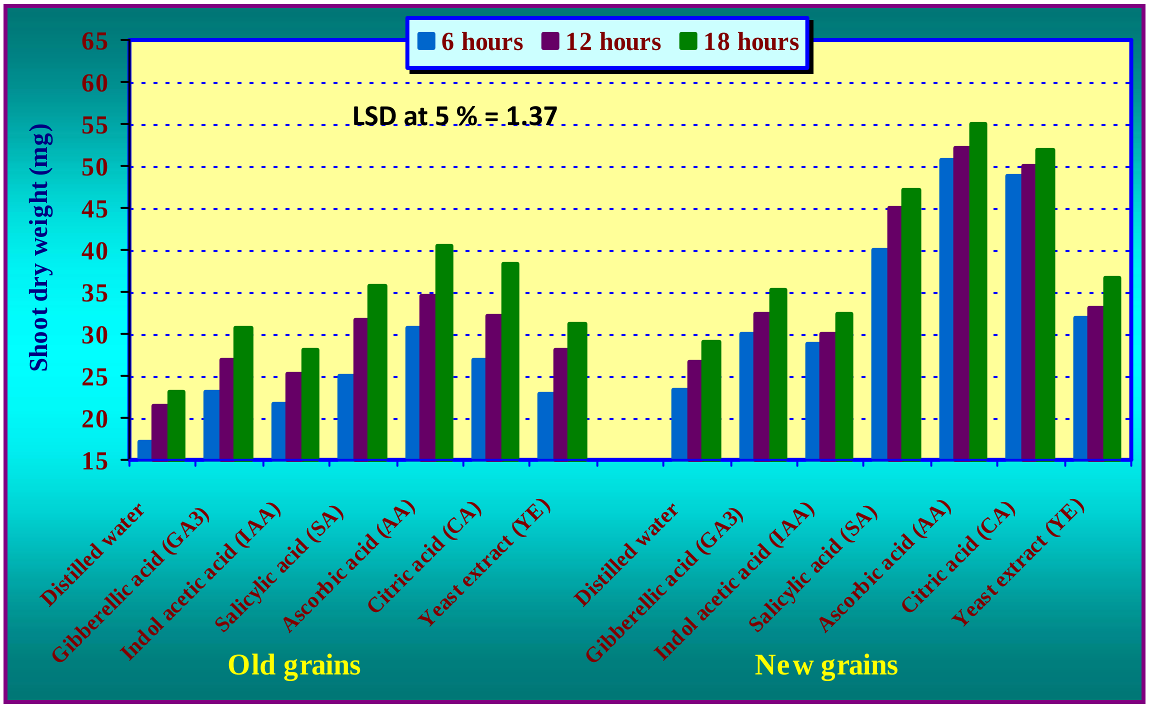

Fig. 10: Plumel dry weight (mg) as affected by the interaction among types of maize grains, soaking treatments and times of soaking.

Conclusion

For maximizing seedlings parameters of old or new harvested grains maize hybrid TWC 352, it could be recommended that soaking in antioxidant substances such as ascorbic acid or citric acid or salicylic acid or natural substances such as yeast extract at the rate of 100 ppm of each one for 18 hours.

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