

The Integrate Effect of Seed Soaking in Aqueous Plant Extracts and Ascorbic Acid on Germination and Seedling Characters of Rice Grains under Salinity Stress

El-Shimaa A. M. Abo-El-Kheer¹; S. B. Gad² and W. A. E. Abido³

¹Department of Agricultural Botany, Mansoura University, Egypt

²Department of Agricultural Zoology, Mansoura University, Egypt

³Department of Agronomy, Mansoura University, Egypt

Corresponding author: El-Shimaa A. M. Abo- El-Kheer

Email Address: dr_shymaa@mans.edu.eg



ABSTRACT

In the recent years using non-traditional sources such as aqueous extracts plant and ascorbic acid are considered as one of the challenges for enhancing germination character and seedling parameters of rice grains. Thus, a laboratory experiment was conducted to study the integrate effect of four plant extracts *i.e.* turmeric (*Curcuma longa*), sorrel (*Rumexvesicarius*), grapes (*Vitisvinifera*), and roselle (*Hibiscus sabdariffa*) in comparison with ascorbic acid (ASA) on germination characters and seedling parameters of rice under salinity stress, using Randomized Complete Block Design (RCBD) with four replications. Data revealed that there was a noticeable improvement in germination percentage and seedling vigor index as compared to control treatments treatment due to grains soaking technique. In general, plant tested extracts and ascorbic acid improved tested criteria in varying degrees as well as improvement was more pronounced in the absence of salinity. The water extract of roselle ranked the first in improving the tested measurements and recorded the maximum values 89.4 and 67.2%; 5.8 and 4.8 cm; 9.08 and 8.30 cm; 14.88 and 13.1 cm; 1330.2 and 880.3; 294.6 and 284.2 mg; 186.8 and 181.0 mg; 37.6 and 36.8 mg; 27.4 and 26.4 mg of germination percentage, shoot length, root length, seedling length, seedling vigor index, shoot fresh, root fresh, shoot dry and root dry weights, respectively in the absence or presence of salinity. While control and ASA treatments recorded the lowest values in this context under salinity stress.

Keywords: Rice, abiotic stress, plant extracts, ascorbic acid, salinity stress, germination, seedling parameters.

INTRODUCTION

Rice (*Oryza sativa* L. Poaceae) is considering the most important and oldest of food cereal crop all over the world. Hundreds of millions of people around the world depend on food made from rice grains. The rice cultivation is successful in fertile clay lands with good drainage, while saline sandy land or alkaline or poor drainage is inappropriate.

Seed soaking application is a low-cost and risk procedure that may be one of the possible options to mitigate the salinity effect in agricultural land (Iqbalet *et al.*, 2011). Plant extracts contain many compounds including organic acids, Aldehydes, aromatic aromatic acids, flavonoids, alkaloids, terpenoids, steroids and some toxic gases. Plant extracts have been reported to stimulate the growth and yield of plants (Rama Rao, 1991), enhance antioxidant properties, and develop tolerance to salinity stress (Zhang *et al.*, 2003). Moreover, seed soaking with optimal concentration of phytohormones has been shown to be beneficial to germination characters and seedling parameters of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Bahrani and Pourreza, 2012).

Salinity significantly affects the various stages of growth and development of plants. Salinity has reached a level of 19.5% within all irrigated land agriculture worldwide (FAO, 2005). One of the mainly significant abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity (Almansouriet *al.*, 2001), that are widespread problems around the world (Soltaniet *al.*, 2006). Physiological plant functions are related to the soil type and its physical and chemical properties (Kandeler, 2007). Saline soil is the soil that contains the largest amount of soluble and insoluble salts that inhibit the natural growth of plant crops (Paul, 2012). Salinity limits varied between agricultural land as affected by natural soil properties. The salinity of the agricultural water at high temperature and low humidity increase evaporation rate and precipitation rate which leads to the deposition of salts and increase the concentration of salinity in the soil, especially in

the surface layer where it is spread (Misra and Dwivedi, 2004). Rice is an agricultural plant whose resistance to salinity is moderate (Maas and Hofman, 1977), where rice responds to salinity as other tolerant crops by undergoes osmotic modification with the accumulation of salts and some organic substances, especially pulins and sugars (Termaat *et al.*, 1985 and Munns and Termaat, 1986). Salinity decrease seed germination rate as salinity negatively affects the transport of the representative substances and hormonal imbalance causes excessive accumulation as well as increasing salt concentration decreases the yield of hay and grain in rice plant (Alam and Azmi, 1990).

Therefore, the present investigation was conducted to study the integrate efficacy of four plant extracts *i.e.* turmeric, sorrel, grapes, and roselle as well as ascorbic acid on rice seeds (*Oryza sativa*) under salinity stress under laboratory conditions.

MATERIALS AND METHODS

Experimental Design and treatments:

Under controlled conditions, a laboratory experiment was conducted using Randomized Complete Block Design (RCBD) with four replications to evaluate the efficacy of four plant extracts *i.e.* turmeric (*Curcuma longa*), sorrel (*Rumexvesicarius*), grapes (*Vitisvinifera*), and roselle (*Hibiscus sabdariffa*) in comparison with ascorbic acid (ASA) on rice seeds germination characters and seedling parameters under salinity stress. Four plant extracts of rhizomes of turmeric, leaves of sorrel and grapes as well as the calyx of roselle were collected and botanical defined. About 100 g of fresh samples were weighed separately and cut into small pieces (5-7 cm), then placed in a conical flask and added 500 cm³ of water and boiled for 30 minutes then left to cool. Before cultivation, seeds were external sterilized with 2% sodium hypochloride for 10 min after that rinsed with distilled water and air dried (Basra *et al.*, 2013). A total of 48 Petri dishes (11 cm) were set at a laboratory bench and brushed with rice grains. Each Petri dish containing 25 grains of healthy rice grains which were soaking previously in the plant extracts and ascorbic acid at the rate of 50 ppm for

about 48 hours then transferred and planted in the Petri dishes. Petri dishes were divided into two groups, the first group was irrigated with distilled water as needed while the second group was once irrigated with 10 ml of NaCl at 5.25 ppm. Each treatment was repeated four times. Eight petri dishes with non soaking rice seeds were left as a control, four were irrigated with water only and the other four were treated with a one-time salt solution. The grains considered to have germinated when shoot extended to more than 2mm from the grains. After 10 days from sowing 10 seedlings were harvested and washed with tap water to estimate germination and seedling parameters. All data of the following parameters were recorded according to the International Rules of Seed Testing Association (ISTA, 2015).

Studied characters

1- Germination percentage (GP %): was recorded on the 10th day after sowing and calculated using the formula:

$$GP (\%) = \frac{\text{Number of germinated grains}}{\text{Number of total grains}} \times 100$$

2- Shoot and root lengths (cm) were measured on ten seedlings randomly taken from each replicate and the mean length of seedlings was calculated.

3- Seedling vigor index: was calculated according to Abdul-Baki and Anderson (1973) using the following formula:

$$\text{Seedling vigor index} = \text{Germination percentage} \times \text{Seedling length (cm)}$$

4- Shoot and root fresh weight (mg/10 seedlings) were measured on ten seedlings randomly taken from each replicate (The harvested plants were partitioned into roots and shoots).

5- Shoot and root dry weights (mg/10 seedlings) were measured using the same seedlings taken for the determination of fresh weight were placed in paper bags and dried at 70 °C until constant weight in an oven, then the plant parts were weighed again.

Data Analysis:

Data were subjected to analysis of variance (ANOVA) according to Gomez and Gomez (1984), followed by Duncan's multiple range (DMR) tests at

0.05% level of significance to compare treatments means using Duncan (1955).

RESULT AND DISCUSSION

Data stated in Table 1 and Figs. 1&2 documented the efficacy of four plant extracts *i.e.* turmeric, sorrel, grapes, and roselle in comparison with ascorbic acid on germination percentage and seedling parameters of rice grains under salinity stress. There was a marked improvement in seed germination percentage and seedling parameters as compared to control treatment. In general, plant tested extracts and ascorbic acid improved tested criteria in varying degrees and improvement was more pronounced in the absence of salinity. Data revealed that there were significant differences between factors under studies on germination and seedling parameters except seedling vigor index. The treatment with roselle extract was the first to achieve a significant ($P < 0.05$) increase in germination and seedling parameters, whether in the absence or presence of salinity with values (89.4 and 67.2%), high seedling length (14.88 and 13.1 cm) as well as seedling vigor index (1330.2 and 880.3), respectively. These results were in accordance with (Dubey, 1984) who revealed that salinity is one of the main environmental stresses which negatively affects seeds germination and the reduce in germination as salinity decrease in the amount of water uptake or the toxic effect of accumulated sodium and chloride ions. The efficacy of salinity on germination might attributed to a decrease hormone delivery throughout the seedling which inhibit the growth (Kord and Khalil, 1995). The obvious superiority of roselle extract may be due to its chemical composition. Roselle leaves are a good source of polyphenolic compounds *i.e.* neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid and flavonoid compounds such as quercetin, kaempferol and their derivatives (Zhen, 2018). Roselle leaves might contain some growth promoting and other bio-active substances that needs to isolation of active compounds from leaves extract should be determination by spectral for their structure.

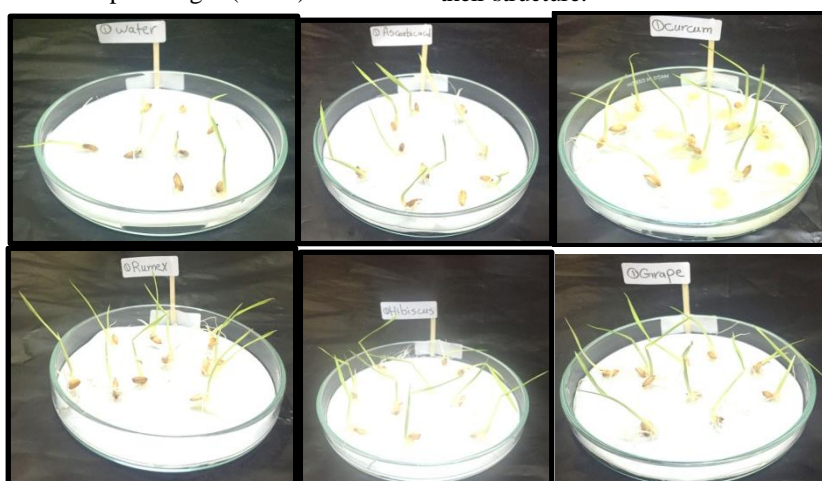


Fig.1. Rice seedlings in petri dishes after soaking in water, Ascorbic acid and, four plant extracts for (48 hrs) before germination,(1), means free salt treatments. Control is water(1); Ascorbic -acid (1) 50ppm; Curcum(1); Rumex; Grape(1) and Hibiscus(1).

Data in table (2) show the efficacy of four plant extracts. *i.e.* turmeric, sorrel, grapes, and roselle in comparison with ascorbic acid on fresh and dry weights of

rice seedlings under salinity stress. There was a marked improvement in both dry and wet weight for shoot and root with differ degrees. The water extract of roselle recorded

similar results as it ranked first in improving the tested measurements with values 294.6 and 284.2; 186.8 and 181.0; 37.6 and 36.8; 27.4 26.4 for shoot fresh, root fresh, shoot dry and root dry respectively in the absence or presence of salinity. While the treatment with ASA has the lowest values in this context comparing to soaking in water. In addition, the process of seeds soaking improved the seedling growth (seedling length, fresh and dry weight) of rice under salinity stress. This data were in accordance with Abdelraouf (2017) who stated that presoaking of sugar beet seeds with the GA₃, ABA, or ASA showed no significant effect to alleviation salt stress on sugar beet seedlings. The interaction between salt

levels and presoaking treatments was significant for whole plant and root fresh and dry weights, shoot/root ratio on fresh weight basis, whole plant and root moisture content and chlorophyll content index, while non-significant for shoot fresh and dry weights, shoot height. On the other hand, Singh and Dara, 1971, mentioned that presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation.

Table 1. Efficacy of four plant extracts in comparison with ascorbic acid on rice seed germination seed seedling vigor characters under salinity stress.

Treatments	Germination percentage(GP %)	Shoot Length(cm)	Root Length(cm)	Seedling length(cm)	Seedling vigor index
S0 + Water	58.0± 17.3c	3.9± 0.9 e	6.2± 0.2 f	10.1±0.3d	585.8
S1 + Water	32.3± 14.6 e	3.0± 0.1 f	5.1± 0.08 g	8.1±0.2c	261.9
S0+ASA	58.0± 17.3b	3.9± 0.2c	6.2± 0.2 c	10.1±0.5b	585.8
S1+ASA	32.3± 14.6 ab	3.0± 0.03 c	5.1± 0.08 c	8.1±0.2c	261.9
S0+Curcuma	67.0± 17.3 b	4.9± 1.2 bcd	7.1± 0.3 e	12±0.8a	804.0
S1+Curcuma	44.3± 14.4 d	4.0± 0.3 e	6.1± 0.1 f	10.1±0.6	447.7
S0+Rumex	73.3±15.30 b	5.1± 0.5 bc	8.2± 0.3 bcd	13.3±0.5a	975.3
S1+Rumex	55.6± 16.1 c	4.3± 0.5 de	7.6± 0.3 de	11.9±0.6b	662.4
S0+Grape	70.2± 10.5 b	5.5± 0.6 ab	8.6± 0.1 ab	14.1±0.7a	990.5
S1+Grape	51.7± 12.2 cd	4.5±0.5 cde	8.0± 0.2 cd	12.5±0.8a	646.8
S0+ Roselle	89.4± 7.4 a	5.8±0.8 a	9.1± 0.1 a	14.9±0.5 a	1330.2
S1+ Roselle	67.2± 10.4 b	4.8± 0.6 cd	8.3± 0.1 bc	13.1±0.6a	880.3

S0= no salinity, S1= with salinity, ASA= Ascorbic acid, mean values in each column followed by the same letter(s) did not differ at P< 0.05 according to Duncan's multiple- range test.

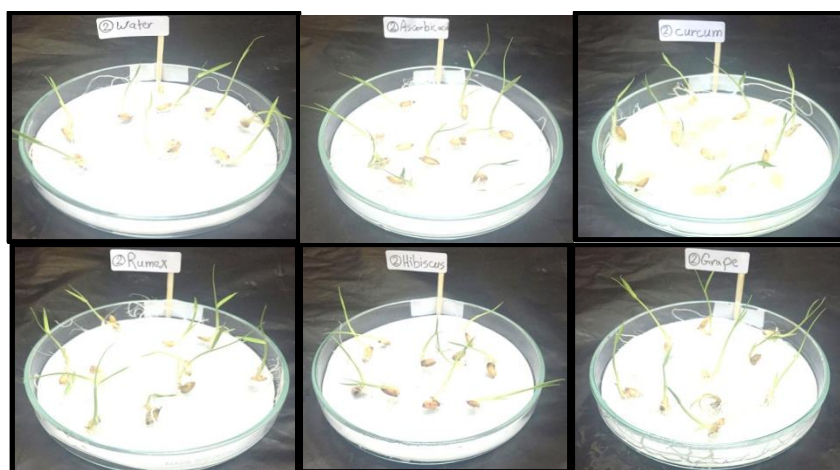


Fig. 2. Rice seedlings in petri dishes after soaking in water, Ascorbic acid and, four plants extracts for (48 hrs) before germination, (2) means that is under salinity stress with (NaCl2). Control is water(2); Ascorbic -acid (2) 50ppm; Curcum(2); Rumex;Grape(2), Hibiscus(2).

Table 2. Efficacy of four plant extracts in comparison with ascorbic acid on fresh and dry weights of rice seedlings under salinity stress.

Treatments	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot Dry weight (mg)	Root Dry weight (mg)
S0 + Water	193.0± 2.5 c	128.0 ± 1.1 g	22.3 ± 0.8bc	21.0±0.5 de
S1 + Water	181.3 ± 3.4 c	117.6±1.7 f	21.3± 0.8 c	20.3± 0.3 e
S0+ASA	193.0± 2.5 c	128.0± 1.1 ef	22.3± 0.8 bc	21.0± 0.5 de
S1+ASA	181.3± 3.4 c	117.6± 1.7 f	21.3± 0.8 c	20.3±0.3 e
S0+Curcuma	203.3±1.4 c	151.6± 0.6 cd	23.3± 0.3 bc	23.6± 0.3 cd
S1+Curcuma	194.0± 2.08 c	144.6± 2.7 de	22.3±0.8 bc	22.0± 1.1 de
S0+Rumex	218.3± 1.4 bc	156.6± 1.6 cd	24.6± 0.3 bc	23.6± 0.3 cd
S1+Rumex	199.3± 2.4 c	151.3± 1.7 cd	24.3± 0.8 bc	22.6± 0.3 cd
S0+Grape	266.0± 21.7 ab	174.5± 7.5 ab	34.0±3.7a	27.2± 1.6 a
S1+Grape	257.7± 19.4 ab	168.2± 7.2 bc	30.7± 3.4 ab	24.7± 1.1 bc
S0+ Roselle	294.6± 17.5 a	186.8± 5.2 a	37.6± 3.05 a	27.4± 0.6 a
S1+ Roselle	284.2± 16.3 a	181.0± 5.3 ab	36.8± 2.7 a	26.4± 0.5 ab

S0= no salinity, S1= with salinity, ASA= Ascorbic acid, mean values in each column followed by the same letter(s) did not differ at P< 0.05 according to Duncan's multiple- range test.

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التأثير المتكامل للنقع في المستخلصات النباتية وحمض الاسكوربيك على الإنبات وصفات البادرة لحبوب الارز تحت تأثير الإجهاد الملحي

الشيمااء عبد الله ابو الخير¹، سمير برهام جاد² و وليد أحمد المعداوي عبيدو³

¹ قسم النبات الزراعي، كلية الزراعة، جامعة المنصورة، مصر

² قسم علم الحيوان الزراعي، كلية الزراعة، جامعة المنصورة، مصر

³ قسم المحاصيل، كلية الزراعة، جامعة المنصورة، مصر

أجريت تجربة معملية بقسم النبات – كلية الزراعة – جامعة المنصورة - مصر بهدف دراسة تأثير معاملات نقع حبوب الارز في بعض المستخلصات النباتية وهي (الكرم – الحميض – العنب – الكركديه) والنقع في حامض الأسكوربيك بتركيز 50 جزء في المليون بالإضافة للنقع في الماء على إنبات ونمو بادرات الارز تحت ظروف الإجهاد الملحي. نفذت التجربة في تصميم القطاعات الكاملة العشوائية في أربع مكررات. أظهرت النتائج المتحصل عليها أن هناك تحسن ملحوظ في نسبة إنبات حبوب الارز وكذلك معامل قوة البادرات مقارنة بالكنترول. سجلت المستخلصات النباتية تحت الدراسة وكذلك حمض الأسكوربيك تحسن واضح في معايير الاختيار بدرجات متفاوتة، وكان التحسن أكثر وضوحاً في غياب الملوحة. سجل المستخلص المائي لنبات الكركديه أفضل النتائج حيث احتل المرتبة الأولى في تحسين القياسات المختبرة بقيم 89.4 و 67.2؛ 5.8 و 4.8؛ 9.1 و 8.3؛ 14.9 و 13.1؛ 1330.2 و 880.3؛ 294.6 و 284.2؛ 186.8 و 181.0؛ 37.6 و 36.8؛ 27.4 و 26.4 لكلا من نسبة الإنبات، طول الريشة، طول الجذير، طول البادرة، دليل قوة البادرة، الوزن الغض للريشة والجذير وكذلك الوزن الجاف للريشة والجذير على التوالي في حالة وجود غياب أو وجود الملوحة. في حين سجلت معاملة النقع في حامض الأسكوربيك بتركيز 50 جزء في المليون أقل القيم للصفات تحت الدراسة مقارنة بالنقع في الماء.