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Effect of Soaking Treatments and Temperature During Germination on Germinability and Rice (*Oryza sativa* L.) Seed Quality

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ABSTRACT

Plant breeders are still doing best for identifying efficient screening tools for overcome heat temperature during germination without any adverse effect on seed viability. The aims of study was to determine the efficiency of soaking old seeds (natural ageing for two years) in some antioxidants namely; calcium chloride, selenium, zinc sulfate in addition to water and dry seed as control under different temperature regimes namely; 15, 20, 25, 30 and 35 ^{o}C. The results revealed that low temperature negatively affected seed quality especially germination percentage and seedling establishment whereas, high temperature gave the highest values to germinability. Calcium chloride 1% was more efficient to all studied traits followed by 50 ppm selenium compared with control (dry seed).The interaction between factors revealed that 1% calcium chloride more efficient at different temperature regimes (low and high temperatures).The results suggest that soaking old seeds in calcium chloride for 24 h can mitigate effects of different temperature regimes and keep quality of old rice seeds. Also, can save costs and time of farmers.
Keywords; temperature stress; natural ageing; germination; viability; antioxidants; peroxidase.

INTRODUCTION

Germination is one of the most critical phases in the growth cycle of plants, because it determines seedling establishment and final yield of the crops. Production rate of rice crop should increase by 1% per year not only for the world's growing population food needs but also due to adverse climatic conditions (Sass *et al.,* 2002). Demands of seed fluctuates and sometimes there may be a surplus of seeds (carry over seed) which need to store up to 2-3 sowing seasons in addition the high price of seeds. Hence, proper seed treatments are needed to overcome this problem. Seedling vigor and seed germination are important factors for obtaining a good plant stand and high yields of a crop. Germination seed is highly dependent on temperature as temperature is one of the basic requisites of this process. However, the range of temperature in which seeds perform better germination depends on crop species. Kazim *et al.,* 2013 reported that germinability is most important step in plant development and the temperature of environment should below 42 °C otherwise it may be delay or totally inhibit germination, depending on the cultivars. Temperature of soil is one of the major environmental factors that influence not only the proportion of germinated seeds, but also the rate of emergence and seedling growth. Abiotic stress seriously affects the productivity of agriculture worldwide. The most important feature of pre sowing seed for long time is based on maintaining viability and seedling establishment above minimum standards. Temperature during germination is becoming the major concern for plant scientists worldwide due to the changing climate. Beebe *et al.,* 2011 show that climate change is expected to worsen abiotic factors globally and adaptation strategies need to be established for target crops to specific environments. Annual maximum and minimum temperature have increased by 0.35 °C and 1.13 °C for the period of 1979-2003. (Peng *et al.,* 2004). Each 1°C increase in temperature during crop growing period, grain yield of rice declined by 10% resulting in a 40- 50% gap of the attainable yield. The result of sharp decrease in cultivated land, shortage supply of water and continuous increase in food demand, farmers are forced to cultivate the rice in marginal environment with high temperature (Prasad *et al.,* 2006) which ultimately leads to reduced yield. High temperature during seed development delaying in germination, seed vigor and dry mass reduction (Wahid *et al.,* 2007). Germination seeds and seedling establishment stage play vital role for sustainable cropping, and are more sensitive to high temperatures during germination. (Dias *et al.,* 2011) show assessing varying degree of stress tolerance at different developmental steps. As a result of the complexity of heat stress, there is need to develop quick and fast screening tests for heat tolerance and plant breeders are still in quest for identifying such efficient tools for detecting heat tolerance indices at effect of temperature during germination stages in rice seedlings. The objectives of study were to study the effect of some soaking substances on improvement of old rice seeds under different temperature degrees during germination.

MATERIALS AND METHODS

Seed source and storage conditions

Rice cultivar seeds (cv. Sakh101) were obtained from the Central Administration for Seed Production (CASP), Agricultural Research Center, Egypt after natural ageing for two years (old seed season 2014 and 2015) in jute bags at room temperature. Experiments were conducted in Seed Technology Section in Mansoura, Dakahlya province, Agricultural Research Center, Field Crops Research Institute, Egypt during 2016. Factorial experimental design in randomized complete block design was used in three replications.

Seed soaking

To perform seed soaking treatments, the seeds were soaked in (50 ppm selenium, 1% calcium chloride, 1% zinc sulphate and distil water for 24 h at room temperature. Unsoked seeds were used as control.

Temperature during germination

The soaked seeds were incubated in growth chamber under different temperature degrees (15, 20, 25, 30 and 35 ^{o}C). 50 seeds as eight replicates of each treatment were placed in petri dishes. The following parameters were measured:

1- Germination percent was recorded by counting normal seedlings two weeks after sowing according to ISTA (1999).
2- Seedling length (cm) of five normal seedlings were recorded two weeks after sowing.
3- Seedling dry weight (mg) was measured after drying five normal seedlings in hot-air oven at 85 ^oC for 12 hours according to (Krishnasamy and Seshu 1990).
4- Seedling Vigor Index (SVI) was calculated according to the following equations of Abdul-Baki (1980) SVI= germination percentage x seedling length, Seedling
5-Vigor Index II= seedling dry weight x germination percentage.
6- Rate of germination : was measured according to the following formula of Bartllet (1937).

$$GR = \frac{a + (a + b) + (a + b + c) \ldots\ldots (a + b + c + m)}{n (a + b + c + m)}$$

Where a, b, c are number of seedlings in the first, second and third count, m is number. of seedlings in final count, n is the number of counts.
7- Germination energy (GE) was recorded on the 4th day of germination test. It is the percentage of germinated seeds at 4 days to the total number of seeds tested (Ruan and Tylkowska 2002).
8- Mean germination time (MGT) was calculated by using the following equation of (Ellis and Roberts, 1981).

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

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

Pot experiment

Rice seeds were sown and irrigation was applied whenever required. Data regarding seedling emergence, were measured during 14 days of sowing. The activity of peroxidase was estimated by the method of (Ghanati *et al.,* 2002). Activity of peroxidase enzyme was determined by the oxidation of guaiacol in the presence of hydrogen peroxide (H_2O_2).Absorbance was recorded at 470 nm for 1 min with spectrophotometer. Data were statistically analyzed through MSTAT-C computer software. Means of treatments were compared using the LSD according to (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Germination percent, seedling length, seedling dry weight and seedling index had significant effects by temperature degrees (Table 1). The lowest germination percentage was obtained at temperature degree (15 ^OC) whereas; the highest germination percentage was recorded at 35 ^OC. Raising temperature degrees during germination stage increased seedling length up to 25^OC. The highest vigor index was recorded at 25 ^oC. This may be due to low temperature stress is one of abiotic stresses and that delay germination. Temperature prevailing during germination directly interferes with amylase activity (Salwa, 2013). Seed soaking with calcium chloride ($CaCl_2$ 1%) had positive effects on germination percentage and other traits followed by selenium (50ppm). The effect may be due to $CaCl_2$and selenium improve antioxidant activity (Salwa, 2013; Yousof 2013).

Data in Table (2) show the effect of treatments on rate of germination, germination energy, mean germination time, field emergence as well as activity of peroxidase enzyme. The lowest means of the studied traits were recorded at 15 ^oC, whereas, the highest values were recorded at 25, 30 and 35 ^oC. Germination rate was (0.545) at 35 ^oC compared with (0.426) at 15. Peroxidase activity is an indicator of rapid germination and speed of growth and its highest activity was recorded at 35 ^oC. Kazim *et al.*, (2013) show that temperature degree make oxidative stress by generating free radicals and alteration of membrane protein components.

Table 1. Effect of temperature during germination and soaking substances on germination percentage, seedling length, seedling dry weight, seedling vigor index I and seedling vigor index II.

Treatments	Germination percentage	Seedling length	Seedling dry weight	Seedling vigor index I	Seedling vigor index II
Temperatures during germination					
15 ^oC	69	6.0	0.031	418	2.0
20 ^oC	84	18.0	0.043	1524	3.0
25 ^oC	85	24.7	0.054	2097	5.0
30 ^oC	87	16.7	0.060	1460	5.0
35 ^oC	89	17.6	0.070	1565	6.0
F test	**	**	**	**	**
LSD at 5%	8.9	2.5	0.010	206.0	5.0
Soaking substances					
Dry seed	73	14.9	0.058	1138	3
Water	76	16.6	0.061	1321	4
$ZnSo_4$ 1%	90	17.5	0.120	1623	5
Se 50 ppm	83	15.7	0.107	1316	5
$CaCl_2$ 1%	91	18.3	0.116	1665	7
F test	**	*	*	**	**
LSD at 5 %	4.9	1.9	0.011	150.7	3.5
Interactions					
A X B	*	NS	*	*	**

Decay the membrane lipids through autocatalytic peroxidation leads to decline cell membrane integrity and becomes more fluid under high temperature as compared to unstressed conditions. Seed treated with calcium chloride, selenium and zinc sulphate enhanced germination rate and other traits. This may be due to the potential of effects of $CaCl_2$ under stressful environment. Also, $CaCl_2$ acts as a positive factor in enhancing germination percentage and field emergence, and improved the germination attributes resulted in better early seedling growth (Yari *et al.,* 2012). The beneficial influence of Ca^{+2} ion on root growth of rice seedlings likely due to competition between Ca^{+2} and Na^{+1} leading to reduced level of internal Na^{+1}(Lin and Kao,1995). Calcium chloride, selenium and zinc sulphate application improved the germination under stress and reduced germination time hence promoted early seedling establishment and synchronized growth (Taiz and Zeiger 2002).

Table 2. Effect of temperature during germination and soaking substances on rate of germination, germination energy, mean germination time, field emergence and peroxidase activity

Treatments	Rate of germination	Germination energy	Mean germination time	Field emergence	Peroxidase activity 9
Temperatures during germination					
15 °C	0.426	48	4.8	60	1.05
20 °C	0.427	50	3.9	68	1.25
25 °C	0.533	73	3.9	82	1.40
30 °C	0.499	90	4.0	80	1.43
35 °C	0.545	91	3.5	88	1.43
F test	**	**	**	**	**
LSD at 5%	0.030	8.0	0.2	5.0	0.023
Soaking substances					
Dry seed	0.453	57	4.2	56	1.01
Water	0.480	69	4.1	73	1.11
$ZnSo_4$ 1%	0.481	74	4.0	80	1.13
Se 50ppm	0.497	75	3.8	81	1.20
$CaCl_2$ 1%	0.518	77	3.9	88	1.22
F test	**	**	*	**	**
LSD at 5 %	0.021	7.8	0.1	3.0	0.019
Interactions					
A X B	**	*	*	**	**

Data in Table (3) show the effects of interaction between temperature during germination and seed soaking treatments on germination percentage. Seed soaking with calcium chloride gave the highest germination percentage (97%) at 35°C whereas the lowest value (53%) was recorded at 15°C and these results show that rice seed which soaked in $CaCl_2$ 1% achieved a good values in mitigating adverse effects of high temperature and promoted germination characters. Also, due to the influence Ca^{+2} on membranes and enhanced antioxidant proteins like SOD enzyme. Ca^{+2} play important role as a cofactors in the activities of numerous enzymes (Taize and Zeiger, 2002).

Regarding peroxidase activity calcium chloride recorded the highest values (1.49 mg) followed by selenium (1.47mg) at 30 °C. On the other hand, dry seed recorded the lowest values (1.0 mg) at 15 °C. This may be due to the role of calcium chloride and selenium on alleviating the damage to rice seedlings and peroxidase protective enzymes to resist the damage of free radicals and play an important role in defense mechanism in stress conditions. These results are in agreement with those by Defang and Xinrong, (2012) who revealed that antioxidants improved the enzyme activity of POD compared with the control and slow down the damage of the plasma membrane.

Table 3. Effect of the interactions between germination temperatures and soaking substances on germination percentage.

Temperature degree / Soaking substances	15°C	20 °C	25°C	30°C	35°C
Dry seed	53	57	66	68	74
Distil water	58	68	80	83	93
$ZnSo_4$ 1%	71	70	81	86	94
Se 50 ppm	78	74	92	83	95
$CaCl_2$1%	85	72	94	94	97
F test			**		
LSD at 5 %			4.5		

Table 4. Effect of interactions between temperature during germination and soaking substances on peroxidase activity of rice seedlings.

Temperature degree / Soaking substances	15°C	20 °C	25°C	30°C	35°C
Dry seed	1.00	1.15	1.33	1.36	1.39
Distil water	1.05	1.27	1.37	1.42	1.42
$ZnSo_4$ 1%	1.06	1.16	1.36	1.45	1.46
Se 50 ppm	1.08	1.28	1.39	1.40	1.47
$CaCl_2$1%	1.09	1.40	1.43	1.47	1.49
F test			**		
LSD at 5 %			0.1		

Data in Table 4 shows the interaction between temperature during germination and soaking treatments on peroxidase activity. The data revealed that the low values recorded at 15°C with untreated seed whereas, the highest values were recorded at temperature degree 35 °C with calcium chloride. This may be due to good activities of some antioxidant enzymes increase during temprature treatment. Enzyme activities increase during temperature treatment may play important role in defense against that particular stress. POD and SOD activities increased and then decreased gradually with the duration of low temperatures treatment time in rice seedlings (Foyer and Noctor 2011).

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تأثير معاملات النقع والحرارة أثناء الإنبات على حيوية وجودة تقاوي الأرز

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يسعى مربي النباتات إلى إيجاد أدوات ووسائل فعالة لتحمل الحرارة فى المراحل المبكرة من حياة النبات دون التأثيرات الضارة على حيوية التقاوى، لذلك أجريت هذه الدراسه في تجربة عاملية فى قطاعات كاملة العشوائية في ثلاثة مكررات بمعامل وحدة بحوث تكنولوجيا البذور بالمنصورة - قسم بحوث تكنولوجيا البذور- معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية خلال عام 2016 وذلك لتقييم تأثير نقع تقاوي الأرز القديمة (تدهور طبيعي لمدة عامين) في بعض مضادات الأكسدة مثل كلوريد الكالسيوم 1% والسلينيوم 50 جزء في المليون وسلفات الزنك 1% بالإضافة إلى النقع فى الماء والمعاملة بدون نقع لمدة 24 ساعة وذلك تحت ظروف درجات حرارة مختلفة خلال الانبات ونمو البادرات (15-20-25-30-35 درجه مئوية). لتحسين أداء البادرات والصفات المتعلقة بها مثل نسبة الإنبات وصفات الحيوية ونشاط إنزيم البيروكسيديز. ويمكن تلخيص أهم النتائج المتحصل عليها فيما يلي: أشارت النتائج إلى أن الحرارة المنخفضة أثرت سلبا على إنبات وجودة تقاوي الأرز بينما الحرارة المرتفعة كان لها تأثير ايجابي على إنبات ونمو البادرات.أعطى كلوريد الكالسيوم بتركيز 1% أعلى كفاءة لجميع الصفات تحت الدراسة تلاه النقع في السلينيوم 50 جزء في المليون بالمقارنة بالكنترول (بدون نقع).اشارت النتائج ان التفاعل بين معاملات النقع في مضادات الاكسدة ودرجات الحرارة المختلفة (منخفضه ومرتفعة) أعطي أعلى القيم عند النقع لمدة 24 ساعة في كلوريد الكالسيوم بتركيز 1% لما للكالسيوم دور في تقليل التاثيرات الضارة للحرارة.**لذا** توصي الدراسة بمعاملة تقاوي الأرز القديمة بكلوريد الكالسيوم 1% أوالسلينيوم 50 جزء في المليون أو الزنك 1% كأحد المواد المشجعة علي الإنبات تحت ظروف درجات الحرارة المختلفة وذلك لتقليل الوقت والتكلفة علي المزارعين بدلا من شراء تقاوي جديدة عالية الثمن.