The Effect of Sodium Hypochlorite (NaOCl) and Heat Treatments on Seed Germination of Rice: An Approach to Restore Seed Viability

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MS designed the study, performed the research and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS, BKS, MNA and NM managed the analyses of the study and given guidance for the study. All authors read and approved the final manuscript.

ABSTRACT

Dormancy in rice serves as a mechanism of survival by protecting the seed from germinating in the mother plants; however, it becomes a problem in germination during sowing in soil or under in vitro conditions. This study was conducted to determine the effect of heat treatment and sodium hypochlorite (NaOCl) treatment of seeds on dormancy alleviation. The seeds included both freshly harvested seeds and one-year-old stored seeds, which were tested for germination after different types of seed treatments. Both the treatments increased the germination percentage in seeds, however, it was lesser in the case of old seeds. The best results were obtained from 2% NaOCl treatment for 24 hrs in new seeds, i.e. 92.84±0.103 % germination percentage (GP). However, the higher GP in old seeds were obtained from 48 hrs of heat-treated seeds i.e. 82.9±0.509 % GP. The results of the experiment revealed that rice seeds start to lose viability within a year due to seed

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dormancy, but this can be reversed with proper measures. These methods of breaking seed dormancy can be considered effective to break seed dormancy and improve seed germination in rice.

Keywords: Seed germination; breaking dormancy; rice; sodium hypochlorite; heat treatment.

1. INTRODUCTION

Rice is the most important staple food crop of about 60% of the world's population. Rice like any other arable cereals produces orthodox seeds, which can be dried and stored at low temperatures to prolong viability. However, due to a plethora of reasons such as poor seed production environment, seed maturity, storage temperature, relative humidity, seed moisture content, they lose viability, even within one year after harvesting [1]. The term seed viability refers to the capability of a seed to germinate and produce a normal seedling and used synonymously with germination capacity. Seed viability also denotes the degree to which seed is alive i.e. metabolically active and possesses enzymes that are capable of catalysing metabolic reactions needed for germination and seedling growth [2]. When the viable seeds cannot germinate even sown under optimal moisture, oxygen and soil conditions are called dormant. There are several reasons for this temporary suspension of growth such as hard impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover [3]. In nature, dormancy can be an advantage for some species because it renders resistance to pre-harvest sprouting and prevents germination until favourable conditions for plant development prevail. However, it is problematic when utilizing rice species in research and breeding [4].

Seed viability is normally at its highest at the time of physiological maturity, although physiological conditions within the inflorescence of the parental plant may not permit germination. Furthermore, seed viability gradually declines with time after physiological maturity. As a result, an effective methodology to recover low-viability germplasm is essential to preserve the genetic base found within existing collections. It was found that the respiration rate is higher in the fresh seeds of rice than the old ones. But, the failure of germination in old seeds is not due to increasing carbon dioxide concentration inside or the nature of the husk acting as a barrier or the old seeds becoming dead. The primary reason was detected to be the presence of germination inhibitors in old seeds. The leachings from the non-viable old seeds inhibited the growth of roots in new seeds indicating the presence of germination inhibitor in rice seeds [5].

There have been several reports of treatments to break seed dormancy in rice using dry heat hot water, high humidity, KNO₃, NaOCl etc. as pre-treatments before germination [6,7,8]. They prove to be effective in directly influencing seed dormancy and improving seed germination and seedling growth to an extent. However, there has been no study regarding the most suitable technique for improving seed germination by breaking seed dormancy. Therefore, in this article we compare the influence of dormancy breaking treatments for both freshly harvested seed and old seeds from storage by analysing the seed germination capability.

2. MATERIALS AND METHODS

This study was conducted in 2019 at the Department of Agricultural Biotechnology, Faculty of Agriculture, Bidhan Chandra Krishi Vishwavidyalaya, India. The germination of rice seeds (Oryza sativa L. var. Kshitish/IET-4094) was carried out inside the plant growth chamber (make is LabX PGC – 24, BIO-TECHNICAL RESOURCES, WB). The healthy seeds (visible) were collected based on their harvesting time, i.e. old seeds (more than 1 year old than the harvesting time) and new seeds (seeds from the previous season i.e. less than 6 months old). The heat treatments include drying of seeds for 0 to 96 hr @ 40-50°C with 40% relative humidity. The sodium hypochlorite (NaOCl) treatments include soaking seeds in 0, 2, 4, 6% (w/v) no aerated solution of NaOCl (Merck) for 24 hr at 25°C. The experiment was arranged in a randomized complete block design with four replications. The ratio of seed weight to solution volume was 1 : 5 (g/ml). The test weight of heat-treated seeds was also noted. The germination tests were conducted on 200 seeds (each from both old and new seed lot) as four replicates of 50 seeds each on top of two moist filter papers in 8 cm Petri dishes at a range of 30 °C to 25°C temperature with relative humidity ranging from 96 to 100% [9]. The seedlings were evaluated according to
the rules of the International Seed Testing Association [10]. Seedlings which produced normal root and shoots were considered to have germinated (normal germination). Germination was defined as the point when the radicle was visible about 2 mm length and counted daily for 14 days. The first counts of germination were made on day 7, and ungerminated seeds were dehulled to remove any dormancy and tested for another 7 d before the final counts were taken. Seeds, which remained ungerminated but became soft at the end of the testing period were considered dead. Data from the treatments were analyzed by the SPSS statistical software. The ANOVA and Duncan tests were used to compare treatment groups to find out whether they showed any statistically significant differences with a significance level (α) set at 0.05.

3. RESULTS

According to the analysis of variance (ANOVA), the effects of different concentrations of sodium hypochlorite and heat treatments on germination percentage (GP) and test weight (TW) were significant (Table 1). The mean comparison of NaOCl treatment (Table 2) indicated that the highest GP in new seeds belonged to 2% NaOCl treatment (92.84±0.103 %) and for old seeds it is with 4% NaOCl treatment (81.68±0.185). The new seeds showed higher GP in both treated and untreated seeds. In the case of heat treatment (Table 3), the highest GP was observed in the new seeds with 72 hrs of treatment (92.78 ± 0.124 %) and for old seed it was 48 hrs of treatment (82.9 ± 0.509 %). In both of the types of seeds, the GP increased with NaOCl treatment and duration of heat treatment, but the extent of improvement varied concerning the source. However, the test weight of seeds drastically reduced with heat treatment in both new and old seeds.

4. DISCUSSION

4.1 NaOCl Treatment

Sodium hypochlorite (NaOCl), the active ingredient in household bleach, is routinely used as a seed disinfectant in studies involving seed-borne pathogens, biological control, endophytes and seed germination as well as in commercial applications [11,12]. Findings from numerous studies have shown that NaOCl pre-treatment of seeds can influence viability, germination and often relieve dormancy in seeds of many species [6]. It was reported that roots and shoots of rice respond differently to hypochlorite, with shoot growth being more responsive. Sodium hypochlorite stimulated rice seedling growth directly as opposed to an indirect effect related to the elimination of microbial contaminants or alleviation of dormancy [12]. The dilute solutions of NaOCl enhanced seedling growth, and inactivated a germination inhibitor present in rice seed hull [13]. These varying results may be caused by the differences in treatment duration and NaOCl concentrations used [14]. It was also suggested that the germination studies that involve seeds possessing physiological dormancy are more affected by pretreatments using NaOCl than seeds with physical dormancy [15].

<table>
<thead>
<tr>
<th>NaOCl concentration</th>
<th>New seed germination (%)</th>
<th>Old seed germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>82.26D±0.150</td>
<td>61.72D±0.551</td>
</tr>
<tr>
<td>2%</td>
<td>92.84A±0.103</td>
<td>77.48C±0.206</td>
</tr>
<tr>
<td>4%</td>
<td>91.92B±0.166</td>
<td>81.68A±0.185</td>
</tr>
<tr>
<td>6%</td>
<td>90.28C±0.159</td>
<td>79.64B±0.194</td>
</tr>
</tbody>
</table>

*Significant at P=0.05 level
Table 3. Effect of heat treatment on test weight and germination

<table>
<thead>
<tr>
<th>Heat treatment duration</th>
<th>New seed test weight (g)</th>
<th>Old seed test weight (g)</th>
<th>New seed germination (%)</th>
<th>Old seed germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>25.55±0.052</td>
<td>22.72±0.035</td>
<td>82.26E±0.150</td>
<td>61.72E±0.551</td>
</tr>
<tr>
<td>24 hr</td>
<td>24.26±0.028</td>
<td>22±0.034</td>
<td>86.74C±0.218</td>
<td>73.76C±0.563</td>
</tr>
<tr>
<td>48 hr</td>
<td>21.58±0.020</td>
<td>21.89±0.007</td>
<td>90.38B±0.136</td>
<td>82.9A±0.509</td>
</tr>
<tr>
<td>72 hr</td>
<td>21.40±0.015</td>
<td>21.64±0.0125</td>
<td>92.78A±0.124</td>
<td>79.52B±0.235</td>
</tr>
<tr>
<td>96 hr</td>
<td>20.91±0.047</td>
<td>21.53±0.011</td>
<td>85.96D±0.181</td>
<td>71.68D±0.185</td>
</tr>
</tbody>
</table>

NaOCl was an effective treatment for stimulating germination or breaking dormancy in some species, such as *P. convolvulus* and *S. vaccaria*. For these species, NaOCl might have increased the permeability of the seed coat to oxygen and loss of germination inhibitors. NaOCl treatment of seeds for a longer duration than the optimum result in a decrease of germination due to disintegration of the seed or the effect of NaOCl on seed biochemical and metabolism processes. [16].

It was earlier reported that the treatment of seeds by 2% NaOCl could increase the germination percentage of *Oryza sativa* L. and increasing the concentration leads to a decrease in the germination [12,17]. In our study, we observed a significant increase in rice germination percentage in both old and new seeds (Table 2). The new seeds required 2% NaOCl and old seeds required 4% NaOCl to express their highest germination potential. However, old seeds were expressing lesser germination, which may be due to the presence of germination inhibitory elements and dormancy. Even though they responded better after NaOCl treatment. These results were following Mahajan et al [18], who suggested NaOCl treatment to cause major changes in seed metabolism that influence the seed germination process. Several studies also demonstrated that NaOCl treatment might either promote or inhibit or be ineffective at the seed germination stage in different species [19].

### 4.2 Dry Heat Treatment

Rice seed treatment with either dry heat or steam is often found to be beneficial for breaking seed dormancy and promoting germination [1]. In our experiment, we found 40-50°C heat treatment to be beneficial for improving seed germination. However, the effect of this treatment varied with the treatment duration and among old and new seeds. This suggests that heat treatment directly improves seed germination and also alleviates seed dormancy, since old seeds also responded to a degree of treatments. Similar results were also observed in few studies. It was reported that an increase in the germination of both intact and hulled seeds of wild rice and cultivated rice species after dry heat treatment at 50°C [4]. Moreover, drying rice seeds (20 new-improved varieties) in oven at 50°C for 48 hours continued by soaking seeds in water for 48 hours were found to be an effective method for breaking dormancy [8,20].

### 5. CONCLUSION

The results of this study demonstrate the effect of heat treatment and NaOCl treatment in improving seed germination. These treatments also break the seed dormancy and restore seed viability, which are otherwise not able to germinate even under standard or ideal condition. However, the level of treatment effect varies among the old and new seeds revealing the loss of viability in rice seeds.

### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors. The authors gratefully acknowledge the DBT-JRF fellowship Fellow ID: DBT/2016/BCW/685, New Delhi, India to Monoj Sutradhar for financial support throughout the term.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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