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*Full Length Research Paper*

# Effects of gibberellic acid treatment and light conditions on germination of true potato seed

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**The effects of gibberellic acid treatment and light conditions on the germination of true potato seed were investigated. The treatments included pre-soaking with different concentrations of $GA_3$ for 24 h and the addition of different concentrations of $GA_3$ to the MS medium. Different light conditions were also examined. One set of each treatment was placed in 16 hours of light and 8 hours of dark. The other set was placed in 24 h of dark for a week and then placed in the same condition as that of the first set. The percentage of seed germination scored two weeks after sowing was analyzed using the three-way analysis of variance. No interaction between two or three factors was observed, but all three factors were individually significant at a 99% confidence level. Consequently, individual analysis of the three factors revealed that 1) the effect of pre-soaking with 4,500 ppm of $GA_3$ was the best, but not significantly different from that of 500 and 1,500 ppm; 2) the effect of 0.5 ppm of $GA_3$ in the medium was the best, but not significantly different from that of 1 ppm; and 3) the effect of dark condition for one week after sowing was highly significant.**

**Key words:** Germination, gibberellic acid, light condition, potato (*Solanum tuberosum* L.), seed dormancy, true potato seed.

## INTRODUCTION

The cultivated potato *Solanum tuberosum* L., which originated from the Andean region of South America, is one of the most important food crops in the world and is ranked fourth in world food production after wheat, corn and rice. It is also on the top of the list of root crops, followed by cassava, sweet potato and yam. In fact, true potato seed (TPS) does not need to be produced for commercial and/or consumable purposes. In conventional potato production, potatoes are propagated vegetatively by means of tubers. When new potato varieties are bred, vegetative clones, tubers are propagated and released for commercial production. However, extensive studies on the use of TPS to produce seeds or ware potatoes have been carried out in many countries because the accumulation of pathogens, physiological decline, low multiplication rates, limited availability, high costs for storage and transport of high quality tuber seeds are the major constraints for successful potato production (Horton and Sawyer, 1985; Manrique, 1994; Upadhya, 1994; Struik and Wiersema, 1999).

True potato seed (TPS) is the name given to botanical seeds produced in the berries of potato plants (Struik and Wiersema, 1999) and results of sexual reproduction, which are an important procedure for potato breeding. In conventional potato breeding, TPS is essential to generate genetic populations and the TPS of wild potato species is often used to generate new varieties (Salaman, 1970; van der Vossen et al., 2003, 2005; Park et al., 2005a, 2005b, 2009; Foster et al., 2009; Pel et al., 2009). When TPS is used for potato breeding, seed dormancy and a low rate of germination are the major obstacles to accelerating breeding processes of new potato varieties. Seeds of many plant species, especially those growing in temperate regions are dormant at the time of mature seeds, which prevents germination, and specific temperatures are usually required to overcome dormancy

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(Baskin and Baskin, 1998; Bewley et al., 2006). In potatoes, seed dormancy starts when seeds are mature in the berry and these seeds will not germinate. Seed dormancy usually lasts for approximately 6-9 months (Struik and Wiersema, 1999). The period of seed dormancy is related to many factors such as seed moisture content (Simmonds, 1968; Pallais, 1995), temperature (Simmonds 1968; Howard, 1980; Pallais 1995), genotype (Pallais et al., 1991), etc. Although seed dormancy can be overcome, seed germination rate will decrease depending on the period of seed storage. Previously, a few methods for the release of TPS dormancy and enhancing seed germination have been developed and involve pre-sowing treatments with $GA_3$, $KNO_3$, $K_3PO_4$, and priming, conditions of storage, light and temperature, etc. (Spicer and Dionne, 1961; Bewley and Black, 1982; Malagamba, 1988; Pallais et al., 1991; Pallais, 1989, 1995; Struik and Wiersema, 1999; Bamberg, 1999, 2000; Xie et al., 2008). However, most of these factors were independently investigated and some of them are time- and cost-ineffective and laborious.

Since $GA_3$ has been shown to be a key factor in controlling seed dormancy and germination in many plant species including potatoes (Amen, 1968; Wareing and Saunders, 1971; Taylorson and Hendricks, 1977; Bhargava, 1997; Kucera et al., 2005; Pawlowski, 2009), $GA_3$ is often used to break seed dormancy and to improve seed germination in many plant species (Pallais et al., 1991; Karam and Al-Salem, 2001; Bahrani et al., 2008; Zeinalabedini et al., 2009; Deng et al., 2010; Zeng et al., 2010). In addition, 1,500 or 2,000 ppm of $GA_3$ has been routinely used to improve seed germination in potatoes before sowing. Zeinalabedini et al. (2009) applied $GA_3$ in agar medium under *in vitro* conditions even though most of the $GA_3$ application was conducted as a pre-sowing treatment. Therefore, in this study, we investigated the effects of gibberellic acid treatment and light conditions on the germination of TPS and determined the best combination of treatment.

## MATERIALS AND METHODS

### Seed materials

Two-year stored true potato seeds (TPS) of three different mapping populations P06808, P06820 and P06837, which were kindly provided by Dr. Y.-E. Park of Highland Agriculture Research Center, Pyongchang, Republic of Korea, were used for testing seed germination. The three mapping populations were derived from crosses between Atzimba and Ranger Russet, between Mira and Ranger Russet and between Superior and Ranger Russet, respectively.

### Seed treatments

20 to 30 seeds were used for each treatment and this depended on the number of seeds in the three populations. All TPS samples were sterilized in 70% ethanol for 1 min and 1.5% sodium hypochlorite for 5 minutes and rinsed in sterilized water three times before treatments. Three different treatments were used in this study (Table 1). The first treatment (A) was the pre-soaking with $GA_3$ at concentrations of 0(a1), 500(a2), 1500(a3) and 4500(a4) ppm for 24 h. The second treatment (B) was the addition of different concentrations of $GA_3$ (0(b1), 0.5(b2) and 1(b3) ppm) to the MS medium. The third treatment (C) was the light conditions. One set (c1) of the first and second treatments was placed in 16 h of light and 8 h of dark. Another set (c2) was placed in 24 h of dark for a week and then placed in the same condition as that of the first set. All TPS sample were sown in MS-based medium with 3% sucrose and placed in a tissue culture room at 25°C. All combinations of the three factors are shown in Table 1.

### Statistical analyses

Counts of the emergence of shoots and roots regarding seed germination were made every 24 h for 17 days, commencing the day after sowing. The curve of root emergence to check the percentage of seed viability and germination was analyzed. To analyze the effects of each treatment, the percentages of seed germination scored two weeks after sowing were analyzed using three-way analysis of variance (ANOVA). After defining the effects of the three factors and the interactions between three factors, t-test and Duncan's multiple-range test (DMRT) were applied to analyze the effects of each treatment.

## RESULTS AND DISCUSSION

TPS has widely been used because of the several drawbacks of vegetative propagation and the necessity of TPS for breeding purposes (Salaman, 1970; Horton and Sawyer, 1985; Manrique, 1994; Upadhya, 1994; Struik and Wiersema, 1999). Although TPS results from sexual propagation, which is problematic in regards to seed dormancy and the low rate of germination, studies to improve seed germination are rare. Therefore, three independent factors were examined in this study using three different TPS populations. Since $GA_3$ and abscisic acid (ABA) are known to control seed dormancy in many plant species, TPS samples were treated with $GA_3$ prior to sowing and added to the medium (Taylorson and Hendricks, 1977; Zeinalabedini et al., 2009). In addition, light conditions were also varied.

### Seed germination

The first emergence of roots occurred under the dark condition (c2) five days after sowing and shooting was mostly observed one to three days after rooting. However, the emergence of shoots was not observed in some of the seeds after the emergence of roots. Overall, the percentage of seed germination was dependent on the three treatments and genotypes as indicated by Pallais et al. (1991). The percentage of root emergence during the experimental period is shown in Figure 1. All of rooting were dramatically increased between seven and nine days and were maximal around two weeks after sowing.

**Table 1.** The percentage of seed germination collected two weeks after sowing.

| Treatment | | | Replication | | | Average |
|---|---|---|---|---|---|---|
| Pre-soaking[a](A) | Medium[b](B) | L/D[c](C) | 1[d] | 2 | 3 | |
| | $GA_3$-0 | Light(c1) | 36.0 | 25.0 | 4.5 | 21.8 |
| | (b1) | Dark(c2) | 88.0 | 25.0 | 65.0 | 59.3 |
| $GA_3$-0 | $GA_3$-0.5 | Light(c1) | 64.0 | 45.0 | 40.0 | 49.7 |
| (a1) | (b2) | Dark(c2) | 83.3 | 65.0 | 72.7 | 73.7 |
| | $GA_3$-1.0 | Light(c1) | 64.0 | 45.0 | 44.0 | 51.0 |
| | (b3) | Dark(c2) | 84.0 | 55.0 | 52.4 | 63.8 |
| | $GA_3$-0 | Light(c1) | 64.0 | 30.0 | 70.0 | 54.7 |
| | (b1) | Dark(c2) | 64.0 | 60.0 | 56.7 | 60.2 |
| $GA_3$-500 | $GA_3$-0.5 | Light(c1) | 52.0 | 60.0 | 73.3 | 61.8 |
| (a2) | (b2) | Dark(c2) | 87.5 | 75.0 | 86.7 | 83.1 |
| | $GA_3$-1.0 | Light(c1) | 66.7 | 45.0 | 70.0 | 60.6 |
| | (b3) | Dark(c2) | 76.0 | 70.0 | 80.0 | 75.3 |
| | $GA_3$-0 | Light(c1) | 52.0 | 70.0 | 23.3 | 48.4 |
| | (b1) | Dark(c2) | 60.0 | 65.0 | 63.3 | 62.8 |
| $GA_3$-1,500 | $GA_3$-0.5 | Light(c1) | 92.3 | 85.0 | 73.3 | 83.5 |
| (a3) | (b2) | Dark(c2) | 84.0 | 85.0 | 83.3 | 84.1 |
| | $GA_3$-1.0 | Light(c1) | 80.0 | 70.0 | 86.7 | 78.9 |
| | (b3) | Dark(c2) | 80.0 | 75.0 | 83.3 | 79.4 |
| | $GA_3$-0 | Light(c1) | 45.5 | 52.6 | 53.3 | 50.5 |
| | (b1) | Dark(c2) | 86.4 | 70.0 | 80.0 | 78.8 |
| $GA_3$-4,500 | $GA_3$-0.5 | Light(c1) | 86.4 | 85.0 | 86.7 | 86.0 |
| (a4) | (b2) | Dark(c2) | 90.5 | 85.0 | 86.7 | 87.4 |
| | $GA_3$-1.0 | Light(c1) | 79.2 | 65.0 | 76.7 | 73.6 |
| | (b3) | Dark(c2) | 100.0 | 80.0 | 80.0 | 86.7 |
| Average | | | 73.6 | 62.0 | 66.3 | 67.3 |

[a] Pre-soaking of 0(a1), 500(a2), 1,500(a3) and 4,500(a4) ppm of $GA_3$ for 24 h on TPS. [b] Application of 0(b1), 0.5(b2) and 1.0(b3) ppm of $GA_3$ in MS medium. [c] Light indicates 16 h light and 8 h dark condition (c1) and dark indicates 24 h dark condition for a week and then 16 h light and 8 h dark condition (c2). [d] Replications 1, 2 and 3 indicate three different populations P06808, P06820 and P06837.

The overall average percentage of root emergence was 6.3 and 73.3%, seven and nine days after sowing, respectively. Two weeks and 17 days after sowing, the average percentage of root emergence was 79.0 and 80.3% and the percentage of seed germination was 67.3 and 75.1%, respectively. The maximum number of root emergence was achieved two weeks after sowing. These results indicate that TPS of any genotypes should germinate before two weeks. In this study, the emergence of both root and shoot was subjected to further statistical analyses as an indicator of seed germination.

## Statistical analyses

For statistical analysis, the data on seed germination collected two weeks after sowing were used (Table 1). Three different mapping populations derived from different crosses were used as replications in this study. The overall average percentage of seed germination was 67.3%. The average percentage of seed germination for each treatment was ranged from 21.8 to 87.4%. The highest percentage of seed germination was observed for samples pre-soaked with 4,500 ppm $GA_3$ (a4), containing 0.5 ppm $GA_3$ in the medium (b2) and incubated in the dark for one week (c2). The lowest percentage of seed germination was observed for samples without pre-soaking (a1) of $GA_3$ and without $GA_3$ in the medium (b1) and incubated for 16 h in light from the beginning (c1) (Table 1).

The effects of three different treatments were statistically analyzed using three-way ANOVA (Table 2).

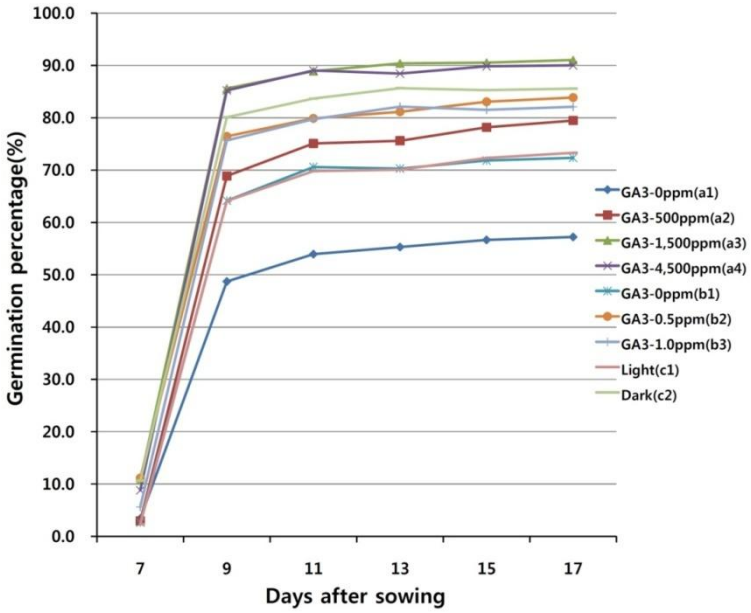


**Figure 1.** Overall percentage of emergence of root regarding seed germination and viability for each treatment.

**Table 2.** Analysis of variance (ANOVA).

| Source of variance (*SV*)[a] | Degree of freedom (*df*) | Sum of square (*SS*) | Mean of square (*MS*) | *F*-test | P (%)[b] |
|---|---|---|---|---|---|
| Replication | 2 | 1643.4 | | | |
| A | 3 | 5907.6 | 1969.2 | 7.9** | 20.1 |
| B | 2 | 6129.3 | 3064.7 | 12.2** | 31.3 |
| C | 1 | 3789.0 | 3789.0 | 15.1** | 38.7 |
| AB | 6 | 356.7 | 59.5 | $0.2^{ns}$ | 0.6 |
| AC | 3 | 869.7 | 289.9 | $1.2^{ns}$ | 3.0 |
| BC | 2 | 437.0 | 218.5 | $0.9^{ns}$ | 2.2 |
| ABC | 6 | 946.1 | 157.7 | $0.6^{ns}$ | 1.6 |
| Error | 24 | 6019.4 | 250.8 | | 2.6 |
| Total | 49 | 26098.3 | | | |

[a] A, B and C indicate three different treatments referred from Table 1. [b] Percentage of each factor contribution on total variation. [ns] and [**] indicate no significant and significant at P<0.01, respectively.

**Table 3.** Effect of pre-soaking with $GA_3$ on the germination of TPS.

| Treatment | $GA_3$-0 (a1)[a] | $GA_3$-500 (a2) | $GA_3$-1,500 (a3) | $GA_3$-4,500 (a4) |
|---|---|---|---|---|
| Germination (%) | $53.2\pm5.3^{bbc}$ | $65.9\pm3.4^{ab}$ | $72.9\pm3.9^{a}$ | $77.2\pm3.4^{a}$ |

[a] Each treatment is corresponding to the data displayed in Table 1. [b] The values represent means ± standard error. [c] Mean values with a different letter are significantly different at P=0.01 by DMRT.

The effect of all three different treatments was highly significant in regards to seed germination (P < 0.01). No interactions between the two factors, AB, BC and AC and three factors, ABC were detected. When the percentage of each factor contributing to seed germination was investigated, they were determined to be 20.1, 31.3 and 38.7% for the pre-soaking with $GA_3$ (A), adding $GA_3$ to the medium (B) and light conditions (C), respectively. Subsequently, the effects of each factor were separately examined by t-test and Duncan's multiple-range test (DMRT) (Tables 3, 4, 5). In regards to pre-soaking with $GA_3$ (A), pre-soaking with 4,500 ppm $GA_3$ (a3) for 24 h

**Table 4.** Effect of adding $GA_3$ to the MS medium on the germination of TPS.

| Treatment | $GA_3$-0 (b1)[a] | $GA_3$-0.5 (b2) | $GA_3$-1.0 (b3) |
|---|---|---|---|
| Germination (%) | 54.6±4.3[b bc] | 76.2±3.0[a] | 71.2±2.9[a] |

[a] Each treatment is corresponding to the data displayed in Table 1. [b] The values represent means ± standard error. [c] Mean values with a different letter are significantly different at P=0.01 by DMRT.

**Table 5.** Effect of light conditions on the germination of TPS.

| Treatment | Light(c1)[a] | Dark(c2) |
|---|---|---|
| Germination (%) | 60.0±3.5[b bc] | 74.5±2.4[a] |

[a] Each treatment is corresponding to the data displayed in Table 1. [b] The values represent means ± standard error. [c] Mean values with a different letter are significantly different at P=0.01 by t-test.

was most effective at a 1% significant level, that is, at a 99% confidence level, but not significantly different from pre-soaking with 500 ppm $GA_3$ (a1) and 1,500 ppm $GA_3$ (a2) for 24 h (Table 3). For the addition of $GA_3$ to the MS medium (B), the addition of 0.5 ppm $GA_3$ (b2) to the MS medium was most effective at a 1% significant level, but not significantly different from adding 1.0 ppm of $GA_3$ (b3) to the MS medium (Table 4). Lastly, for the different light conditions (C), 24 h of dark for a week and then placement in 16 h of light and 8 h of dark (c2) was more effective (P=0.01) than 16 h of light and 8 h of dark from the beginning (c1) (Table 5).

Although several factors have already been shown to be involved in overcoming seed dormancy and in increasing the seed germination rate, it is necessary to determine the best combination of treatments. As shown in this study, pre-soaking with 1,500 ppm $GA_3$ for 24 h, addition of 0.5 ppm $GA_3$ to the MS medium and incubation in the dark for a week after sowing can improve the germination rate of TPS and consequently the efficiency of potato breeding.

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