Seed Res., Vol. 40(1): 67-72 June 2012

# Effect of priming on seed germination attributes of pansy (*Viola* x *Wittrockiana*) under laboratory conditions

**RISHU SHARMA, B.S. DILTA, H.S. BAWEJA AND B.P. SHARMA**

Department of Floriculture and Landscaping, College of Horticulture, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan 173 230 (H.P.)

rishu.sharma155@gmail.com

**ABSTRACT** Pansy (*Viola x Wittrockiana* Gams.) is a very important bedding plant and widely grown as a winter annual in India. The poor and slow rate of seed germination is a major problem in pansy; its seeds are very costly especially the $F_1$ hybrids. Therefore, seed germination studies were carried out in pansy seeds with polyethylene glycol (PEG-6000) @ 300 g/l, $GA_3$ @20 mg/l, biopriming with *Trichoderma viridi* @ 8 g/kg of the seed, hydropriming (with water) and control (non-primed) for two consecutive years (2010 and 2011). The germination percentage increased tremendously in both the years of study with all the priming treatments in comparison to non-primed seeds and can also be evidenced by pooled analyses of the two years data. Among all the priming treatments, $GA_3$@20 mg/l had recorded the highest results w.r.t. germination percentage (93.12%), seedling length (6.55 cm), seedling vigour index (609.6), seedling fresh weight (15.80 mg), seedling dry weight (1.50 mg). The lowest germination percentage (60.75%), seedling length (3.15 cm), seedling vigour index (195.3), seedling fresh weight (10.30 mg), seedling dry weight (0.78 mg) recorded in non-primed or control seeds. The minimum seedling moisture content (91.20%) was recorded in PEG-6000 @300 g/l and maximum seedling moisture content (94.85%) was recorded in non-primed or control seeds.

**Key words**: Pansy, seed priming, seed germination, seedling vigour, PEG, $GA_3$

Pansy is a large group of hybrid plants belonging to the family Violaceae, cultivated as garden flowers. Modern pansies are derived from viola tricolor hybridised with other *Viola* species and hybrid referred to as *Viola* x *Wittrockiana* [1]. In flower language, pansy is considered as the symbol of free thoughts [2]. Pansy is an annual or short lived perennial widely grown as a bedding plant and occasionally produced for cut flowers [3]. The uses of pansy includes: herbal medicine, in syrup and honey making. The leaves and flowers of pansy are the rich sources of vitamin A and C besides grown as flowering potted plants. Commercial importance of pansies can be realized as the most popular fall bedding plant [4]. Pansies are cold tolerant plants [5]. One of the problems associated with pansy growing is poor and slow rate of seed germination. Even under the optimum conditions, the germination percentage is low (50-60%) [6]. Thus accounts for huge monetary losses as its seeds are very costly. Temperature required for germination of pansy is 17°-21°C.

Seed priming (controlled hydration followed by redrying) has been used to reduce germination time, harmonize germination, improve germination rate and improve the crop establishment in many crops under sub-optimal temperature conditions [7]. Priming salvia seed with PEG-6000 @–0.8 MPa for 10 days at 15°C improved germination of cultivars [8]. Keeping all this in view, the present studies were planned to know the effect of priming treatments on seed germination parameters and to work out the best priming treatment for improving seed germination in pansy besides other important parameters of commercial importance.

Received: May 2012 Revised: June 2012 Accepted: June 2012

## MATERIALS AND METHODS

*Seed material*

The disease free and uniform seeds of *Viola x Wittrockiana* Gams., "Formula mixture", were used in present study. For all the treatments, healthy and uniform seeds were selected by removing the light and damaged seeds, following critical observation. Different seed lots containing 100 seeds each were made for treatment with different priming agents.

*Seed priming treatment*

Seed priming was carried out by treating the seeds with different priming agents as: For priming with PEG-6000, the seeds were kept in 9 cm Petri dish on Whatmann No.1 filter paper and were moistened with 5 ml solution of PEG-6000 @300 g/l. For $GA_3$ priming, the seeds were treated in same way as PEG 8000 with 5 ml of $GA_3$ solution prepared @20 mg/l. For hydropriming, seeds were moistened with 5 ml distilled water. The moisture must be at the level to ensure only one surface absorption of priming solution. All the Petri dishes with different treatments were kept at 23°C in incubator for 24 hr. For biopriming of seed, the commercially available powder (formed bio-agent) of *Trichoderma viridi* was taken in required quantity *i.e.* 8 g/kg of seed and the suspension of bio-agent was prepared by mixing the required quantity of bio-agent in 100 ml of distilled water and then pansy seeds were treated for 24 hr at 23°C in that particular suspension.

After 24 hr of priming treatment, the seeds were taken out and washed with distilled water for 2-3 times to remove excess of chemical and spread in a thin layer on blotting paper for drying under room conditions to attain original moisture content. Another required quantity of untreated seeds was used as control.

*Germination experiment*

The seed germination studies in pansy were conducted in two consecutive years (2010 and 2011). After seed priming, the seeds were kept in 9 cm Petri dishes on filter paper moistened with water at constant temperature of 17.5°C. The treatments in the germinator were arranged in completely randomized design, having five treatments with four replications, each containing 100 seeds. To study the germination parameters, daily counts on germination were made by counting the seeds germinating on each day at fixed time. All the parameters were calculated according to the standard formulae and pooled analyses was done for two years. The data were subjected to CRD analysis using Assex software and the results were computed accordingly.

1. **Seed germination percentage:** The number of normal seedlings were counted at the end of 10th day as count of germination and expressed in percentage.

2. **Seedling length (cm):** 10 seedlings were taken randomly and length was measured with the scale in centimetres by adding radical and plumule length.

3. **Seedling fresh weight** (mg/seedling): Ten normal seedlings used for measuring seedling length were taken and weighed, the average weight was calculated and expressed in mg/seedling.

4. **Seedling dry weight** (mg/seedling): Ten normal seedlings used for measuring fresh weight were wrapped in butter paper and dried in a microwave oven for 3 min. Then the seedlings were removed and allowed to cool in a desiccator for 30 min before weighing and expressed in mg/seedling (average of 10 seedlings).

5. **Moisture content of seedling (%):**

$$mc\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100$$

6. **Vigour index:** The vigour index (VI) was calculated by adopting the method suggested by Dezfuli *et al.* [9] and expressed in pure number.

VI = Germination (%) x Seedling length (cm)

## RESULTS AND DISCUSSION

Results presented in table 1 showed that all the parameters under study were influenced by seed

priming treatments and exhibited better results over control in both years of study. According to pooled mean of the data for both the years, priming gave higher values for all the parameters in comparison to control.

*Seed germination (%)*

The data presented in table 1 indicated that germination percentage was maximum in $T_2$ ($GA_3$ @20 mg/l) in both the years (*i.e.* 94.25% in 2010 and 92.00% in 2011, respectively) and these results were found to be statistically at par with $T_1$ (PEG-6000 @300 g/l). However, minimum germination percentage was observed in $T_5$ (non-primed or control) in both the years (*i.e.* 60.25% in 2010 and 61.25% in 2011, respectively). The pooled data also showed the similar trend *i.e.* maximum germination percentage (93.12%) in $T_2$ ($GA_3$ @20 mg/l) which was found to be statistically at par with $T_1$ (PEG-6000 @300 g/l) and minimum germination percentage (60.75%) in $T_5$ (non-primed or control). The increased germination of pansy seeds following priming with $GA_3$ and PEG might be due to the fact that these priming treatments could have induced quantitative changes in biochemical contents of the seeds, improved membrane integrity and metabolism of seeds as well as synthesis of some proteins (globulins and cruciferin) than the non-primed seeds [10, 11]. The priming of pansy seeds with $GA_3$ might have increased the germination percentage due to the reason that the application of gibberellins especially $GA_3$ increased the amino acid contents in embryo besides releasing some hydrolytic enzymes required for digestion of endospermic starch when seeds renew growth at germination [12]. Seed hydropriming at -10 bars for 3hr significantly exhibited higher germination percentage, speed of germination, root length and seed vigour in amaranth cultivars [13].

*Seedling length*

The data presented in table 1 indicated that

**Table 1 Effect of seed priming on germination percentage and seedling vigour**

| Treatment | Germination (%) | | | Seedling length (cm) | | | Seedling fresh wt(g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | Pooled | 2010 | 2011 | Pooled | 2010 | 2011 | Pooled |
| $T_1$ (PEG-6000 @300 g/l) | 89.00 (70.78) | 90.00 (71.29) | 89.50 (71.29) | 5.18 | 6.55 | 5.86 | 14.00 | 15.19 | 14.60 |
| $T_2$ ($GA_3$ @20 mg/l) | 94.25 (76.16) | 92.00 (73.62) | 93.12 (74.89) | 6.23 | 6.86 | 6.55 | 16.00 | 15.60 | 15.80 |
| $T_3$ (Biopriming @8 g/kg of seeds) | 85.00 (67.25) | 85.50 (67.72) | 85.25 (67.49) | 4.05 | 6.18 | 5.11 | 12.29 | 14.50 | 13.40 |
| $T_4$ (Hydropriming) | 82.00 (64.93) | 80.25 (63.73) | 81.12 (64.33) | 2.98 | 5.45 | 4.21 | 11.30 | 13.94 | 12.62 |
| $T_5$ (Control) | 60.25 (50.95) | 61.25 (51.56) | 60.75 (51.26) | 2.13 | 4.18 | 3.15 | 9.80 | 10.80 | 10.30 |
| Mean | 82.10 (66.02) | 81.80 (65.68) | - | 4.11 | 5.85 | - | 12.68 | 14.01 | - |
| $CD_{(0.05)}$ | 5.44 | 6.86 | - | 0.69 | 0.54 | - | 1.07 | 1.55 | - |
| $CD_{(0.05)}$ for pooled data | | | | | | | | | |
| Treatments | 2.95 | | | 0.55 | | | 0.90 | | |
| Year | NS | | | 0.35 | | | 0.57 | | |
| Treatments x Year | NS | | | 0.79 | | | 1.28 | | |

*Firures in parentheses are square root transformed values

**Table 2. Effect of seed priming on seedling fresh weight, seedling dry weight and seedling moisture content**

| Treatment | Seedling dry weight (mg) | | | Vigour index | | | Moisture content (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | Pooled | 2010 | 2011 | Pooled | 2010 | 2011 | Pooled |
| $T_1$ (PEG-6000 @300 g/l) | 1.23 | 1.59 | 1.41 | 461.0 | 590.5 | 525.8 | 91.20 (9.55) | 89.49 (9.46) | 90.35 (9.51) |
| $T_2$ ($GA_3$ @20 mg/l) | 1.33 | 1.68 | 1.50 | 586.6 | 632.6 | 609.6 | 91.67 (9.57) | 89.25 (9.45) | 90.46 (9.51) |
| $T_3$ (Biopriming @8 g/kg of seeds) | 1.05 | 1.50 | 1.27 | 344.7 | 528.6 | 436.7 | 91.44 (9.56) | 89.63 (9.47) | 90.54 (9.52) |
| $T_4$ (Hydropriming) | 0.72 | 1.42 | 1.07 | 244.3 | 437.2 | 340.8 | 93.56 (9.67) | 89.83 (9.48) | 91.70 (9.58) |
| $T_5$ (Control) | 0.50 | 1.08 | 0.79 | 131.5 | 259.1 | 195.3 | 94.85 (9.74) | 90.10 (9.49) | 92.47 (9.62) |
| Mean | 0.97 | 1.45 | - | 353.6 | 489.6 | - | 92.55 (9.62) | 89.66 (9.47) | - |
| $CD_{(0.05)}$ | 0.20 | 0.18 | - | 61.52 | 97.28 | - | 0.09 | NS | - |
| $CD_{(0.05)}$ for pooled data | | | | | | | | | |
| Treatments | 0.13 | | | 55.14 | | | 0.05 | | |
| Year | 0.08 | | | 34.86 | | | 0.03 | | |
| Treatments x Year | NS | | | NS | | | 0.07 | | |

*Firures in parentheses are square root transformed values

seedling length was recorded maximum in $T_2$ ($GA_3$ @20 mg/l) in both the years (*i.e.* 6.23 cm in 2010 and 6.86 cm in 2011, respectively) and found to be significant over other priming treatments in 2010, but statistically at par with $T_1$ (PEG-6000 @300 mg/l) in 2011. However, minimum seedling length was observed in $T_5$ (non-primed or control) in both the years (*i.e.* 2.13 cm in 2010 and 4.18 cm in 2011, respectively). The pooled data showed the similar trend *i.e.* maximum seedling length (6.55 cm) in $T_2$ ($GA_3$ @20 mg/l) and found to be significant over all other treatments. The increased seedling length following priming with $GA_3$ might be due to the increased rate of cell division in the root and shoot tips incited by the application of $GA_3$ [11]. Seed priming with $GA_3$ @10 ppm showed highest germination percentage as well as higher radical length and plumule length in blackgram and horsegram seeds [12].

*Seedling fresh weight*

The data presented in table 1 indicated that maximum seedling fresh weight was recorded in $T_2$ ($GA_3$ @20 mg/l) in both the years (*i.e.* 16.00 mg in 2010 and 15.60 mg in 2011, respectively) and found to be significant over all other priming treatments in 2010, but statistically at par with $T_1$ (PEG-6000 @300 g/l) in 2011. However, minimum seedling fresh weight was observed in $T_5$ (non-primed or control) in both the years (9.80 mg in 2010 and 10.80 mg in 2011). The pooled data showed the similar trend *i.e.* maximum seedling fresh weight (15.80 mg) in $T_2$ ($GA_3$ @20 mg/l) and found to be significant over all other treatments and minimum seedling fresh weight (10.30 mg) in $T_5$ (Non-primed or control).

*Seedling dry weight*

The data presented in table 1 indicated that maximum seedling dry weight was recorded in $T_2$ ($GA_3$ @20 mg/l) in both the years (*i.e.* 1.33 mg in 2010 and 1.68 mg in 2011, respectively) and found to be statistically at par with $T_1$ (PEG-6000 @300 g/l) in both the years. However, minimum seedling dry weight was observed in $T_5$ (Non-primed or control) in both the years (*i.e.* 0.50 mg in 2010 and 1.08 mg in 2011, respectively). The pooled data showed the similar trend *i.e.* maximum seedling dry weight (1.50 mg) in $T_2$ ($GA_3$ @20 mg/l) and found to be statistically at par with $T_1$ (PEG-6000 @300 g/l), whereas minimum seedling dry weight (1.08 mg) in $T_5$ (Non-primed or control) seeds.

The increased seedling fresh weight and seedling dry weight following priming with $T_2$ ($GA_3$ @20 mg/l) and $T_1$ (PEG-6000 @300 g/l) might be ascribed with the fact that $GA_3$ and PEG-6000 enhances the water uptake of the seedlings which might have activated the enzymes with an accompanying mobilization of reserve materials ending in transport of the reserve materials in the embryo by osmotic conditioning and thus stronger seedlings were obtained as a result of embryo growth [15].

*Seedling vigour index*

The data presented in table 1 indicated that maximum seedling vigour index was recorded in $T_2$ ($GA_3$ @20 mg/l) in both the years (*i.e.* 586.6 in 2010 and 632.6 in 2011, respectively) and found to be significant over all other priming treatments in 2010, but statistically at par with $T_1$ (PEG-6000 @300 g/l) in 2011. However, minimum seedling vigour index was observed in $T_5$ (Non-primed or control) in both the years (131.5 in 2010 and 259.1 in 2011). The pooled data showed the similar trend *i.e.* maximum seedling vigour index (609.6) in $T_2$ ($GA_3$ @20 mg/l) and found to be significant over all other treatments and minimum seedling vigour index (195.3) in $T_5$ (Non-primed or control). Higher seedling vigour with $T_2$ ($GA_3$ @20 mg/l) was attributed to the increased seedling length caused by the rapid cell division and cell growth with same treatment of priming [13].

*Seedling moisture content*

The data presented in table 1 indicated that seedling moisture contents were maximum in $T_5$ (Non-primed or control) in both the years (*i.e.* 94.85% in 2010 and 90.10% in 2011, respectively). However, minimum seedling moisture contents (*i.e.* 91.20% in 2010 and 89.25% in 2011, respectively) were recorded in $T_1$ (PEG-6000 @300 g/l) and $T_2$ ($GA_3$ @20 mg/l), respectively. On pooled analysis of the data, the maximum seedling moisture contents (92.47%) was found in $T_5$ (Non-primed or control) and was statistically at par with $T_4$ (Hydropriming), whereas minimum seedling moisture content (90.35%) was found in $T_1$ (PEG-6000 @300 g/l) and was statistically at par with $T_2$ ($GA_3$ @20 mg/l) and $T_3$ (Biopriming). These studies were correlated with another study, where moisture content of newly-germinated brussels sprout seeds was reduced to 16% without loss of viability and these low-moisture-content germinated (LMCG) seeds produce greater number of seedlings, emerged more uniformly and more rapidly than the non-germinated dry seeds, leading to larger, more uniform seedlings for subsequent transplanting [16]. Moisture contents of the seedlings may increase or decrease inconsistently under different salt concentrations [17].

From this study it was concluded that seed priming had exhibited positive effects on germination characteristics of pansy seeds such as germination percentage, seedling length, seedling vigour index, seedling fresh weight, seedling dry weight and seedling moisture contents under laboratory conditions in both the years of study. Therefore, seed priming can be utilized as measure to overcome the problem of poor and low seed germination in pansy; and can be very economic for the growers as the higher seed cost will be compensated by the higher germination values. Priming with $GA_3$ @20 mg/l has been proved to be the most effective and economical for enhancing the seed germination of pansy.

## REFERENCES

1. PANSY WIKIPEDIA (2011). The Free Encyclopedia.

2. GAYLOR AL (1997). Pansy for your thoughts. *Freethought Today*

3. CARPENTER WJ AND BOUCHER JF (1991). Priming improves high temperature germination of pansy seed. *Hort Sci* **26**(5): 541-44

4. BAILEY DA (1998). *Commercial Pansy Production*. North Carolina State University, Leaflet 521

5. POST K (1949). Florist Crop Production and Marketing. Orange Fudd, New York 33-35

6. JOERGENSEN KENNETH (2004). Starting with Seeds: Pansy in Seeds & Plants

7. AFZAL I, ASHRAF S, QASIM M, BASRA SMA AND SHAHID M (2009). Does halopriming improve germination and seedling vigor in marigold. *Seed Sci Technol* **37**: 436-45

8. CARPENTER WJ (1989). *Salvia splendens* seed pre-germination and priming for rapid and uniform plant emergence. *J Amer Soc Hort Sci* **114**(2): 247-50

9. DEZFULI PM, SHARIF-ZADEH F AND JANMOHAMMADI MOHSEN (2008). Influence of priming techniques on seed germination behaviour of maize inbred lines (*Zea mays* L) *ARPN J Agricultural and Biological Science* **3**(3): 22-25

10. RAO MSL, KULKARNI S, LINGARAJU S AND NADAF HL (2009). Biopriming of seeds: potential tool in the integrated management of *Alternaria blight* of sunflower. *Helia* **50**: 107-14

11. VARIER A, KURIAKOSE A AND DADLANI M (2010). Subcellular basis of seed priming. *Curr Sci* **99**(4): 450-56

12. CHAUHAN JS, TOMAR YK, SINGH I, N ALI, SEEMA AND DEBARATI (2009). Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. *J Amer Sci* **5**(5): 79-84

13. MOOSAVI A, TAVAKROL RA AND AYNEHBAND A (2009). Effect of seed priming on germination characteristics polyphenol oxidase and peroxidise activities of four amaranth cultivars. *J Food Agri and Environ* **7**(3&4): 353-58

14. AMOOAGHAIE R AND VALIVAND M (2011). Combined effect of gibberellic acid and long time osmopriming on seed germination and subsequent seedling growth of *Klussia odoratissima* Mozaff. *African Journal of Biotechnology* **10**(66): 14873-80

15. MUHAMMAD JAMIL AND EUI SHIK RHA (2007). Gibberellic acid ($GA_3$) enhance seed water uptake, germination and early seedling growth in sugar beet under salt stress. *Pakistan J Biol Sci* **10**: 654-58

16. FINCH-SAVAGE WE (1998). Comparison of brussels sprout seedling establishment from ungerminated and low-moisture-content germinated seeds. *Ann Appl Biol* **113(2):** 425-29

17. MUHAMMAD ZAHIR AND HUSSAIN FARRUKH (2010). Effect of NaCl salanity on the germination and seedling growth of some medicinal plants. *Pakistan J Bot* **42**(2): 889-97.