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STUDY ON INFLUENCE OF OSMOPRIMING BY POLYETHYLENE GLYCOL 6000 ON GERMINATION OF PAPAYA SEEDS

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ABSTRACT

The experiment aimed to ascertain how osmo-priming by polyethylene glycol 6000 (PEG 6000) influences the germination of papaya seeds. Seeds were primed with six strengths of polyethylene glycol (PEG 6000) as priming media for 24 hours at room temperature (distilled water served as the control, PEG 6000 other concentrations were 5%, 10%, 15%, 20% and 25%). Completely Randomized Design with three replications for the experiment was used, and each replication had 6-7 seeds. Coco peat, garden soil, and compost were combined and prepared in the following ratio of 2:1:1 for the potting mixture before planting the prepared seeds. On the basis of specific occurrences, such as seed germination percentage, number of leaves, length of leaves, length of roots, number of rootlets, seedling height, and total chlorophyll content, data were gathered on days 20, 25, and 30 days after sowing. According to the findings of the variance analysis, different osmotic potentials significantly affected the amount of seedling vigour, germination percentage, and total chlorophyll content. Among the treatments he 15% polyethylene glycol 6000 (T4) seed treatment increased seedling parameters and raised germination percentage as compared to control
Keywords **:** Osmo-priming, papaya seed, polyethylene glycol, germination, observation.

Introduction

The papaya (*Carica papaya* L.) is a tasty tropical fruit that belongs to the Caricaceae family and is regarded as the "wonder fruit of the tropics". Papaya is a tropical and it thrives in areas with summertime temperatures between 35-38°C. Papaya seedlings can be ready after 60 days of seed sowing. June – September is the best time for sowing however planting in rainy days should be avoided.

The dormant embryos and slowly permeable seed coverings of papayas lead their seeds to germinate slowly. Effective seed germination is crucial for productivity. Large financial losses may result from uneven or poor germination and subsequent inhomogeneous seedling growth. Many crops, including maize, wheat, rice, and canola, benefit from seed priming treatments because they result in enhanced seed germination and establishment (Basra *et al*., 2005; Ghiyasi *et al*., 2008a, b). It has been effectively proven that seed priming increases germination and emergence in the seeds of many crops, especially in the seeds of vegetables and small-seeded grasses (Dell Aquila and Tritto, 1991). Seed priming, an affordable and practical approach, is used to achieve consistent seed development in crops. It has positive benefits on crop productivity, maturity, release photo- and thermo-dormancy, nutrient uptake, and water use efficiency.

Osmopriming uses an osmotic solution with a low water potential instead of using just water. Water enters the seed slowly because of the low water potential of osmotic solutions, which permits progressive seed imbibition and stimulation of the early stages of germination but prevents radicle protrusion. The water potential of a priming agent usually ranges from -1.0 MPa to -2.0 MPa. However, water potential levels and the length of the priming treatment should always be adjusted to the species, cultivar, and occasionally seed lot. Polyethylene glycol (PEG), mannitol, sorbitol, glycerol, and inorganic salts including NaCl, KCl, KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, and $CaCl_2$ are among the several substances utilized in the osmopriming process. The most common chemical employed in osmopriming treatment is PEG, mainly owing to its specific characteristic. It has been demonstrated that seed priming with PEG is an efficient way to increase seed germination, seedling emergence, and stress tolerance in a variety of crop plants under challenging circumstances such as salt, water, cold, and nano-ZnO stressors (Lutts *et al*., 2016).

Thus, in the present study osmopriming with PEG 6000 was done in papaya seeds to facilitate germination and enhance other growth-related parameters.

Materials and Methods

Seed Collection and Study Area

Fresh organic, mature papayas (*Carica papaya*) were purchased from offline stores of local market, from which seeds has been extracted for the experiment. The experiment has been executed in pot culture atmosphere, at Department

of Horticulture, Institute of Agricultural Science, University of Calcutta, India.

Seed Extraction

Papaya fruits were cut and thereafter seeds were carefully extracted. Further washing was carried out in running water to make them free from the pulp adhered to their surface.

Soil Preparation

At first, the soil was collected from the nearest pond-side area of the premises of Tarak Nath Palit Campus, University of Calcutta, and then mixed with well-decomposed compost and coco peat (which were purchased from an online shopping store) were added to the mixture for better result. The mixture was left for three days so that the microorganisms in the mixture development becomes enough and then finally the medium was ready to use.

Treatment and Experimental Design

The experiment comprised six different treatments viz. T1: Seed treatment with 0% Polyethylene Glycol (only water as control), T2: Seed treatment with 5% Polyethylene Glycol, T3: Seed treatment with 10% Polyethylene Glycol, T4: Seed treatment with 15% Polyethylene Glycol, T5: Seed treatment with 20% Polyethylene Glycol and T6: Seed treatment with 25% Polyethylene Glycol. The experiment was laid down in Completely Randomized Design (Gomez and Gomez, 1984; Sheoran *et al*., 1998) method with these six treatments having 3 replications for each with 5-6 seeds in each pot. The pots were filled with 50% coco peat, 25% garden soil, and 25% compost in each of them. The seeds of papaya were soaked in treatments of polyethylene glycol 6000 (mentioned above) for a period of 24 hours and then were taken out on filter paper to dry out under room temperature. Seeds were planted about 10 to 15 mm deep into the soil. Further data were recorded on the basis of specific incidents such as germination percentage of seed, number of leaves, length of leaves, length of roots, number of rootlets, and seedling height. Every day the pots were exposed out in the sun for 6 to 7 hrs. Irrigations were done on daily basis in the evening until all the seeds germinate and then in an interval of 2 days. Other cultural practices like weeding and plant protection measures were done uniformly in every pot.

Observation

Three major observations were done on 20, 25, 30 DAS (Days after Sowing).

Germination percentage

Sowing dates and soaking durations and the interaction of sowing dates and soaking durations significantly affect the germination percentage of papaya seeds. The following formula was used to calculate the percentage of germination. Similar was done by Nichols (1968).

$$GP=(S/T) \times 100$$

Where, S- The number of germinated seed.

T- Total number of seeds.

Number of leaves

The number of leaves per plant in each treatment was recorded in randomly selected plants at every 5 days interval after germination and then the mean number of leaves was calculated. A similar procedure was done by Damalas (2019).

Length of leaves

The average size and area of the leaves were measured by a scale. The length of leaves was determined in centimeters. The average measurement of five randomly selected leaves from each treatment was subjected to statistical intervention (Tania *et al*., 2019).

Length of roots

The length of roots was measured by scale from the base of the shoot to tip of the roots. The root length was expressed in centimeters. Root length is a better parameter in relating absorption of water and nutrients by roots. (Eisvand *et al*., 2011).

Number of rootlets

Counting of number of rootlets were done. A similar experiment was conducted by Singh (2017).

Total length of seedling:

Final length of each seedling was measured by measuring from the shoot tip to the root tip by scale. (Chiu *et al*., 2002).

Chlorophyll content:

The chlorophylls are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. Chlorophylls are extracted in 80% acetone and the absorption at 663 nm and 645 nm are read in a spectrophotometer. The work on chlorophyll content calculation was done by Arnon, (1949).

Results and Discussion

Number of leaves

From the experimental results presented in Table-1 that the collected highest mean number of leaves at 20 DAS was 4.33 observed in the treatment of T2-5% Polyethylene glycol 6000 and T6-25% Polyethylene glycol 6000 where as a constant data has been observed as 4.00 in the treatment T1 (control: water soaked for 24 hours), T3-10% polyethylene glycol 6000, T4-15% polyethylene glycol 6000, and T5- 20% polyethylene glycol 6000. At the next observation on 25 DAS the highest mean number of leaves was 5.33 observed in the treatment of T1 (control: water soaked for 24 hours) and lowest mean number was 4.00 observed in the treatment of T2- 5% polyethylene glycol, and T3-10% polyethylene glycol 6000. Likewise at the observation recorded on 30DAS, it was obtained that T4-15% polyethylene glycol 6000 had the maximum number of leaves of 5.67, which was jointly followed by T2-5% polyethylene glycol 6000 and T3-10% polyethylene glycol 6000 with 5 number of leaves each. T1(control: 0% polyethylene glycol for 24 hours) was found with the least number of 4 mean leaves.

Table 1 : Number of leaves of the papaya seedlings influenced by osmopriming

Treatments	20 DAS	25 DAS	30 DAS
T1	4.00	5.33	4.00
T2	4.33	4.00	5.00
T3	4.00	4.00	5.00
T4	4.00	4.67	5.67
T5	4.00	5.00	4.67
T6	4.33	4.33	4.33
CD	**NA**	**0.734**	**0.734**
SEm±	**0.192**	**0.236**	**0.236**

Length of leaves

Variations in the length of the leaves were observed under varied polyethylene glycol 6000 concentrations. The initial observation was done on 20 DAS, and the treatment T6-25% polyethylene glycol 6000 had the highest mean leaf length of 2.10 cm, and the treatment T3-10% polyethylene glycol 6000 was with the lowest mean leaf length of 1.57 cm. However, the data of 25 and 30 DAS showed that treatments T2-5% polyethylene glycol 6000, T4-15% polyethylene glycol 6000, T5-20% polyethylene glycol 6000 improved significantly as compared to the first finding. At 25 DAS, T4-15% polyethylene glycol 6000 documented the maximum length of leaves with 2.13 cm, which was followed by T6-25% polyethylene glycol 6000 and T2-5% polyethylene glycol 6000 with 2.10 and 2.03 cm length of leaves respectively. At the end of the experiment T4-15 % polyethylene glycol 6000 gave promising result with 2.17 cm length of leaves. However, treatments like T5-20% polyethylene glycol 6000, T2-5% polyethylene glycol 6000 and T3-10% polyethylene glycol 6000 showed good values.

Table 2 : Length of leaves (cm) of the papaya seedlings influenced by osmopriming

Treatment	20 DAS	25 DAS	30 DAS
T1	1.67	1.80	1.83
T2	1.97	2.03	1.90
T3	1.57	1.83	1.90
T4	1.90	2.13	2.17
T5	1.97	1.97	2.10
T6	2.10	2.10	1.77
CD	**NA**	**0.199**	**0.254**
SEm±	**0.116**	**0.064**	**0.082**

Number of rootlets

The quantity of rootlets changed noticeably between different replications in different pots. Table 3 shows that on 20 DAS, treatment T5- 20% polyethylene glycol 6000 and T6-25% polyethylene glycol 6000 had the highest mean number of rootlets of 8.00, whereas treatment T3-10% polyethylene glycol 6000 had the lowest value of 6.67. Another finding on 25 DAS showed that treatment T6-25% polyethylene glycol 6000 had the highest mean number of rootlets of 8.33 which was closely followed by T1 (control: 0% polyethylene glycol for 24 hours) and T5- 20% polyethylene glycol 6000, where both documented 8.00 number of rootlets. The treatment T4-15% polyethylene glycol 6000 had the lowest mean number at 7.33. Whereas, the data collected on 30 DAS shows good hike on number of rootlets where highest mean number of rootlets was 10.67 in treatment T4- 15% polyethylene glycol 6000 succeeded by T2- 5% polyethylene glycol 6000 with 10.33 rootlets and lowest was 7.67 in treatment T1 (control: 0% polyethylene glycol for 24 hours).

Table 3 : Number of rootlets of the papaya seedlings influenced by osmopriming.

Treatments	20 DAS	25 DAS	30 DAS
T1	7.33	8.00	7.67
T2	7.33	7.67	10.33
T3	6.67	7.67	9.67
T4	7.67	7.33	10.67
T5	8.00	8.00	8.67
T6	8.00	8.33	9.00
CD	**NA**	**NA**	**0.948**
SEm±	**0.430**	**0.360**	**0.304**

Length of roots

It is being observed from experimental results shown in Table-4, that the highest mean root length of 7.30 cm was observed in treatment T5- 20% polyethylene glycol 6000, whereas, the lowest mean root length of 5.23 cm was observed in treatment T1(control: 0% polyethylene glycol for 24 hours) on 20 DAS. Next observation was recorded on 30 DAS where highest mean root length was 9.47 cm on treatment T4-15% polyethylene glycol 6000, which was followed by T2-5% polyethylene glycol 6000 and T3-10% polyethylene glycol 6000 with 8.50 and 8.07 cm length of roots. The lowest mean root length was recorded as 5.77 cm in treatment T5-20% polyethylene glycol 6000.

Table 4. : Length of roots (cm) of the papaya seedlings influenced by osmopriming

Treatments	20 DAS	30 DAS
T1	5.23	5.97
T2	5.37	8.50
T3	5.40	8.07
T4	6.27	9.47
T5	7.30	5.77
T6	7.13	7.27
CD	**1.223**	**1.294**
SEm±	**0.393**	**0.415**

Total length (cm) of seedlings

It is being observed from experimental results shown in Table-5, that highest mean total length of seedling of 9.00 cm was observed in treatment T3- 10% polyethylene glycol 6000, whereas, the lowest mean total length of seedling of 7.30 cm was observed in treatment T1 (control: 0% polyethylene glycol for 24 hours) on 20 DAS. Next observation was recorded on 25 DAS where a noticeable increase in the seedling length was found as compared to 20 DAS. Here the highest mean total length of seedling was 10.00 cm in treatment T3-10% polyethylene glycol 6000, which was very closely followed by the treatments T4-15% polyethylene glycol 6000 and T5- 20% polyethylene glycol 6000 with seedling length being 9.37 and 9.07 cm respectively. The lowest mean total length of seedling was recorded 8.00 cm in treatment T2- 5% polyethylene glycol 6000. Data recorded on 30 DAS however showed the highest mean total length of seedling of 10.27 cm in treatment T6-25% polyethylene glycol 6000 but treatments like T5-20% polyethylene glycol 6000, T2-5% polyethylene glycol 6000, T3-10% polyethylene glycol 6000 and T1 (control: 0% polyethylene glycol for 24 hours) recorded a proper total seedling length at the end of the study.

Table 5 : Total length (cm) of the papaya seedlings influenced by osmopriming

Treatments	20 DAS	25 DAS	30 DAS
T1	7.30	8.77	9.10
T2	7.40	8.00	9.70
T3	9.00	10.00	9.40
T4	8.20	9.37	8.00
T5	7.57	9.07	10.07
T6	8.37	8.87	10.27
CD	**0.992**	**1.042**	**0.846**
SEm±	**0.319**	**0.334**	**0.271**

Germination percentage

According to the experimental results shown in Table 6, on 20 DAS treatment T4-15% polyethylene glycol 6000 had the highest mean percentage of germination, which was recorded at 63.49%, and treatments T2-5% polyethylene glycol 6000 and T6-25% polyethylene glycol 6000 had the lowest mean percentages, which were both recorded at 50.79%. On 25 DAS, the treatment T4-15% polyethylene glycol 6000 had the highest mean germination percentage of 82.53% while the treatment T2-5% polyethylene glycol 6000 had the lowest mean percentage of 57.14%. Lastly the experimental results observed on 30 DAS shows highest mean germination percentage as 87.30% in treatment T4-15% polyethylene glycol 6000, whereas lowest mean germination percentage was 65.00% in treatment T2-5% polyethylene glycol 6000. From the experimental data from the table 6, it is thus evident that treatment T4 i.e., 15% polyethylene glycol for 24 hours showed great result in germination percentage, where as other treatments also did perform well.

Table 6 : Germination percentage of the papaya seedlings influenced by osmopriming

Treatments	20 DAS	25 DAS	30 DAS
T1	55.55	61.90	66.66
T2	50.79	57.14	65.00
T3	60.31	76.19	82.53
T4	63.49	82.53	87.30
T5	61.90	74.60	80.95
T6	50.79	74.60	82.53
CD	**NA**	**16.025**	**15.744**
SEm±	**3.666**	**5.144**	**5.054**

Total Chlorophyll content

Table 7 shows the data of total chlorophyll content (mg/ml) of the papaya seedlings where the highest chlorophyll content was recorded as 11.94 mg/ml in treatment T1 (control: 0% polyethylene glycol for 24 hours) followed by treatment T5-20% polyethylene glycol 6000 with 10.51 mg/ml and lowest in treatment T2-5% polyethylene glycol 6000 with 7.40 mg/ml on 20 DAS. Whereas on 30 DAS the highest total chlorophyll content was recorded as 9.05 mg/ml in treatment T4- 15% polyethylene glycol 6000 which was immediately followed by T2-5% polyethylene glycol 6000 with 9.00 mg/ml and the lowest total chlorophyll content was 7.21 mg/ml in treatment T6-25% polyethylene glycol 6000. From the table it was thus seen that treatment T4- 15% polyethylene glycol 6000 and T2-5% polyethylene glycol 6000 showed standard accumulation in the total chlorophyll content at the last day of observation.

Table 7 : Total chlorophyll content (mg/ml) of the papaya seedlings influenced by osmopriming.

Treatment	20 DAS	30 DAS
T1	11.94	8.19
T2	7.40	9.00
T3	7.77	8.61
T4	9.85	9.05
T5	10.51	8.84
T6	8.75	7.21
CD	**2.231**	**NA**
SEm±	**0.716**	**0.401**

This study has shown that seed priming is a successful and efficient tactic for increasing seedling vigour, and the number of leaves. Data from 20 days after sowing (Table 1) showed that the maximum mean number of leaves was 4.33, and that number climbed to 5.33 by 25 days after sowing. And observation on the first 30 days after sowing shows that 5.67 was the greatest mean number of leaves. As a result, there was a progressive increase in the mean greatest number of leaves. Great results are shown by treatments T2 (5 % polyethylene glycol for 24 hours), T3 (10 % polyethylene glycol for 24 hours), and T4 (15 % polyethylene glycol for 24 hours). Similar outcomes were observed in an experiment to determine the impact of soyabean seed osmopriming with PEG 6000 carried out by Sadeghi *et al*. (2011). Effect of osmopriming on the growth of the papaya seedling showed remarkable benefit on the length of leaves (Table 2). The observation on 30 DAS showed maximum mean length of leaves of 2.17 in treatment T4- 15% polyethylene glycol 6000 and the minimum was 1.77 in treatment T6- 25% polyethylene glycol 6000. Ghobadi *et al*. (2012) reported similar results on his experiment hormonal priming (gibberellic acid) and osmopriming on wheat seeds, where the results showed an increase in the number of leaves and length of leaves, because of the treatments. Papaya seedlings also showed an appreciable effect of osmopriming on the number of rootlets, length of roots and total length of seedlings (Table 3, Table 4, Table 5). The number of rootlets per seedling was increased with progressing days. Overall highest mean number of rootlets was 10.67 in the treatment of T4- 15% polyethylene glycol 6000 on 30 DAS. The maximum mean length of root was 9.47 cm which was observed in the treatment of T4- 15% polyethylene glycol 6000 on 30 DAS. The effect of seed priming on the total growth of seedling of papaya was observed by the data of Table 5, where almost all the treatments showed a significant gain in the total length of the seedlings. In the study of Mouradi *et al*. (2016) on osmopriming of Moroccan alfalfa seeds, which were treated with polyethylene glycol (PEG 6000) (-0.6 MPa) for 24 hours Also showed higher growth of seedlings than treated one.

Seed priming have been demonstrated as a successful and effective strategy to improve germination of papaya seeds. Significantly higher germination percentage was observed in osmoprimed seeds as compared to non-primed seeds which indicated a positive effect of seed priming in influencing the seed germination process (Table 6). The highest germination percentage of 87.30% was recorded in the treatment T4-15% polyethylene glycol 6000. Salehzade *et al*. (2009), also concludes similar results in his study of how priming in seed is influencing germination and development attributesof wheat (*Triticum aestivum* L.), that seeds which were osmoprimed with polyethylene glycol (PEG 8000) and KNO_3 solutions for 12 hr, improved germination and seedling vigour than control. Total chlorophyll content of the papaya seedlings influenced by osmopriming also shows good values. At the end of the study T2-15% polyethylene glycol 6000 was obtained with the maximum total chlorophyll content of 9.05 mg/ml and T6-25% polyethylene glycol 6000 demonstrated the least content of 7.21 mg/ml. In the study of Anwar *et al*. (2020), in seed priming as promising technique, resulted that seed priming with GA_3 and KNO_3 synergistically promoted the chlorophyll contents, photosynthesis and nutrients uptake in cucumber seedlings, thus leading to improve plant growth parameters.

Conclusion

Osmopriming by Polyethylene glycol PEG 6000 can be used to increase seedling growth. In the major observation, these following consequences were evident to justify the above statement. A higher mean number of leaves, 5.67 was recorded in the treatment T4-15% polyethylene glycol 6000, whereas the lowest was 4.00 in the treatment T1 (control). This denotes that treatment T4-15% polyethylene glycol 6000 gives satisfactory results which show increasing number of leaves. The maximum mean length of leaves was observed as 2.17 cm in the treatment T4-15% polyethylene glycol 6000 and the lowest was 1.77 cm in the treatment T6-25% polyethylene glycol 6000. The highest mean number of rootlets was 10.67 again in the treatment T4-15% polyethylene glycol 6000 and the lowest, 7.67 was observed in the treatment T1 (control). The maximum length of roots of the papaya seedlings was observed 9.47 cm in the treatment T4-15% polyethylene glycol 6000 and minimum mean length of roots was 5.77 in the treatment T5-20% polyethylene glycol 6000. Overall treatment T2, T3, T4 i.e., 5%, 10%, 15% of polyethylene glycol 6000 respectively gave good results in length of roots. The highest in total length of papaya seedlings was recorded as 10.27 cm in the treatment T6- 25% polyethylene glycol 6000 and the lowest was 9.10 cm in the treatment T1 (control). Germination percentage reveals maximum progress during the experiment. The highest percentage of germination was 87.30 % in the treatment T4-15% polyethylene glycol 6000. Among the six treatments, T3, T4, T5, and T6 i.e., 10%, 15%, 20%, and 25% polyethylene glycol PEG 6000 gave great response in germination. Total chlorophyll content on 20 DAS marks the highest value as 9.05 mg/ml in the treatment T4- 15% polyethylene glycol 6000. Thus, from the entire experiment conducted it can be concluded that all the treatments performed well, however T4- 15% polyethylene glycol 6000 gave remarkable response in terms of germination and growth.

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