Effect of seed treatment on germination and physiology of custard apple (Annona squamosa L.) at seedling stage

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Abstract
An investigation was conducted to see the effect of GA3 and cow urine on growth and physiology of custard apple. The result evinced that the gibberellic acid at 200 ppm (T3) recorded minimum days taken to germination (15.75), days taken to 50% germination (10.50) germination percentage (86.67%), significantly maximum length of seedling (25.17cm) where recorded in treatment T3, maximum fresh and dry weight (4.43and 0.81 g) of shoots respectively were obtained at 150 days after sowing under treatment T3. The highest number of roots per seedling where found in treatment T3 i.e (55.75), The maximum LAI (1.29) and LAD (3701.38 cm2 days) was noted under treatment T3 (GA3 200 ppm), The maximum mean survival percentage of seedlings (83.82%), seedling vigour index I (0.81cm) and seedling vigour index II (77.03g) was recorded under treatment T3.

Keywords: Seed germination Fresh weight, dry weight, LAI, LAD, Length of seedling, Number of leaves per seedling, Seedling vigour index I and II, Survival percentage

1. Introduction
Custard Apple (Annona squamosa L.) belongs to the family Annonaceae and one of the finest fruits introduced in India from Tropical America. It is also found in wild form in many parts of India. It is also known as sugar apple or sweetsop in English while in Hindi ‘Sharifa’ and ‘Sitaphal’ are it’s common name. In Bengal and Assam it is known as ‘Ata’ , Anusa’ in Gujarat’, ‘Seetapandu’ in Telagu, ‘Sitapalam’ in Tamil and ‘Sirpha’ in Malayalam. In India, Custard Apple occupies an area of 29.87 thousand ha with production of 228.37 MT and the productivity is 12 TH (Horticulture Statistics at a Glance-2018). In M.P., custard apple occupies an area of 2.74 thousand ha with production of 37.00 MT. its productivity is about 23.91 MT (Horticulture Statistics at a Glance-2018).
It is found growing almost in all the tropical and sub tropical regions mostly in wild form. It grows very well even on a shallow soil. It also sheds off leaves during stress period to minimize the moisture loss from plant tissues through transpiration and thus a most appropriate fruit crop for rainfed region. It is considered as beneficial for cardiac disease, diabetes, hyperthyroidism and cancer. It contains about 28-55% of edible portion consisting of 73.30% moisture, 1.60% protein, 0.30% fat, 0.70% mineral matter, 23.90% carbohydrates, 0.20% calcium, 0.40% phosphorus, 1.0% iron, 12.40-18.15% sugar and 0.26-0.65% acidity with calorific value of 105 K cal/100g.
Setten and Koek-Noorman (1992) [13] observed that Annonaceae seeds undergoing dispersal have a small embryo that is considered underdeveloped and immature. Seed germination of custard apple is uneven and irregular making sexual propagation difficult. Much experimental evidences support the concepts that specific endogenous growth promoting and inhibiting compounds are involved directly in the control of seed development, dormancy and germination (Black, 1980). Custard apple requires 35-50 days for potential germination (Hernandez, 1983). Irregular germination, in custard apple seeds may be due to dormancy or due to hard seed coat. Very limited work has been carried out on this aspect in India and in different parts of the world indicating, the utility of GA3 from 150-500ppm and chemicals for
getting better germination of custard apple seeds (Banker, 1987; Stino et al., 1996, Pawshe et al., 1997; Ratan and Reddy, 2004) [3, 8, 12]. Therefore, pre treatment of custard apple seed with water, different organics and chemicals is very important to improve germination. Considering the above problem the investigation was conducted to find out effect of water soaking, PGR and chemicals on seed germination and survivability of custard apple.

2. Material and Method
The present experiment entailed “Effect of seed treatment on germination and growth of custard apple at seedling stage” was carried out at Shed net House, Department of Horticulture G.H Raisoni University Saikhedha M.P during September, 2018 to March, 2019. It was laid out in completely Randomized Design (CRD). The experiment was done to find out the influence of gibberelic acid on germination, growth and survival of custard apple. The experiment consist of ten treatment which replicate four time i.e. T1 (GA3 125 ppm), T2 (GA3 150 ppm), T3 (GA3 200 ppm) and T4 (GA3 250 ppm), T5 (Cow urine 10 %), T6 (Cow urine 20%), T7 (Cow urine 30%), T8 (Cow urine 40%), T9 (Water 100%) and T10 (Control). Fresh seed of Custard apple was collected, extracted and soaked in 125 ppm, 150 ppm and 200 ppm, 250 ppm, of gibberelic acid solution and cow urine of 10%, 20%, 30%, 40%, respectively was weighed in an electrical weighing balance and conical flask for preparation of solution. The seeds were soaked in water and as well as in GA3 and Cow urine for 24 hours and grown in polybags under the polyhouse. Polybags having a length of 15 cm and diameter of 10 cm with 200 gauge thickness were used and filled with the soil and FYM was added for manure purpose. Treated seed of Custard apple were sown in polythene bags of 15 X 10 cm size filled One seed per poly bag was sown at 2-2.5 cm depth on 17th July 2018. Watering was done using rose can regularly Necessary plant protection measures were taken. The growth parameters were recorded at 60, 90,120 and 150 days after sowing. Five plants were randomly selected for observations and mean value was computed. The data were analyzed using standard statistical methods. (Panse and Sukhatme,1985). The length was measured with meter scale, width was with vernier calipers and weight with electronic weighing machine. The germination in each treatment was recorded at 60 days after sowing. Number of seedlings were counted and expressed as germination percentage

| Treatment details |
|-------------------|
| Notation | Concentration | Remark |
| T1 | GA3- 125ppm | Seed soaking for 24hrs |
| T2 | GA3-150ppm | Seed soaking for 24hrs |
| T3 | GA3-200ppm | Seed soaking for 42hrs |
| T4 | GA3-250ppm | Seed soaking for 24hrs |
| T5 | Cow urine 10% | Seed soaking for 24hrs |
| T6 | Cow urine 20% | Seed soaking for 24hrs |
| T7 | Cow urine 30% | Seed soaking for 24hrs |
| T8 | Cow urine 40% | Seed soaking for 24hrs |
| T9 | Water 100% | Seed soaking for 24hrs |
| T10 | Control | Without soaking |

3. Result and Discussion
3.1 Days taken to start germination
The data pertaining to days taken to start germination revealed that almost all the treatments showed significant effect on days taken to start germination of seed over treatment T10 (control). The minimum days (15.75) were taken to germinate the seed of custard apple under treatment T3 which was found statistically at par with treatment T1 (17.50), T2 (21.25), T4 (20.75). The maximum days (36.00) were taken to start germination under treatment T10 (control). The increase in germination was due to GA3. These findings are supported by Dhankhar (1996) [3].

3.2 Days taken to 50% germination
The data pertaining to days taken to 50% germination clearly showed that all the treatments significantly affected the days taken to 50% germination of seed over treatment T10 (control). GA3 concentration of 200 ppm in T3 recorded minimum days (10.50) for 50% germination which was statistically at par with treatment T1 (11.75), T2 (15.50). The maximum days (27.75) to achieve 50% germination were noted under treatment T10 (control). Similar results have been reported by Meena et al. (2003) [6] in papaya seed treated with GA3 for germination and found maximum percentage of germination. The findings of Venkatrao et al. (2005) [15] are also close to the conformity with the present findings

3.2.1 Percentage of germination in each treatment at 60 days
Data revealed that almost all the treatments showed significant effect on percentage of germination of seed at 60 DAS over treatment T10 (control). The maximum percentage of seeds germination (86.67%) was noted at 60 days after sowing under treatment T3 (GA3 200 ppm) which was found statistically at par with treatment T2 (73.59), T5 (68.96), T6 (66.86), T7 (67.80), T8 (67.26), T9 (67.03). Whereas, the minimum percentage of seeds germination (62.83%) was under noted treatment T10(control). These findings may be due to GA3, which would have triggered the activity of specific enzymes that promoted early germination, such as α-amylase, which have brought an increase in availability of starch assimilation. Similar findings were reported by Wagh et al. (1998) [16]. And Plant growth regulators and some chemicals are widely used in increasing the seed germination percentage and for healthy growth. Similar findings were reported by Parmar et al. (2016) [7].

3.2.2 Leaf Area Index (LAI) and Leaf Area Duration (LAD)
The result of present study indicated that in seed treated with different dose of GA3 and cow urine for 24hrs. Leaf Area is an important input in physiological and agronomic studies such as in various transpiration models, characterization of crop growth, LAI etc. (Thakur and Kaur, 2001) [14]. The effect of gibberelic acid showed significant effect on Leaf Area Index and Leaf Area Duration. The maximum LAI (1.29) and LAD (3701.38 cm2 days) were noted under treatment T3 (GA3 200 ppm). Whereas, the minimum LAI (0.46) and LAD (2128.35 cm2 days) were obtained under treatment T10. This was higher ascribed to higher magnitude increases in parameter associated with the LA. The finding was supported by Setten and Koek-Noorman (1992) [9].

3.2.3 Length of seedling (cm)
That various treatments showed significant effect on length of seedling over control treatment (T10) at 150 days after sowing. The maximum seedling height of (25.17cm) were recorded when seeds soaked in 200 ppm concentration of
GA3 treatment (T3), whereas, minimum height of seedlings (13.09 cm) was recorded under control treatment (T10). Gibberellins are well known for inter-nodal cell elongation, thereby leading to increase in seedling length. These findings are supported by Ratan and Reddy (2004) [12].

3.3 Fresh and dry weight of shoot and roots (g)
A perusal of data indicated that the fresh weight of shoots was significantly affected by different treatments over treatment T10 (control). The maximum fresh weight of shoots (4.43g) was recorded under treatment T3 and found significantly at par with rest of the treatment, whereas, minimum fresh weight of shoots (2.06 g) was noted under treatment T10 (control). It is also clear that dry weight of shoots was significantly affected by the various treatments. The maximum dry weight of shoots was (0.81g) was recorded under treatment T3 which was found statistically at par with T6 (0.69), T1 (0.68), T2 (0.63), T4 (0.60), while minimum (0.51g) in treatment T10. It is evident from data that all the treatments significantly affected the fresh weight of shoots over treatment T10 (control). The maximum fresh weight of shoots (4.43g) was recorded under treatment T3 which was significantly superior over rest of the treatments and minimum fresh weight of shoots (2.06g) was noted under treatment T10 (control). The various treatments also showed great influence on dry weight of shoots over treatment T10 (control) at 150 days after sowing. The maximum dry weight of shoot (0.81 g) was recorded under treatment T3 which was found to be significantly superior over rest of the treatments and minimum dry weight of shoot (0.51 g) was noted in treatment T10. Increase in fresh weight of shoots is due to the influence of GA3 on different plant parts, which could be due to its effect in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity and permeability of cell membrane leading to enhanced growth. Increase in the dry weight of different plant parts due to improved mobilization of nutrients due to the application of GA3, which promotes plant growth and development. The findings are agreement with the findings of Rahemi and Baninasab (2000) [10]. A part from that at 400 ppm maximum polyembryony is observed which gives rise to maximum biomass per plant. This result is in agreement with the findings of Hore and Sen (1995) in which he reported that at 400 ppm maximum polyembryony is found in acid lime. Plant growth regulators and some chemicals are widely used in increasing fresh and dry weight of shoot. Similar findings were reported by Parmar et al. (2016) [7].

3.4 Number of roots per seedling
The data revealed that various treatments showed significant effect on number of roots over treatment T10 (control) after 150 days. The maximum number of (55.75) roots per seedling was recorded under treatment T3 which was at par with treatment T4 (47.28), T5 (49.50), T6 (51.25) and T7 (55.50cm) while minimum number of root was recorded under treatment T10 (29.75). Vigorous root growth due to GA3 might have resulted in more production of photosynthates and their translocation through phloem to the root zone, which might be responsible for improving the root growth. Similar findings were reported by Wagh et al. (1998) [16].

3.5 Seedling vigour index I
The result of present study indicated that in seed treated with different dose of GA3 and cow urine for 24 hrs. The present research reveals that the effect of gibberellic acid showed significant effect on seedling vigour index I. The maximum seedling vigour index I (1816.55cm) was recorded under treatment T3 and minimum (522.21cm) was recorded under treatment T10 i.e. control, which was at par with treatment T1 (850.00), T2 (799.20), T8 (706.64).

3.6 Seedling vigour index II
The result of present study indicated that in seed treated with different dose of GA3 and cow urine for 24 hrs. In the present study, significantly maximum seedling vigour index II (77.03g) was computed under treatment T3 (GA3 200 ppm) and minimum seedling vigour index II (26.14g) was recorded under treatment T10. The results are in conformity with the results of Gurung et al. (2014) [6].

3.7 Survival percentage of seedlings
The data revealed that various treatments have great influence on survival percentage of seedling over treatment T10 (control) at 150 DAS. Data indicates that maximum survival percentage (83.82) was recorded under treatment T3 which was found statistically at par with rest of the treatment whereas minimum survival percentage of (63.59) under treatment T10 (control) at 150 days after sowing. The finding was supported by Meena et al. (2003) [8]. Survival percentage in aonla 100 days old seedlings were significantly highest in the 1% KNO3 treatment. The finding was supported by Purby and Meghwal (2005) [9]

Table 1: Influence of gibberellic acid and cow urine on days taken to start germination (days) and Days taken to 50% germination

| Treatment | Treatment details | Days taken to start germination | Days taken to start 50% germination |
|-----------|-------------------|---------------------------------|------------------------------------|
| T1        | 125 PPM GA3       | 17.50                           | 11.75                              |
| T2        | 150 PPM GA3       | 21.25                           | 15.50                              |
| T3        | 200 PPM GA3       | 15.75                           | 10.50                              |
| T4        | 250 PPM GA3       | 20.75                           | 16.50                              |
| T5        | 10 % Cow urine    | 22.00                           | 17.25                              |
| T6        | 20 % Cow urine    | 22.50                           | 19.25                              |
| T7        | 30 % Cow urine    | 20.50                           | 15.00                              |
| T8        | 40 % Cow urine    | 22.50                           | 15.50                              |
| T9        | 100 % Water       | 29.50                           | 22.50                              |
| T10       | Control           | 36.00                           | 27.75                              |
| SEm±      |                   | 0.69                            | 0.68                               |
| CD at 1%  |                   | 1.99                            | 1.97                               |

Table 2: Influence of gibberellic acid and cow urine on Percentage of germination in each treatment at 60 DAS

| Treatment | Treatment details | Percentage of germination in each treatment at 60 DAS |
|-----------|-------------------|-----------------------------------------------------|
| T1        | 125 PPM GA3       | 65.84                                               |
| T2        | 150 PPM GA3       | 73.59                                               |
| T3        | 200 PPM GA3       | 78.63                                               |
| T4        | 250 PPM GA3       | 73.20                                               |
| T5        | 10 % Cow urine    | 68.96                                               |
| T6        | 20 % Cow urine    | 66.86                                               |
| T7        | 30 % Cow urine    | 67.80                                               |
| T8        | 40 % Cow urine    | 67.26                                               |
| T9        | 100 % Water       | 67.03                                               |
| T10       | Control           | 62.83                                               |
| SEm±      |                   | 0.95                                                |
| CD at 1%  |                   | 2.73                                                |
### Table 3: Influence of gibberellic acid and cow urine on leaf area duration at 150 DAS

| Treatment | Treatment details | Leaf area duration at 150DAS |
|-----------|-------------------|-----------------------------|
| T1        | 125 PPM GA3       | 3206.60                     |
| T2        | 150 PPM GA3       | 3318.92                     |
| T3        | 200 PPM GA3       | 3701.38                     |
| T4        | 250 PPM GA3       | 3345.83                     |
| T5        | 10 % Cow urine    | 2975.55                     |
| T6        | 20 % Cow urine    | 3076.62                     |
| T7        | 30 % Cow urine    | 3490.93                     |
| T8        | 40 % Cow urine    | 3017.82                     |
| T9        | 100 % Water       | 2509.05                     |
| T10       | Control           | 2128.35                     |
| SEm±      |                   |                             |
| CD at 1%  |                   |                             |

### Table 4: Influence of gibberellic acid and cow urine on Leaf area index at interval of 120 & 150 days after sowing

| Treatment | Treatment details | Leaf area index at interval of 120 & 150 days after sowing |
|-----------|-------------------|----------------------------------------------------------|
| T1        | 125 PPM GA3       | 0.51                                                      |
| T2        | 150 PPM GA3       | 0.83                                                      |
| T3        | 200 PPM GA3       | 1.29                                                      |
| T4        | 250 PPM GA3       | 0.79                                                      |
| T5        | 10 % Cow urine    | 0.62                                                      |
| T6        | 20 % Cow urine    | 0.80                                                      |
| T7        | 30 % Cow urine    | 1.11                                                      |
| T8        | 40 % Cow urine    | 0.81                                                      |
| T9        | 100 % Water       | 0.59                                                      |
| T10       | Control           | 0.46                                                      |
| SEm±      |                   | 0.07                                                      |
| CD at 1%  |                   | 0.21                                                      |

### Table 5: Influence of gibberellic acid and cow urine on length of seedling(cm) at 150 days after sowing

| Treatment | Treatment details | length of seedling(cm) at 150 days after sowing |
|-----------|-------------------|-----------------------------------------------|
| T1        | 125 PPM GA3       | 13.16                                         |
| T2        | 150 PPM GA3       | 17.73                                         |
| T3        | 200 PPM GA3       | 25.17                                         |
| T4        | 250 PPM GA3       | 17.74                                         |
| T5        | 10 % Cow urine    | 24.33                                         |
| T6        | 20 % Cow urine    | 24.24                                         |
| T7        | 30 % Cow urine    | 20.88                                         |
| T8        | 40 % Cow urine    | 20.22                                         |
| T9        | 100 % Water       | 17.75                                         |
| T10       | Control           | 13.09                                         |
| SEm±      |                   | 1.43                                          |
| CD at 1%  |                   | 4.13                                          |

### Table 6: Influence of gibberellic acid and cow urine on number of roots per seedling at 150 DAS

| Treatment | Treatment details | Number of roots per seedling at 150 days |
|-----------|-------------------|-----------------------------------------|
| T1        | 125 PPM GA3       | 42.00                                    |
| T2        | 150 PPM GA3       | 46.15                                    |
| T3        | 200 PPM GA3       | 55.75                                    |
| T4        | 250 PPM GA3       | 47.28                                    |
| T5        | 10 % Cow urine    | 49.50                                    |
| T6        | 20 % Cow urine    | 51.25                                    |
| T7        | 30 % Cow urine    | 55.50                                    |
| T8        | 40 % Cow urine    | 41.69                                    |
| T9        | 100 % Water       | 31.00                                    |
| T10       | Control           | 29.75                                    |
| SEm±      |                   | 2.05                                      |
| CD at 1%  |                   | 5.91                                      |

### Table 7: Influence of gibberellic acid and cow urine on fresh weight of shoot dry weight of shoot (g) 150 DAS

| Treatment | Treatment details | Fresh weight of shoot at 150 DAS (gm) | Dry weight of shoot at 150 DAS (gm) |
|-----------|-------------------|---------------------------------------|-----------------------------------|
| T1        | 125 PPM GA3       | 4.13                                   | 0.68                              |
| T2        | 150 PPM GA3       | 3.82                                   | 0.63                              |
| T3        | 200 PPM GA3       | 4.43                                   | 0.81                              |
| T4        | 250 PPM GA3       | 3.28                                   | 0.80                              |
| T5        | 10 % Cow urine    | 3.31                                   | 0.59                              |
| T6        | 20 % Cow urine    | 3.22                                   | 0.59                              |
| T7        | 30 % Cow urine    | 3.82                                   | 0.59                              |
| T8        | 40 % Cow urine    | 2.71                                   | 0.54                              |
| T9        | 100 % Water       | 2.72                                   | 0.53                              |
| T10       | Control           | 2.06                                   | 0.51                              |
| SEm±      |                   | 0.31                                   | 0.03                              |
| CD at 1%  |                   | 0.89                                   | 0.09                              |

### Table 8: Influence of gibberellic acid and cow urine on Seedling vigour index(cm) and Seedling vigour index II (g) at 150 DAS

| Treatment | Treatment details | Seedling vigour Index I (cm) | Seedling vigour index II (g) at 150 DAS |
|-----------|-------------------|-------------------------------|----------------------------------------|
| T1        | 125 PPM GA3       | 850.00                        | 68.47                                  |
| T2        | 150 PPM GA3       | 799.20                        | 49.10                                  |
| T3        | 200 PPM GA3       | 1816.55                       | 77.03                                  |
| T4        | 250 PPM GA3       | 697.89                        | 37.94                                  |
| T5        | 10 % Cow urine    | 705.12                        | 37.39                                  |
| T6        | 20 % Cow urine    | 680.57                        | 32.30                                  |
| T7        | 30 % Cow urine    | 607.24                        | 33.59                                  |
| T8        | 40 % Cow urine    | 706.64                        | 30.21                                  |
| T9        | 100 % Water       | 698.58                        | 29.41                                  |
| T10       | Control           | 522.21                        | 26.14                                  |
| SEm±      |                   | 60.80                         | 2.19                                   |
| CD at 1%  |                   | 175.61                        | 6.32                                   |

### Table 9: Influence of gibberellic acid and cow urine on Survival percent of seedling at 150 DAS

| Treatment | Treatment details | Survival percent of seedling at 150 DAS |
|-----------|-------------------|----------------------------------------|
| T1        | 125 PPM GA3       | 74.57                                  |
| T2        | 150 PPM GA3       | 69.43                                  |
| T3        | 200 PPM GA3       | 83.82                                  |
| T4        | 250 PPM GA3       | 70.61                                  |
| T5        | 10 % Cow urine    | 70.61                                  |
| T6        | 20 % Cow urine    | 75.75                                  |
| T7        | 30 % Cow urine    | 78.40                                  |
| T8        | 40 % Cow urine    | 75.24                                  |
| T9        | 100 % Water       | 66.22                                  |
| T10       | Control           | 63.59                                  |
| SEm±      |                   | 3.78                                   |
| CD at 1%  |                   | 10.91                                  |

### 4. Conclusion
It can be concluded that treatment T3 (200 ppm GA3 and cow urine for 24hrs) was the best among the all for all the parameters.

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