Original Research Article

In vitro Regeneration of *Corchorus olitorius* var. O-4

Abstract

The aim of this study was to determine how several plant growth regulators, such as 6-benzylaminopurine (BAP), indole acetic acid (IAA), and indole-3-butyric acid (IBA), affected the tossa jute plant's ability to regenerate *in vitro*. However, the most calluses and shoots were produced when the cotyledon-attached petioles of *Corchorus olitorius* var. O-4 were utilized as explants and inoculated in Murashige Skoog (MS) media supplemented with 2.5 mg/L BA + 0.5 mg/L IAA. Two weeks after induction (WAI), the 2.5 mg/L BA + 0.5 mg/L IAA treatment generated five leaves. The IBA therapy at 2.0 mg/L resulted in the regeneration of the majority of roots. The treatment 0.6 mg/L IBA produced the longest roots and the highest proportion of root induction. Regenerated plantlets have a 68.4% survival rate in shaded conditions and an 87.2% survival rate in full sunlight. Thus, in order to genetically alter the organism, an effective technique for Corchorus olitorius var. O-4 in vitro regeneration has been established.

Keywords: Benzylaminopurine, Indole-3-butyric acid, *In vitro* regeneration, Corchorus olitorius

Introduction

Jute (2n=14) is a dicotyledon plant under the Malvaceae family. Bangladesh is the second producer of jute fibre after India and first exporter in the world. In Bangladesh, jute fibre is known as 'golden fibre' (Islam *et al*., 2017). As it is biodegradable in nature, its fibre is economically and environmentally important. It has diversified use, for instances, biodegradable shopping bag, building material, charcoal production (Jahan *et al*., 2022).

Tossa jute has more economic value than other jute genotypes. Bangladesh Jute Research Institute has developed O-4, an enhanced high-yielding tossa jute variety. If the variety is sown early, before March 30th, it will produce flowers. It has been demonstrated that this variety can be grown across the nation in high to medium-high land with loamy to sandy-loamy soils and an effective drainage system. Farmers benefit financially and their lifestyle is improved as a result of high fiber yield, quality, and market price (Bangladesh Jute Knowledge Bank). But due to global warming, increased population rate and reduced agricultural land, tossa jute production is alarming day by day (Kumar *et al.*, 2022). Non-availability of modern varieties with improve plant types including resistance to diseases is also a major constrain. As a result, fast growing, high fibre yield, disease and pest resistant tossa jute variety is crying need.

"Achieving a sustainable improvement in jute productivity in adverse environments can only be accomplished through a continuous supply of new genetic materials" (Aggarwal, 2000). Conventional breeding techniques are time consuming for improvement of a desired variety. *In vitro* regeneration technique of plants is inevitable and important tool in biotechnology for genetic transformation study which facilitate introduction of new traits quite efficiently. This part of work could be a step forward for further genetic improvement.

Phytohormones, another name for plant hormones, are signal molecules that are produced in very small amounts within plants. Hormones in plants regulate every facet of growth and development, starting with embryogenesis (Mendez- Hernandez *et al*., 2019), the regulation of organ size, pathogen defense (Shigenaga *et al*., 2016; Burger *et al*., 2019), stress tolerance (Ku *et al*., 2018; Ullah *et al*., 2018) and

through to reproductive development (Pierre-Jerome *et al*.,2018). 6-Benzylaminopurine, also known as benzyl adenine, BAP, or BA, is a first-generation synthetic cytokinin that triggers reactions in plants, causing them to produce blooms and increase fruit richness through cell division. IAA has a wide range of actions, including causing cell division and elongation, which have an impact on plant growth and development (Silva *et al*., 2019). On a bigger scale, IAA functions as a signaling molecule that is essential for the growth coordination and organ development of plants. In plant tissue culture, a process known as micropropagation uses IBA and other auxins to start root production *in vitro* (Bridgen *et al*., 1992). Since it was well documented that cotyledon explants have a high frequency of regeneration and transformation. (Huda *et al*., 2007). Hence in present study we utilized cotyledon as the source of explant material to optimize the protocol for the regeneration of tossa jute. Considering the above facts, the present investigation has been undertaken to findout the performance of different hormones (BAP, IAA and IBA) with the optimum concentration for *in vitro* plantlets regeneration of *Corchorus olitorius* var. O-4. This attempt has been made to establish an efficient *in vitro* plant regeneration system from different explants of *Corchorus olitorius var.* O-4, which could be used to produce jute transgenics with specific desired traits through Agrobacterium-mediated methods or other advanced techniques.

Materials and Methods

This experiment was carried out in plant tissue culture lab, Molecular Biology Department, Bangladesh Jute Research Institute in March 2022-january 2023. *Corchorus olitorius* var. O-4 (Tossa Jute) seeds were collected from Breeder Seed Department, Genetic Resources and Seed Division, Bangladesh Jute Research Institute, Dhaka 1207.

Seeds were thoroughly cleaned under tab water. Then, seeds were soaked in trix solution for ten minutes. After that, seeds were repeatedly rinsed with diluted water. In the Laminar Air Flow Cabinet, the remaining tasks were completed. The explants were then sterilized for 1 minute with 70% ethanol, followed by 1 minute of distilled water washing. Once more, the explants were sterilized for 10 minutes with 0.1 % $HgCl_2$ (Sarker *et al*., 2007). The explants were then rinsed at least three times with double distilled water. Sterilized seeds were then placed in controlled MS medium without hormone for germination. After germination, 7 days old cotyledon attached petiole inoculated (cotyledon attached

petiole slightly submerged in semi-solid MS media) on 30 ml semi-solid MS (Murashige and Skoog, 1962) media supplemented with various concentrations of BAP (1.0, 1.5, and 2.0, 2.5. 3.0 mg/l) and control treatment (0.0 mg/l) with 0.5 mg/l IAA for callus induction. Callus transferred to the MS medium supplemented with BAP (1.0, 1.5, and 2.0, 2.5. 3.0 mg/l) with 0.5 mg/l IAA. For root induction, four concentrations of ½ MS media + IBA (0, 0.5, 1.0, 1.5, and 2.0 mg/l) were used. The experiments were arranged in Completely Randomized Design (CRD) with five replications. Each of replications consisted of five culture vials. The culture vials were incubated at 22 ± 2°C with 16 hours photoperiod for callus formation, shoot regeneration and root development (Hoque *et al*., 2010).

The culture vials with fully formed plantlets were moved to normal room temperature after 5 weeks. The rooted plantlets were taken out of the culture vials the following two to three days, and the medium that was still attached to the roots was carefully rinsed away with tap water. Individual plantlets were transplanted into plastic containers filled with a 1:1:1 mixture of soil, sand, and cow dung. For seven days following transplantation, a moist, clear poly bag was placed over the plants and pot to avoid desiccation. The plantlets were housed in a shade house for 12 days to lessen unexpected shock. Plantlets were then moved to the field after 12 days.

Results and Discussions

A series of experiments were conducted for callus, shoot and root induction in *Corchorus olitorius* var. O-4).

Callus induction

Approximately 82.54% (Fig. 3, Plate A) callus response were recorded in MS + 2.5 mg/l BAP + 0.5 mg/l IAA (Table 1). In BAP and IAA supplemented medium 14.32 days were required for callus initiation (Fig. 3, Plate B) (Table 1). MS+ 1.0 mg/l BAP + 0.5 mg/l IAA produced lowest number of callus (36.45%) and highest number of days (20.51 days) required for callus induction (Table 1.). According to Ghosh *et al*., 2009, For the induction of callus tissue, MS basal medium supplemented with a combination of vitamins and growth regulators (0.30 mg/L NAA and 0.30 mg/l Kinetin) was

found to be superior than SH (White's medium) medium in tossa jute. This contradicts with the result. This difference might be occurred due to different hormone concentrations.

Table 1. Effect of different concentrations of BAP and IAA on days to callus initiation and percentage of callus induction

SL No.	Treatment BAP+IAA (mg/l)	Days to callus initiation	Percentage of callus induction
1.	0.0+0.5	0 g	0
2.	1.0+0.5	20.51 a	36.45
3.	1.5+0.5	18.12 bc	57.63
4.	2.0+0.5	14.32 cd	71.12
5.	2.5+0.5	7.54 f	82.54
6.	3.0+0.5	13.74de	69.10
7.	3.5+0.5	20.10 ab	43.38
	CV %	3.74	-
	LSD (0.05)	0.6882	-

Figures in a column followed by no letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Shoot induction

When explants were subcultured on MS+2.5 mg/l BAP+ 0.5 mg/l IAA supplemented medium gives highest number of shoots (85.53%) (Fig. 3, Plate C) and the lowest number of days (9.25 days) were required for shoot initiation. MS+ 3.5 mg/l BAP + 0.5 mg/l IAA produces lowest number of shoots (45.38%) followed by highest days to shoot initiation (Table 2). Mondal *et al*. 2018, described that after 7–10 days of dark incubation, micro shoots were similarly induced from epicotyl explants, with the greatest micro shoot (2.30 ± 0.15), on MS media supplemented with 0.1 mg/l IAA and 1.0 mg/l Kin. Which was slightly different from our result and this was likely due to differenences concentraton and types of hormone. According to Sarker *et al*. 2007, 1.0 mg/l BAP+1.0 mg/l IAA gives 80% shoot in *Corchorus capsularis* var. CVL-1. This result also different from our. It might be occurred due to varietal differences.

Table 2. Effect of different concentrations of BAP and IAA on days to shoot initiation and percentage of shoot induction

SL No.	Treatment BAP+IAA (mg/l)	Days to shoot initiation	Percentage of shoot induction
1.	1.0+0.5	18.5 ab	48.50
2.	1.5+0.5	17.3 bc	54.36
3.	2.0+0.5	14.8 cd	71.12
4.	2.5+0.5	9.25 g	85.53
5.	3.0+0.5	12.7 ef	73.10
6.	3.5+0.5	19.9 a	45.38
	CV %	2.54	-
	LSD (0.05)	0.3313	-

Figures in a column followed by no letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT. CV= Coefficient of variation, LSD (0.05) = Least significant difference.

Root induction

Shoots were transferred to half strength of MS medium supplemented with different concentrations of IBA for root induction. The excellent rooting (70%) was obtained when shoots cultured on half strength of MS medium with 0.5 mg/l IBA (Fig. 1). Healthy roots were obtained in this medium after 14 days of culture (Fig. 3, Plate D). The lowest percentage (5%) of root was obtained from ½ strength MS medium (controlled) and highest number of days (30 days) is required (Fig. 2). According to Hoque *et al*. 2010, ½ MS+0.6 mg/l IBA in O-9897 had the highest percentage (45%) of rhoots and the shortest time (13.75 days) needed to induce rhoots. It is almost similar to our result. According to Hossain *et al*., 2024, 52.5% roots were obtained from 1.0 mg/l IBA and roots were initiated after 10 days of induction of shoot in *Corchorus olitorus* var. O-9897. This result was different from our result. It might be due to varietal response. Moreover, MS medium with low osmotic strength is still the best option (Dönmez et al. 2022). Hoque et al. (2010) previously achieved around 45% rooting of variety O-9897 in 13 days on MS medium with 0.6 mg/L IBA. The results showed that 1.0 mg/L IBA on rooting media can start more rooting than 0.6 mg/L IBA in a shorter amount of time (10 days). Huda et al. (2007) reported that 19.3 shoots (53.6%) began rooting in C. olitorius in 8.2 days, which is less than what we found in our study. Furthermore, it has been previously observed that Hibiscus cannabinus var. HC-2 and C. capsularis shoots generated from cotyledonary petioles can root in vitro (Naher et al. 2003).

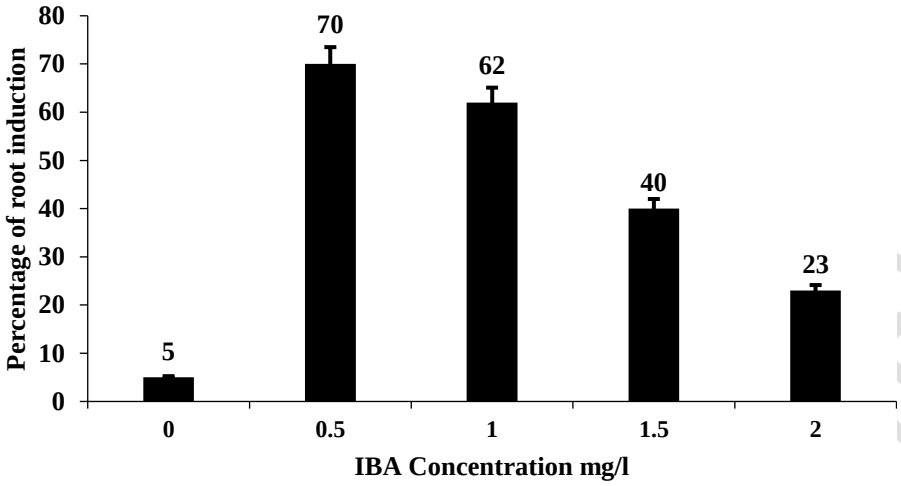

Figure 1. Effect of IBA+ ½ MS strength MS media on percentage of root induction in *Corchorus olitorius* var. O-4

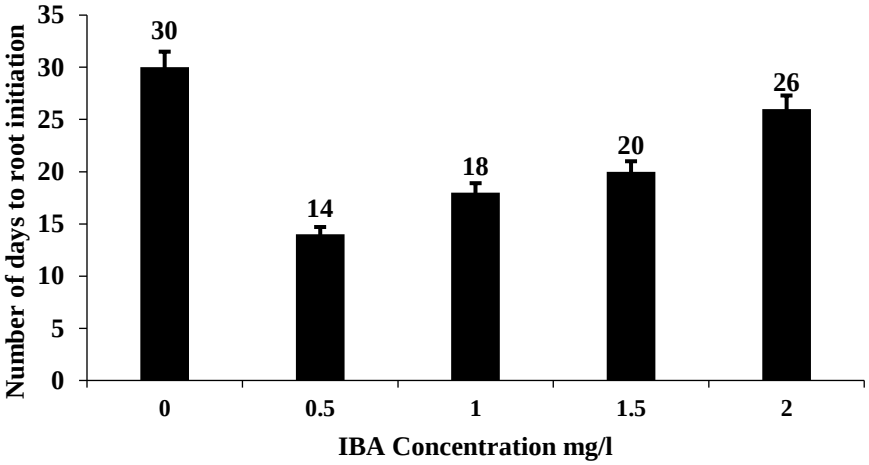

Figure 2. Effect of IBA+ ½ strength MS media on number of days to root initiation in *Corchorus olitorius* var. O-4

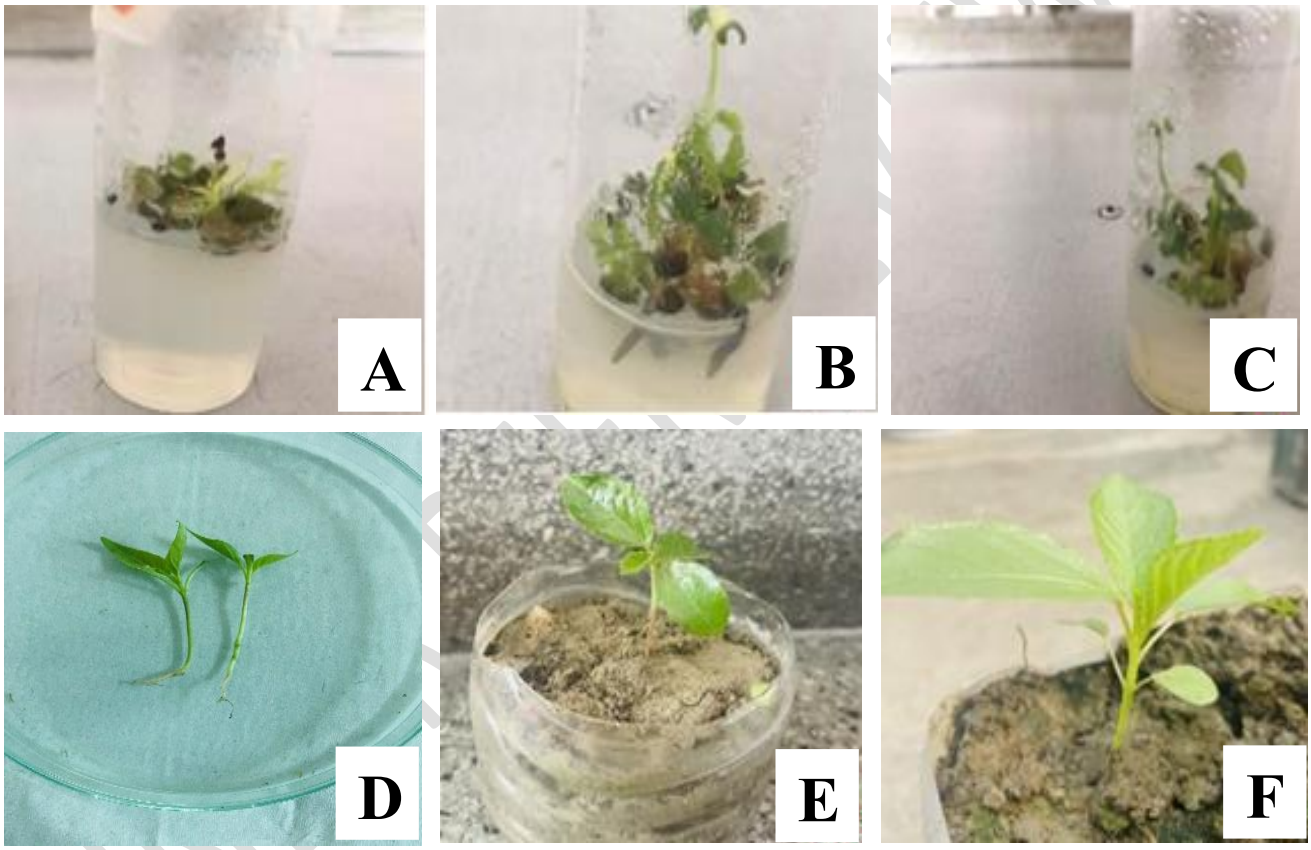

Figure 3. Plate A. Callus growing in MS+ 2.5 mg/l BAP+0.5 mg/l IAA. Plate B. Shoot initiation in MS+ 2.5 mg/l BAP+ 0.5 mg/l IAA. Plate C. Transfer of shoots in ½ MS+0.5 mg/l IBA. Plate D. Root obtained in ½ MS+ 0.5 mg/l IBA Plate E. Acclimatization of plant in shade condition. Plate F. Acclimatization of plant in field condition.

Acclimatization

After five weeks of culture, a sizable number of shoots and roots had grown. Without causing any damage to the roots, the plantlets were carefully taken out of the vial. Before being moved to the field settings, plants were acclimated and toughened in the shade house and growth cabinet. When the plants were initially moved, 15 of them survived in shade conditions (75%) (Fig. 3, Plate E). Ultimately, 12

of the 15 plants that were transplanted under normal atmospheric conditions survived, yielding an 80% survival rate (Fig. 3, Plate F). According to Anand and Rao (2000), 75% of plantlets survived in their original state. 90% of plantlets were found to survive in the shadow house's soil. According to several other studies, 45 (86.53%) of the 52 rooted plantlets transformed into normal plants when exposed to open sunshine; in contrast, Huda et al. (2007) claimed 90% and Islam (2009) succeeded in obtaining 92.5% (37 out of 40) healthy plantlets.

Conclusion

Using a cotyledon-connected petiole for regeneration and multiplication, the current study outlines an efficient in vitro clonal propagation method for Corchorus olitorius var. O-4. This process could be a useful tool for genetic change. The impacts of different Tossa Jute genotypes and the micropropagation of Tossa Jute utilizing different auxin and cytokinine hormone combinations and concentrations can be studied further.

Disclaimer (Artificial intelligence)

Author(s) have declared that no generative AI technologies have been used during the writing or editing of this manuscript.

References

1. Aggarwal, V.K. Breeding Technique of Jute, Kenaf and Mesta. Consultancy Rep. ARMR (Winrock international), BARC, Dhaka. 2000. Pp 25-30.
2. Anand, A. and Rao, C. S. A Rapid *In vitro* Propagation Protocol for *Piper barberi gamble*. A critically endangered plant. *In vitro Cell. Dev. Biol. Plant*. 2000. 36: 61-64.
3. Bridgen, M.P, Masood, Z.H. and Spencer-Barreto, M. "A laboratory exercise to demonstrate direct and indirect shoot organogenesis from leaves of *Torenia fournieri*". *Hort_Technology*. 1992. pp. 320–322.

4. Burger, M. and Chory, J. Stressed out about hormones: How Plants Orchestrate Immunity. *Cell Host & Microbe*. 2019. 26 (2): 163–172.
5. Dönmez, D., Erol, M.H., Biçen, B., Şimşek, Ö. and Kaçar, Y.A. The effects of different strength of MS Media on in Vitro Propagation and Rooting of Spathiphyllum. *Anadolu Tarım Bilimleri Dergisi*, 2022. *37*(3), pp.583-592.
6. Ghosh, P. K. and Chatterjee, A. Regeneration of plants from hypocotyl derived callus tissue of Jute (*Corchorus olitorius* L. var. JRO-632). *J. of Cro. and We.* 2009. 5(2): 78-79.
7. Hoque, M., Nasiruddin, K. M., Haque, G. K. M. N. and Biswas, G. C. J. *In vitro* regeneration performance of *Corchorus olitorius*. *Bangladesh Agril. Univ*. 2010. 8(1): 1–6.
8. Hossain, M. J., Ahmed, B., Mimi, A., Ferdous, R., & Hosen, Q. M. Robust and reliable *in vitro* regeneration of Tossa Jute (*Corchorus olitorius* L. var. O-9897*). Pla. Cel., Tis. and Org. Cul*. 2024. 157(2): 50.
9. Huda, K. M. K., Bhuiyan. M. S. R., Kabir, M. H., Uddin, A. F. M. and Khatum, A. *In vitro* plant regeneration protocol of Tossa Jute (*Corchorus olitorius*). 2007. *Inter. J. of Integ. Bio*. 1(2).
10. Islam, M. M. and Ali, M. S. Economic importance of Jute in Bangladesh: Production, research achievements and diversification. *Int. J. of Eco. Th. and App*. 2017. 4(6): 45-57.
11. Islam, M.S., Kamrul Huda, K.M., Mahmud, F., Akhter Banu, S. and Wang, M.H. Regeneration and Genetic Transformation of Tossa Jute ('Corchorus olitorius L.'). *Australian J.Cro. Sci*. 2009. *3*(5), pp.287-293.
12. Jahan S. M., Hossain, S. and Khan, M. Economic importance of Jute. *Sprin. Nat*. 2022. Pp 1–16
13. Ku, Y.S., Sintaha, M., Cheung, M. Y. and Lam, H. M. Plant Hormone signaling crosstalks between biotic and abiotic stress responses. *Int. J. of Mol. Sci.* 2018. 19 (10): 3206.
14. Kumar, L., Chhogyel, N., Gopalakrishnan, T. Hasan, M. K., Jayasinghe, S. L., Kariyawasam, C. S., Kogo, K. B. and Ratnayake, S. Chapter 4 - Climate change and future of agri-food production. Future Foods-Global Trends, Opportunities, and Sustainability Challenges. 2022. Pp. 49-79.

15. Mendez-Hernandez, H. A., Ledezma-Rodríguez, M., Avilez-Montalvo, R. N., Juárez-Gómez, Y. L., Skeete, A. and Avilez-Montalvo, J. Signaling overview of plant somatic embryogenesis. *F. in P. Sci.* 2019. 10: 77.
16. Mondal, R., Dey, S. Karmakar, G. P. and Mondal, B. A. Development of an efficient micro propagation based *Agrobacterium* mediated genetic transformation protocol in commercial cultivar of Jute (*Corchorus capsularis* L.). *Int. J. of P. Res.* · March 2018. Pp 115-128.
17. Naher, Z., Khatun, A., Mahbub, S., Alim, M.A. and Siddique, A.B. Influence of genotypes on plant regeneration from cotyledons of Corchorus capsularis L. *Biotechnology (Pakistan)*, 2003. *2*(1).
18. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 1962. 15: 473-497.
19. Sarker, R. H., Al-Amin, G.M. and Hoque, M. I. *In vitro* regeneration in three varieties of White Jute (*Corchorus capsularis* L.). *Plant Tissue Cult. & Biotech.* 2007. 17(1): 11-18.
20. Shigenaga, A. M. and Argueso, C. T. No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. Seminars in Cell & Developmental Biology. 2016. 56: 174–189.
21. Teixeira D. S., Jaime, A. "Is BA (6-benzyladenine) BAP (6-benzylaminopurine)?". *Research Gate*. Retrieved. 2019.
22. https://bjkb.gov.bd/varient-detail/18