

## OPTIMIZATION OF HYGROMYCIN DOSES FOR *Corchorus capsularis* SEEDLINGS (CVL-1 AND KOMOLA GENOTYPE)

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### ABSTRACT

The success of *Agrobacterium*-mediated genetic transformation in deshi jute plants (*Corchorus capsularis* L.) depends on the selection of transformants. In the present research, the hygromycin sensitivity test was performed on wild-type seeds to obtain the most effective hygromycin concentration for selection of transgenic. Based on root growth, the most effective concentration of hygromycin was determined 25 mg/l. In this concentration of hygromycin, non-transgenic plants indicated no root elongation, as compared to transgenic plants. A clear decrease of root growth was optimized for the selection of transformants. Thus, for effective optimization and selection of transformants, we can make easy the laborious screening tasks in *in planta* transformation for further verification through polymerase chain reaction (PCR).

**Key words:** Hygromycin, selective marker, *Agrobacterium tumefaciens*, deshi jute.

### Introduction

With increasing negative concerns for the use of synthetic fibre and preferential shift towards natural fibre on environmental and health grounds, the demand of this biodegradable fibre yielding crop is likely to increase in future (Prosanta *et al.* 2014). Due to having its significant role in fiber production, it is important to improve the quality of jute. Considering the importance of jute in Bangladesh and limitation in improving its yield and quality, it is necessary to incorporate genes of desired traits in the local varieties (Sarker *et al.* 2008). Using *Agrobacterium tumefaciens* mediated genetic transformation it is possible to incorporate the specific genes for desirable characters (Sarker *et al.* 2007). During *Agrobacterium* mediated genetic transformation, an important step is involving the selection for transgenic plants through antibiotics (EE *et al.* 2014). Hygromycin is more toxic than the existing antibiotics and has been used as a selective agent in many crop plants (Amareshwari *et al.*, 2019). It is utilized as a selectable marker used in transgenic plants to identify and maintain cells that carry the hygromycin resistance gene. Selectable marker genes encode proteins that confer resistance to antibiotic selection agents and identify the transformed cells from non-transformed cells (Zuraida *et al.*, 2013). Due to the presence of the selection agents after the process of genetic transformation the non-transformed cells were inhibited their root growth. Therefore, the study aimed to determine the most effective hygromycin concentration for the selection of transgenic *Corchorus capsularis* jute plant. Firstly, the effect of different hygromycin concentrations on wild-type seeds, through the hygromycin sensitivity test was tested. This optimum concentration allows a more cost-effective, timesaving and less laborious selection method, which also clearly distinguish between the transgenic and the non-transgenic plants (EE *et al.* 2014).

### Materials and Methods

Seeds of CVL-1 variety and Komola genotypes of white jute (*Corchorus capsularis* L.) collected from Genome Research Center at Bangladesh Jute Research Institute, Dhaka were used in the present investigation.

**Seed sterilization and MS (Murashige and Skoog, 1962) medium preparation:** For better germination and to reduce contamination, seeds were washed through by tap water for 7-8 times. This process was followed by distilled water for 3-4 times. Then seeds were sterilized with 70% ethanol for 45 seconds. After that 0.1% HgCl<sub>2</sub> was used for 15 mins and shaking at 1000rpm. Again washed out the seeds with

distilled water for 7-8 times and then air dry the seeds in laminar at least 30 minutes. MS medium (dephyte) was prepared by ¼th strength; 1% sucrose and 0.6% agar (dephyte) at pH: 5.8.

**Hygromycin concentration:** Hygromycin B (invitrogen) was applied on seeds for eight different concentrations (0mg/L as control, 3mg/L, 5mg/L, 10mg/L, 15mg/L, 20mg/L, 25mg/L, 50mg/L) with five replications.

### Results and Discussion

Sterilized seeds were inoculated on MS medium with different concentrations of hygromycin and incubated at 30°C for 72 hours. Results showed that hygromycin had no effect on the seeds germination, almost 100% of the seeds were germinated which was similar with EE *et al.* (2014) in case of Arabidopsis. In case of CVL-1, control plate was shown 100% long root efficiency of seedlings which was followed by 96%, 83%, 52%, 28% and 6% for 3mg/L, 5mg/L, 10mg/L, 15mg/L and 20mg/L respectively (Table 1).

Table 1. Effect of hygromycin concentration on root growth percentage of the CVL-1 jute seedlings

Sl. no	Hygromycin concentration	Avg. no. of seeds used	No. of long root					Average (%)
			Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	
1	Control	30	30	30	30	30	30	100%
2	3 mg/L	30	29	28	29	29	29	96%
3	5 mg/L	30	25	26	25	24	25	83%
4	10 mg/L	30	16	16	15	16	15	52%
5	15 mg/L	30	8	8	9	8	9	28%
6	20 mg/L	30	2	2	1	2	2	6%
7	25 mg/L	30	0	0	0	0	0	0%
8	50 mg/L	30	0	0	0	0	0	0%

No root growth was found for 25mg/L and 50mg/L, seeds were germinated but root length was too short to control seedlings (Fig. 1). Moreover, Komola genotypes also showed approximately similar results. Here, control plate was also shown 100% long root of seedlings. With the intention of 3mg/L and 5 mg/L hygromycin concentration plate were shown long root efficiency 94% and 84.6% respectively. In case of 10mg/L plate, 15mg/L plate, 20mg/L plate, the effect of hygromycin concentration on root growth percentage were decreased as 34.7%, 11.3% and 5.3% respectively (Table 2). Root length decreased by increasing hygromycin concentration (Fig. 3).

Table 2. Effect of hygromycin concentration on root growth percentage of the Komola genotype seedlings

Sl. no	Hygromycin concentration	Avg. no. of seeds used	No. of long root					Average (%)
			Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	
1	Control	30	30	30	30	30	30	100%
2	3 mg/L	30	28	28	28	29	28	94%
3	5 mg/L	30	25	25	26	25	26	84.6%
4	10 mg/L	30	10	11	10	11	10	34.7%
5	15 mg/L	30	4	4	3	3	3	11.3%
6	20 mg/L	30	1	2	2	2	1	5.3%
7	25 mg/L	30	0	0	0	0	0	0%
8	50 mg/L	30	0	0	0	0	0	0%

By evaluating the effect of hygromycin on jute seed, the presence of 25 mg/L hygromycin completely inhibited root growth, and thus, this concentration is suitable for selection of transgenic plants. The study of EE *et al.* (2014) revealed that the most effective hygromycin concentration at 25mg/L was determined for the screening of transgenic Arabidopsis seeds. This level was lower than 50 mg/L hygromycin used in a previous study Namrata *et al.* (2023). Antibiotics at high levels not only killed the non-transformed cells but also inhibited growth of the transformed cells and plants, which delayed plant regeneration (Wilmerk

and Dons, 1993). Thus, selective agents applied at appropriate concentrations are important in avoiding an undesirable number of untransformed (Zuraida *et al.*, 2013).

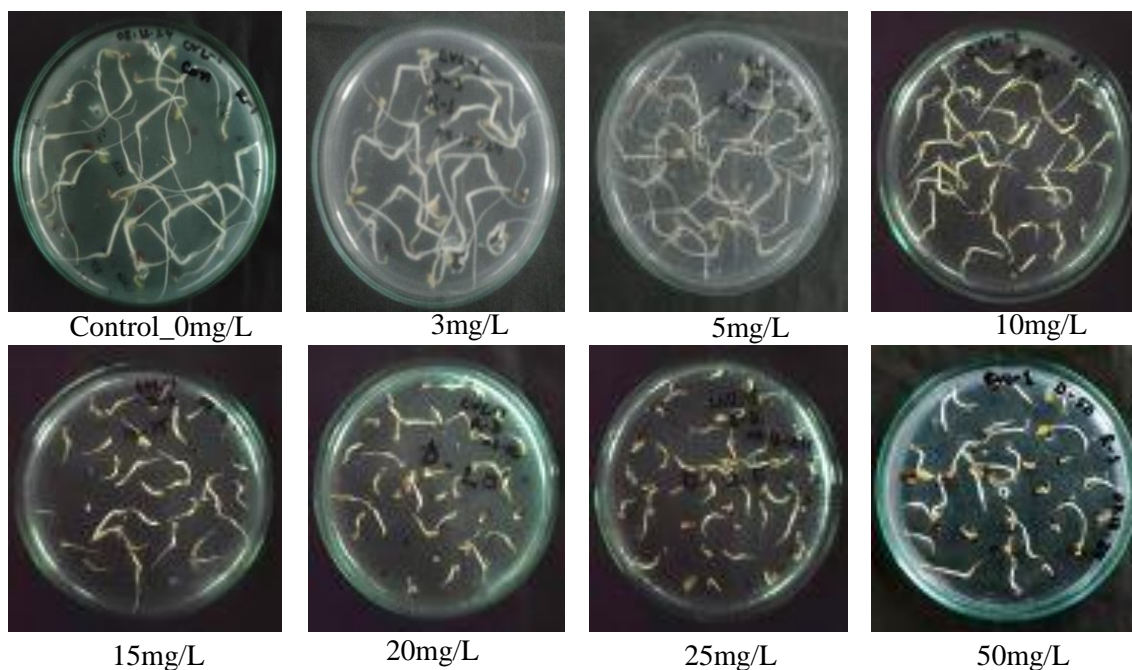


Fig. 1. CVL-1 seedlings with multiple doses of hygromycin concentration shows different root length

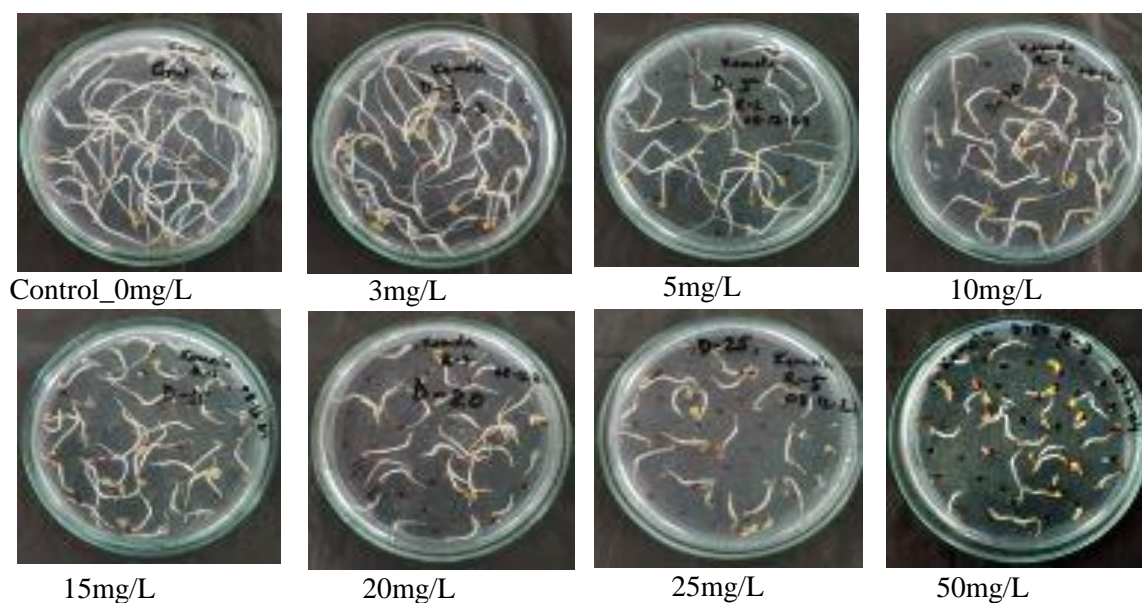


Fig 2. Komola genotype seedlings with multiple doses of hygromycin concentration

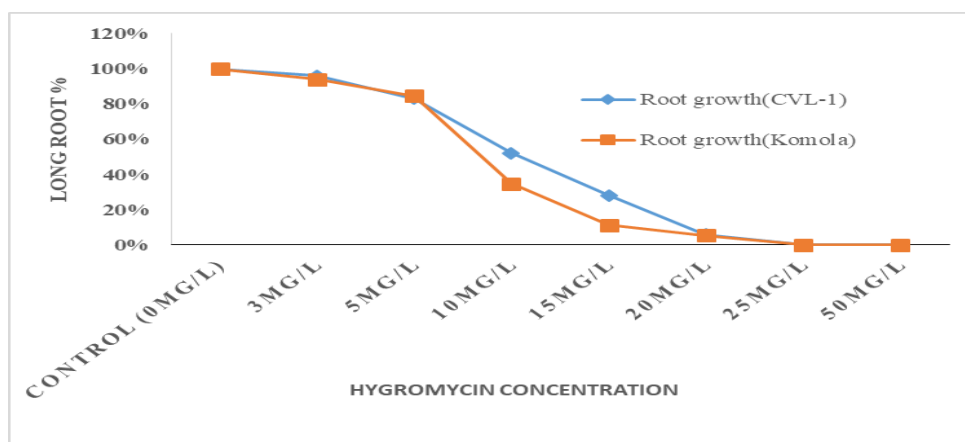


Fig. 3. Root growth of seedlings was continuously decreased by increasing hygromycin concentration in both CVL-1 variety and Komola genotype

### Conclusion

This study provides an analysis of the hygromycin-selectable marker concentration optimization for the selection of positive transformants in *Corchorus capsularis*. The most effective concentration of hygromycin for selection of transgenic was in the range from 20 to 30 mg/L. The concentration within this range (25 mg/L) was manageable to distinguish transgenic *capsularis* jute plants from the non-transgenic, which showed a significant decreased in root length on selection medium.

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