

Comparative Study of Regeneration Potential in Three Rapeseed (*Brassica napus* L.) Genotypes

M. M. Islam*, M. S. Sony¹, T. Z. Khan, R. M. Emon, N. A. Khan and F. Khatun¹

Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA),
Mymensingh-2202, Bangladesh

Key words: Rapeseed, *In vitro* regeneration, Plant growth regulators, Callus induction, Shoot organogenesis, Tissue culture protocol

Abstract

Rapeseed (*Brassica napus* L.) is a major oilseed crop valued for its high content of omega-3 and omega-6 fatty acids; however, its yield potential is frequently limited by various biotic and abiotic stresses. The establishment of an efficient and reproducible *in vitro* regeneration system is therefore essential for genetic improvement of this crop. Explants (hypocotyl, epicotyl, and cotyledonary node) from eight-day-old seedlings were cultured on full-strength MS medium using different concentrations and combinations of plant growth regulators. The study successfully optimized an *in vitro* regeneration protocol for rapeseed cultivars BINA Sarisha-12, RT-35, and RT-38, identifying specific hormonal and explant requirements for each stage. Hypocotyls emerged as the most effective source for callus induction, peaking at 93.19% in BINA Sarisha-12, followed by 91.95% in RT-35, and 90.74% in RT-38., while cotyledonary nodes proved superior for shoot regeneration with a high frequency of 97.78%. Optimal shoot elongation was achieved using 2.5-3.0 mg/l BAP, whereas rooting efficiency was maximized using IBA for BINA Sarisha-12 and a synergistic IBA/IAA combination for the RT genotypes. Cotyledonary nodes were determined as the best explants for shoot regeneration and hypocotyls for callus induction, establish a highly efficient, genotype-specific framework essential for future CRISPR-Cas9 genome editing and genetic advancement in rapeseed.

Introduction

The genus *Brassica* belongs to the family Brassicaceae (formerly Cruciferae) under the order Brassicales; *Brassica* has three categories, namely– the Rapeseed, the Mustard and the Cole. Rapeseed is the top-ranking oilseed crop in Bangladesh and is considered the world's third most important vegetable oil after soybean and palm (Sultana et al. 2021).

*Author for correspondence: <mislambina73@gmail.com>. ¹Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

To achieve self-sufficiency in edible oils, the Bangladesh government has prioritized oilseed research and development, making significant financial investments. As a result, the Bangladesh Agricultural Research Institute (BARI) and the Bangladesh Institute of Nuclear Agriculture (BINA) have released several improved oilseed cultivars (Miah and Mondal 2017). More than 20 mustard cultivars have been released by Bangladeshi universities and agricultural research agencies, including BARI and BINA.

Mustard demand and consumption are expected to increase significantly and this would lead to a 1% annual increase in average mustard productivity from 1.14 tons/ha in 2021 to 1.22 tons/ha in 2030 (USDA Foreign Agricultural Service, 2025). Bangladesh produces a little over 1.2 million metric tons of edible oils from mustard and rapeseed, which only makes up 10% of the total need; the other 90% is imported (USDA, 2025).

This reduced yield is associated with the occurrence of various diseases, insect pest infestation, and non-adaptation of different cultivars across different agro-climatic zones. Furthermore, Bangladesh's changing climate, which includes drought, salt, high temperatures, and waterlogging, makes farming more difficult (Mollika et al. 2011). In other words, plant is susceptible to both biotic and abiotic stress. Bangladesh does not have suitable oilseed varieties for such situations (Miah and Mondal 2017).

Traditional breeding methods are used most often for crop improvement. The primary difficulty lies in a narrow genetic base, sexual barriers with wild relatives, Linkage Drag and, the specific genes needed for resistance to pests and diseases simply do not exist within the species' natural breeding pool. The problems in mustard improvement cannot be solved with conventional plant breeding alone. Similarly, the limitations of conventional breeding are the high genetic rate of segregation due to cross-pollination, and absence of appropriate wild germplasm for hybrid variety of Brassica species (Gurjar et al. 2025). Such procedures are labor-intensive and take around seven to eight years to accomplish one breeding cycle of crossing. Interspecific hybridization is often hindered by genetic incompatibilities between species, limiting the flow of potentially useful traits (Toma and Islam 2023).

The increasing demand for rapeseed-based products has led to a significant focus on research efforts to improve disease resistance, seed yield, and seed quality in *B. napus*. The most effective biotechnological strategy for developing superior cultivars with all desired agronomic qualities, such as increased oil content, improved disease resistance, and shorter growing length, is *in vitro* regeneration (Quezada et al. 2021). Elite *Brassica* cultivars have shorter maturation times and higher yields with superior oil, although with the rising climatic changes across the worlds, breeding for the improvement of these genotypes are still necessary. To facilitate advanced genetic transformation and genome editing techniques in *Brassica napus* L., it is essential to develop effective, genotype-independent regeneration systems (Bhalla and Singh 2008, Chu et al. 2020). The objective of this study is to compare the regeneration potential of different genotypes and to establish an efficient *in vitro* regeneration protocol for three genotypes of *B. napus*.

Materials and Methods

Three genotypes of *Brassica* spp., namely *Brassica napus* var. BINA sarisha12 (released variety), RT-35, RT-38 (advanced lines) were collected from the plant breeding division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and used in the present investigation.

The surface sterilized seeds were inoculated on half strength of MS with sucrose and agar for germination and seedling development. The cultured seeds were kept in dark conditions till the germination. Normally germination took place within 2-3 days of seed inoculation and then was transferred to 16 hours of light conditions at $25 \pm 2^\circ\text{C}$ in growth room.

Explants like cotyledonary node, hypocotyl, and epicotyl were excised from germinating seeds. Six to seven day - old seedlings were used as the source of explant. Isolated explants were cultured on MS supplemented with BAP, NAA, Kinetin and AgNO_3 for regeneration. *In vitro* regenerated shoots were subcultured regularly to fresh medium at an interval of 12-15 days for further multiplication. Elongated shoots were about 2-3 cm elongated and were separated and cultured on rooting medium containing half strengths of MS without hormonal supplement or with IBA and combinations of IBA and IAA. The plantlets with sufficient roots were then transplanted to small pots containing sterilized soil. Pots were covered with transparent perforated polythene bags. After proper hardening, plantlets were transferred to natural environment.

Results and Discussion

Different concentrations of BAP, NAA, Kinetin and AgNO_3 were used in MS to determine the optimum media composition for callus induction and shoot initiation from three varieties of *Brassica* used in this investigation. Table 1 shows, among the different explants used, hypocotyl and epicotyl were found to be the most responsive in terms of percentage of callus induction. As for the, cotyledonary node (CN) explants, they did not show any callus induction under the tested hormonal combinations. Instead, shoots were regenerated directly from the CN explants without an intermediate callus phase, indicating direct shoot organogenesis. In case of BINA sarisha12, RT-35 and RT-38, MS media supplemented with 0.75 mg/l BAP, 0.2 mg/l NAA and 0.5 mg/l Kn and AgNO_3 showed the best response for callus induction. In this case, 93.20% of hypocotyl explants of BINA sarisha12, 91.96% of hypocotyl explants of RT-35 and 90.74% of hypocotyl explants of RT-38 showed callus initiation.

Table 2 shows the effect of different BAP concentrations, explant types, and genotypes on shoot induction in *B. napus*. A highly significant variation ($p \leq 0.05$) was observed among treatments. The highest shoot induction was recorded at 4.0 mg/l BAP across all genotypes, with CN explants consistently performing best, reaching up to 96-98% in BINA sarisha12 and RT-38. Hypocotyl explants also responded well at higher BAP levels, particularly in RT-38 and BINA Sarisha-12 ($\geq 87\%$). In contrast, the hormone-

free media showed no response in hypocotyl and epicotyl explants, while CN explants showed moderate regeneration. Among genotypes, BINA sarisha12 exhibited the highest shoot induction overall, followed closely by RT-38, while RT-35 showed relatively lower responsiveness.

Table 1. Effects of different BAP concentrations, genotypes, and explant types on average callus induction (%) in *B. napus* cultivars.

Genotypes	BAP (mg/l)	HC (%)	EC (%)
BINA sarisha12	0.00	-	-
	0.75	93.20a	88.78a-d
	1.00	92.75a	81.74e-g
	2.00	78.95fg	71.59hi
RT-35	0.00	-	-
	0.75	91.20 ab	85.65b-e
	1.00	91.96 a	85.28 c-e
	2.00	69.14 i	62.23 j
RT-38	0.00	-	-
	0.75	89.37 a-d	90.43 a-d
	1.00	90.74a-c	76.37gh
	2.00	84.69 d-f	68.34 i

Grand Mean = 41.46, LSD (0.05) = 5.76, CV = 8.53, Level of Significance = ***

HC= Hypocotyl, EC = Epicotyl.

Table 2. Effects of different BAP concentrations, genotypes, and explant types on average shoot induction (%) in *B. napus* cultivars.

Genotypes	Hormonal supplements(mg/l)	Average Shoot Initiation (%)		
	BAP (mg/l)	HC (%)	EC (%)	CN (%)
BINA sarisha12	0.00	0.00 i	0.00 i	77.50cd
	3.00	64.73e	46.81 fg	97.78 a
	4.00	87.38 b	46.43 fg	95.88 a
RT-35	0.00	0.00i	0.00i	52.92 f
	3.00	75.93cd	42.62 gh	85.94 b
	4.00	82.28bc	40.56 gh	95.52 a
RT-38	0.00	0.00 i	0.00 i	64.73 e
	3.00	74.51 d	38.16 h	80.91 b-d
	4.00	87.25 b	41.35 gh	96.30 a

Grand Mean = 54.65, LSD (0.05) = 6.83, CV = 7.62, Level of Significance = ***

HC= Hypocotyl, EC = Epicotyl, CN= Cotyledonary node.

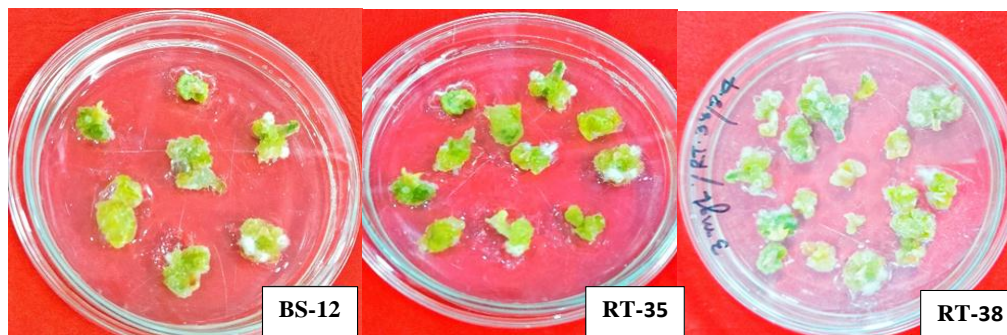


Fig. 1. Callus induction from explants of *B. napus* cultivars (BINA Sarisha12, RT-35, and RT-38).

Table 3 presents the effect of different IBA concentrations, genotypes, and explant types on root initiation percentage in *B. napus*. A significant variation ($p \leq 0.05$) was observed among treatments, with the highest rooting response recorded in BINA sarisha12 treated with 1 mg/l IBA, showing 73.63, 57.94, and 30.26% rooting in CN, HC, and EC explants respectively. The second-highest response was found in RT-38 at the combined IBA treatment (0.5 + 0.3 mg/l), particularly for CN explants (60.62%). In contrast, all genotypes showed negligible or zero rooting under hormone-free conditions. Among the genotypes, BINA sarisha12 performed best overall, followed by RT-38, while RT-35 showed comparatively lower rooting efficiency across treatments.

Table 3. Effects of different BAP concentrations, genotypes, and explant types on root initiation (%) in *B. napus* cultivars.

Genotypes	Hormonal supplements (mg/l)	Average root initiation%		
		HC (%)	EC (%)	CN (%)
BINA sarisha12	0.00	0.00 j	0.00 j	3.71 ij
	IBA= 1 mg/l	57.94 b	30.26 de	73.63 a
	IBA (0.5mg/l + 0.3 mg/l)	10.61 ghi	7.23 hij	9.35 ghi
RT-35	0.00	0.00 j	0.00 j	0.00 j
	IBA= 1 mg/l	10.84 g-i	5.56 ij	15.28 f-h
	IBA (0.5mg/l + 0.3 mg/l)	38.88 d	17.86 fg	49.02 c
RT-38	0.00	0.00 j	0.00 j	8.10 h-j
	IBA= 1 mg/l	12.32 f-i	0.00 j	11.99 f-i
	IBA (0.5 mg/l + 0.3 mg/l)	29.71 e	20.75 f	60.62 b

Grand Mean = 17.54, LSD (0.05) = 8.85, CV =30.80, Level of Significance = ***

Callus formation was strongly influenced by BAP concentration and genotype. The best response occurred at 0.75-1.0 mg/l BAP (with 0.2 mg/l NAA, 2 mg/l Kn, and 5 mg/l AgNO_3). Higher concentrations (2.0 mg/l) reduced callus formation, likely due to hormonal imbalance or tissue stress. These findings agree with Sarkar et al. (2022) and Bhalla and Singh (2008), who reported optimal callusing in *Brassica* at moderate BAP

levels. Hypocotyls produced the highest callus response for all cultivars, which aligns with Cardoza and Stewart (2004), who noted hypocotyls are more competent for dedifferentiation due to juvenile tissue characteristics. Genotype significantly affected response, with BINA sarisha12 performing best, followed by RT-38 and RT-35.

Shoot organogenesis was most successful at 4 mg/l BAP combined with NAA, Kn, and AgNO₃. BINA sarisha12 showed the highest response (76.56%), followed closely by RT-38 and RT-35. The absence of BAP drastically reduced regeneration efficiency, confirming the crucial role of exogenous cytokinins. These results support earlier reports by Bhalla and Singh (2008) and El-Hallous et al. (2014), who identified 3-4 mg/l BAP as optimal for shoot initiation in *Brassica napus*. Cotyledon explants performed best, while epicotyls showed the lowest response.

Root development varied significantly between genotypes and auxin treatments. The highest rooting was achieved using 1.0 mg/l IBA, particularly in BINA sarisha12 (53.94%), supporting findings by Alam et al. (2008) and Darçin et al. (2014), who reported IBA as the most effective auxin for *Brassica* rooting. A combination of IBA + IAA improved rooting in RT-35 and RT-38 but was ineffective for BINA sarisha12, demonstrating genotype-specific hormonal sensitivity

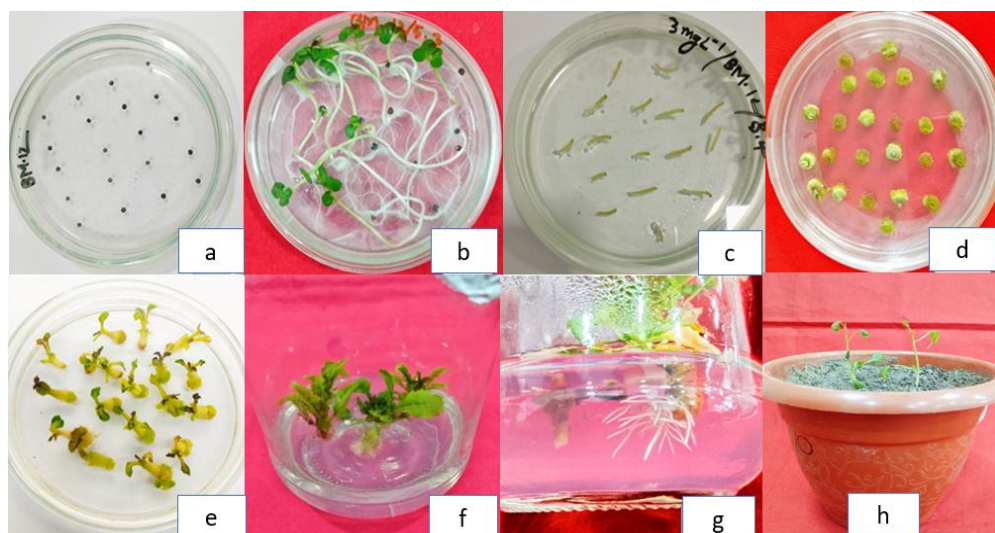


Fig. 2. *In vitro* development of plantlets in *B. napus* variety BINA sarisha12: (a) germination of seeds on half-strength MS medium, (b) seedling development after 8 days, (c) explant obtained from 8-day-old seedlings, (d) callus induction, (e) shoot initiation, (f) developing shoots, and (g) root initiation. (Variety name: BINA sarisha12).

The present study successfully established an optimized *in vitro* regeneration protocol for three rapeseed (*Brassica napus* L.) cultivars, demonstrating that regeneration efficiency is significantly governed by the interaction between genotype and explant type. Among the evaluated cultivars, BINA sarisha12 exhibited the most robust

performance for both shoot and root regeneration, making it the most suitable candidate for subsequent biotechnological applications- such as genetic transformation or genome editing work. The experimental results indicate that hypocotyl explants are generally superior for higher callus induction and cotyledonary nodes are tested superior for achieving high shoot and root regeneration efficiency across the tested rapeseed genotypes. Furthermore, the application of 1.0-2.0 mg/l BAP in combination with low concentrations of NAA proved to be the most effective hormonal treatment for inducing shoot formation. These findings provide a critical baseline for advancing the genetic potential of rapeseed through genome editing experiments. While this study establishes an optimized regeneration protocol for specific rapeseed genotypes, the observed variation in cultivar response highlights a critical need to investigate the underlying genetic mechanisms. Future research must also account for genotype-dependent variation, as significant differences in regeneration efficiency between established varieties and advanced lines necessitate separate optimization of culture conditions. By identifying these molecular triggers, future research can bypass current regeneration bottlenecks, directly enabling the use of CRISPR-Cas9 and other genome-editing tools to develop climate-resilient and high-yielding rapeseed varieties.

References

- Bhalla PL and Singh MB** (2008) *Agrobacterium*-mediated transformation of *Brassica napus* and *Brassica oleracea*. *Nat. Prot.* **3**(2): 181-189.
- Khan I, Ali W, Ali Takar Z, frooqi A and Akhtar W** (2010) Increased regeneration efficiency of *Brassica napus* L. cultivars Star, Westar and Cyclone from hypocotyle and cotyledonary explants.
- Miah M and Mondal M** (2017) Oilseeds sector of Bangladesh: challenges and opportunities, *SAARC J. Agricult.* **15**(1): 161-172. doi: 10.3329/sja.v15i1.33146.
- Mollika SR, Sarker RH and Hoque MI** (2011) *In vitro* plant regeneration in *Brassica* spp. *Plant Tiss. Cult. Biotech.* **21**(2): 127-134. <https://doi.org/10.3329/ptcb.v21i2.10235>
- Toma TI and Islam A** (2023) Efficient *in vitro* Regeneration of *Brassica napus* L. Var. BARI Sorisha-18. *Plant Tiss. Cult. Biotech.* **33**(2): 107-114. <https://doi.org/10.3329/ptcb.v33i2.70436>
- USDA Foreign Agricultural Service** (2025) Bangladesh: Oilseeds and Products Annual. GAIN Report Number: BG2025-0002. Available at: <https://fas.usda.gov/data/bangladesh-oilseeds-and-products-annual-5>
- Sarkar MAR, Sarkar S, Akter N, Rashid SB and Rahman SM** (2022) *In vitro* regeneration of chickpea (*Cicer arietinum* L.) cultivated in Bangladesh. *Plant Tiss. Cult. Biotech.* **32**(2): 205-216. doi: 10.3329/ptcb.v32i2.63554.
- Cardoza V and Stewart CN** (2004) Invited review: Brassica biotechnology: progress in cellular and molecular biology. *In vitro Cell. Develop. Biol. Plant* **40**(6): 542-551.
- El-Hallous EI, Shaaban H and Shaaban HF** (2014) Micropropagation of mulberry (*Morus alba* L.) cv. Al-Taify. *Int. J. Bio-Tech. Res.* **4**(4): 1-10.
- Darçin ES, Kolsarici O and Yildiz M** (2014) Establishment of efficient regeneration protocol for three rapeseed cultivars. *Biotech. Biotechnol. Equip.* **28**(1): 21-26.

- Alam MA, Hossain MR, Paul SK, Sarker SC and Haque MA** (2008) Optimization of callus induction and plantlet regeneration through filament culture of five oilseed *Brassica* species. *J. Agrofor. Environ.* **2**(2): 169-172.
- Sultana S, Mahmud F and Asaduzzaman M** (2021) Assessment of Genetic Diversity of 62 F4 Population of Rapeseed (*Brassica napus* L.) Through Multivariate Analysis. *SAARC J. Agricult.* doi: 10.3329/sja.v19i1.54775.
- Quezada-Martinez D, Nyarko C, Schiessl S and Mason A** (2021) Using wild relatives and related species to build climate resilience in *Brassica* crops. *TAG. Theor. Appl. Gen. Theoretische Und Angewandte Genetik* **134**(3): 1711-1728. doi: 10.1007/s00122-021-03793-3.
- Chu U, Kumar S, Sigmund A, Johnson K, Li Y, Vongdeuane P and Jones T** (2020) Genotype-Independent Transformation and Genome Editing of *Brassica napus* Using a Novel Explant Material. *Front. Plant Sci.* **11**. doi: 10.3389/fpls.2020.579524.
- Gurjar D and Gautam** (2025) Bridging the Genetic Gap: Utilization of Wild Relatives and Landraces in *Brassica juncea* Improvement Programs. *Ann. Res. Rev. Biol.* doi: 10.9734/arrb/2025/v40i72264.

(Manuscript received on 25 March, 2026; revised on 5 May, 2026)