

1 Original Research Article

2 **Development of an efficient plant regeneration**  
3 **system of field mustard (*Brassica campestris*)**

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7 **ABSTRACT**  
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**Aims:** The present study was conducted with a view to develop an efficient protocol for high frequency plant regeneration of *Brassica campestris* for further crop improvement program by biotechnological manipulation and to optimize this system for regeneration of a number of *B. campestris* genotypes.

**Study design:** Completely Randomized Design.

**Place and Duration of Study:** This experiment was carried out in the Genetic Engineering Laboratory of the Department of Genetics and Plant Breeding, Sylhet Agricultural University, Bangladesh during the period of July 2013 to June 2014.

**Methodology:** Cotyledon and hypocotyl explants of *B. campestris* cv. BARI sarisha-12 were cultured on MS medium supplemented with different concentration of 6-Benzylaminopurine (BAP) and  $\alpha$ -Naphthaleneacetic acid (NAA) for callus initiation and shoot regeneration. Later on subsequent subculturing is done for shoot elongation and multiplication. MS medium supplemented with various concentrations of NAA were used for root formation.

**Results:** From a total of 15 different combinations of BAP and NAA tested, the combination of 1.0 mg L<sup>-1</sup> BAP and 0.5 mg L<sup>-1</sup> NAA gave the highest frequency of callus initiation (94.44%) as well as shoot regeneration (63.89%) in case of cotyledon explants where as hypocotyl explants showed 47.62% callus initiation and 19.04% shoot regeneration frequency. Four days old cotyledon explants showed the highest shoot regeneration frequency (72.22%) and higher number of shoots per explant (3.94) than those from older seedling. The shoot regeneration frequency markedly enhanced to 83.33% by the addition of 2.0 mg L<sup>-1</sup> AgNO<sub>3</sub> to the MS medium supplemented with 1.0 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA and this combination also showed the maximum number of shoots per explant (6.86). Shoot regeneration potentiality of five *B. campestris* genotypes were investigated and indicated that this system would be widely applicable to all the genotypes. The regenerated shoots were easily rooted on MS medium supplemented with 0.2 mg L<sup>-1</sup> NAA and the whole plants were transferred to pot soils and grown to maturity.

**Conclusion:** MS medium supplemented with 1.0 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA and 2.0 mg L<sup>-1</sup> AgNO<sub>3</sub> is more efficient for multiple shoot regeneration by using cotyledon explants and it may be utilized for *in vitro* improvement program of *B. campestris*.

9  
10 **Keywords:** *Brassica campestris*; Phytohormons, Cotyledon; Hypocotyl; Regeneration.

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12 **1. INTRODUCTION**

13  
14 Brassicaceae is a family having about 3,000 species grouped into 350 genera including  
15 several types of edible plants [1]. The genus *Brassica* comprises commercially important  
16 vegetables and oilseed crops that are the good source of nutrients and health promoting  
17 phytochemicals [2]. High intake of these crops lessen the risk of age-related chronic  
18 illnesses such as cardiovascular health and other degenerative diseases [3] and also  
19 reduces the risk of several types of cancer [3-5]. Among the oilseed crops, *B. campestris*  
20 has a wide spread global distribution and mostly cultivated as vegetable and oilseed crops in  
21 Europe, Canada and Indian subcontinents.

22 In Bangladesh, *B. campestris* is one of the most important oilseed crops. The climatic and  
23 edaphic factors of Bangladesh are quite favorable for the cultivation of rapeseed and mustard.  
24 The total cultivated area under rapeseed and mustard cultivation is 0.532 million hectares  
25 which produces 0.657 million tons of mustard per year covering only 40% of domestic need  
26 [6]. As a result the country is continuously facing a huge shortage of oils and oilseed and  
27 spending huge amount of foreign currency to meet the country's demand [7]. The poor yield  
28 condition of mustard in Bangladesh might be due to the lack of high yielding variety, poor  
29 cultural and management practices and plant protection measures for raising the crop. As  
30 our land is limited but we have to increase our mustard production within limited land, so that  
31 it is necessary to develop high yielding as well as biotic and abiotic stress resistant *B.*  
32 *campestris* crop varieties to fulfill the domestic need.

33 Conventional breeding programs alone were not successful enough to develop high yielding  
34 crop variety of *B. campestris* due to high degree of segregation upon cross pollination and  
35 unavailability of suitable germplasm as well as it is labor and resource intensive and time  
36 consuming [8]. On the other hand, recent techniques in plant genetic engineering have  
37 opened new avenues for crop improvement by developing transgenic. In this regards a high  
38 frequency plant regeneration system is crucial. *In vitro* techniques have been applied in  
39 *Brassica* from different point of views and organogenesis, somatic embryogenesis and  
40 regeneration were achieved [9-14]. During last decades, considerable efforts have been  
41 made to develop *in vitro* technique for regeneration of *Brassica* spp. During these attempts a  
42 wide variety of explants have been used such as leaves [15]; roots [16]; anther [17-18];  
43 filament [19]; cotyledon [11]; hypocotyls [20] and protoplasts [21]. However, it is proved that,  
44 *B. campestris* is one of the recalcitrant members of Brassicaceae in tissue culture by  
45 studying shoot regeneration from callus [22], leaf discs [23], cotyledons [10], and from  
46 isolated protoplasts [24]. Moreover, various explants like cotyledons [25-27], hypocotyls [27],  
47 stem and leaf segments [28], shoot tips [26], and filaments and anthers [29] have been used  
48 for *in vitro* regeneration of *B. campestris*. However, no report has been found on *in vitro* plant  
49 regeneration of *B. campestris* genotypes grown in Bangladesh except *B. campestris* cv. Tori-  
50 7 which showed low regeneration frequency.

51 Considering the above, this study was carried out to establish an efficient protocol for high  
52 frequency plant regeneration of *B. campestris* genotypes grown in Bangladesh, which is  
53 prerequisite for genetic transformation and to evaluate the genotypic variation for plantlet  
54 regeneration potentiality of *B. campestris*.

## 55 56 2. MATERIAL AND METHODS

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58 This experiment was carried out in the Genetic Engineering Laboratory of the Department of  
59 Genetics and Plant Breeding, Sylhet Agricultural University, Bangladesh during the period of  
60 July 2013 to June 2014. Five *B. campestris* genotypes namely Tori-7, BARI sarisha-6, BARI  
61 sarisha-9, BARI sarisha-12 and BARI sarisha-15 [collected from Bangladesh Agriculture  
62 Research Institute (BARI)] were used to fulfill the objectives of the present investigations.  
63 Among these varieties BARI sarisha-12 was used to standardize the plant regeneration  
64 protocol for *B. campestris* and other genotypes were used to observe their plantlet  
65 regeneration potentiality.

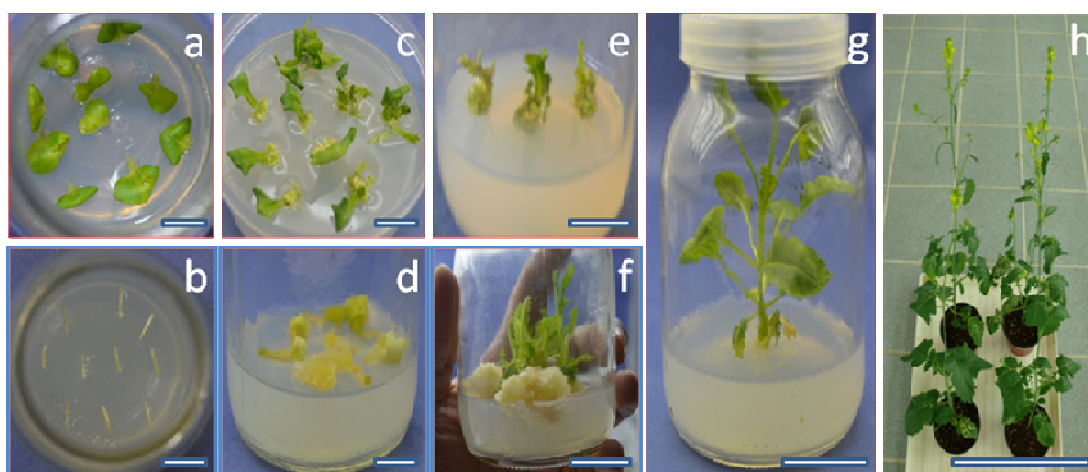
66 The seeds were sterilized in the solution of 70% ethyl alcohol (MERCK, Germany) for 2 min  
67 and 10% Clorox (Sodium hypochlorite, The Clorox Company, Oakland, USA) for 10 min  
68 followed by four rinses in sterile distilled water. The seeds were then placed on germination  
69 medium comprising half strength MS [30] salts and vitamins, 3% sucrose and 1% agar with  
70 a density of 15 seeds per culture vessels and incubated in 25±2°C temperature under 16  
71 hours photoperiod provided by 144W white fluorescent lamps (culture condition).

72 Five days old cotyledon and hypocotyl explants of *B. campestris* cv. BARI sarisha-12 was  
73 cultured on MS media supplemented with different concentration of BAP (0.5, 1.0, 2.0, 3.0  
74 and 4.0 mg L<sup>-1</sup>) and NAA (0.1, 0.2 and 0.5 mg L<sup>-1</sup>) to determine optimal medium for callus

75 **initiation.** Cotyledons along with 1-2 mm petioles were very carefully excised from the  
 76 hypocotyl and apical shoot meristems of seedlings (3 to 7 days old seedlings). The  
 77 hypocotyls were then discarded from the root tip and cut into 4-5 mm length segments. The  
 78 whole procedure was carried out in laminar airflow cabinet. Ten to 15 excised cotyledons  
 79 along with petioles and hypocotyl segments were placed on each culture vessels containing  
 80 50 ml regeneration media. Cotyledons along with petioles were placed in upward direction  
 81 with the petiole in contact with the media whereas hypocotyl segments were placed  
 82 horizontally on the surface of the media (Fig. 1a & b). The culture vessels were sealed with  
 83 parafilm and marked with permanent marker to indicate specific treatment and incubated in  
 84 culture condition.

85 Regeneration media comprised MS salts and vitamins, 3% sucrose, 1% agar and various  
 86 concentrations of 6-Benzylaminopurine (BAP) (0.5, 1.0, 2.0, 3.0 and 4.0 mg L<sup>-1</sup>) and α-  
 87 Naphthalene acetic acid (NAA) (0.1, 0.2 and 0.5 mg L<sup>-1</sup>). To investigate the effect of AgNO<sub>3</sub>  
 88 on shoot regeneration various concentration of AgNO<sub>3</sub> (1.0, 2.0, 3.0, 4.0 and 5.0 mg L<sup>-1</sup>)  
 89 were added with the regeneration media. *In vitro* regenerated shoots were subcultured  
 90 regularly to fresh media at an interval of 12-15 days for further multiplication. About 2-3 cm  
 91 elongated shoots were separated and cultured on rooting medium containing MS salts and  
 92 vitamins, 3% sucrose, 1% agar and different concentration of NAA (0, 0.1, 0.2 and 0.5 mg L<sup>-1</sup>)  
 93 for root formation. When the rooted plantlets became 5-7 cm in length with sufficient root  
 94 system, these were taken out very carefully from the culture vessels with undisturbed rooting  
 95 system and washed gently in tap water to remove agar medium and sucrose traces to  
 96 discourage infection by fungal contamination. The plantlets were then transplanted to  
 97 moistened soil in pots containing sterilized soil and covered with moist polythene bags for  
 98 preventing desiccation. After proper hardening, the plantlets were transferred to natural  
 99 environment.

100 The experiment was arranged in Completely Randomized Design (CRD) with 3 replications.  
 101 Data were recorded on the percentage of callus initiation, percentage of shoot regeneration  
 102 and number of shoots per explant and statistically analyzed to ascertain the significance of  
 103 the experimental results. The mean and standard deviation for all treatments were calculated  
 104 by using MS Excel 2007. The significance and difference between means were evaluated at  
 105 5% level of significance by Duncan's Multiple Rang Test [31] using MSTATC statistical  
 106 software [32].



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108 **Fig. 1. The regeneration process of *B. campestris* cv. BARI sarisha-12. (a) cotyledon**  
 109 **explants, (b) hypocotyl explants, (c and d) shoot regeneration in MS medium**  
 110 **supplemented with 1.0 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA and 2.0 mg L<sup>-1</sup> AgNO<sub>3</sub> from**  
 111 **cotyledon and hypocotyl explants respectively, (e and f) shoot elongation on 1.0 mg L<sup>-1</sup>**

112 <sup>1</sup> BAP, 0.5 mg L<sup>-1</sup> NAA and 2.0 mg L<sup>-1</sup> AgNO<sub>3</sub> medium (g) root induction of regenerated  
 113 shoot on MS medium supplemented with 0.2 mg L<sup>-1</sup> NAA, (h) flowering of regenerated  
 114 plants. **Scale bars represent 5 mm (a, b, c, d), 1 cm (e, f), 2 cm (g), and 10 cm (h).**  
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### 116 3. RESULTS AND DISCUSSION

#### 117 3.1 Optimal media for callus introduction

118 From a total of 15 different combinations tested, cotyledon explants showed the highest  
 119 (94.44%) callus initiation frequency in MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA combination  
 120 and the lowest (33.33%) in MS + 4.0 mg L<sup>-1</sup> BAP + 0.1 mg L<sup>-1</sup> NAA combination whereas  
 121 hypocotyl explants showed the highest (47.62%) callus initiation frequency in MS + 1.0 mg L<sup>-1</sup>  
 122 BAP + 0.5 mg L<sup>-1</sup> NAA combination and the lowest (11.9%) in MS + 4.0 mg L<sup>-1</sup> BAP + 0.1  
 123 mg L<sup>-1</sup> NAA combination (Table 1). A significant difference was found in callus initiation  
 124 frequency between cotyledon and hypocotyl explants and it is clear that cotyledon explants  
 125 showed better performance than the hypocotyl explants. Similar trend in callus initiation was  
 126 also reported previously that cotyledon explants produced higher frequency of celli than the  
 127 hypocotyls [27].  
 128  
 129

130 **Table 1. Frequency of callus initiation from 5 days old cotyledon and hypocotyl**  
 131 **explants of *B. campestris* cv. BARI sarisha-12 on MS media supplemented with**  
 132 **various concentrations of BAP and NAA. Data consist of three replicates, each**  
 133 **comprising 12 explants. The mean values were compared by DMRT. Mean ± SD**  
 134 **followed by same letters are not significantly different at P = 0.05.**

Treatments	Callus initiation frequency (%)	
	Cotyledon	Hypocotyl
MS + 0.5 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	58.33±1.0efg	30.95±0.5cde
MS + 1.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	75.00±1.0bc	33.33±0.5bcd
MS + 2.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	50.00±1.0fgh	23.81±0.5defg
MS + 3.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	44.40±0.5hi	16.67±0.5gh
MS + 4.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	33.33±1.0i	11.90±0.5h
MS + 0.5 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	72.22±0.5bcd	23.81±0.5defg
MS + 1.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	80.50±0.5b	42.86±2.0ab
MS + 2.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	55.56±1.1efgh	21.43±1.0efgh
MS + 3.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	61.11±0.5def	19.05±0.5fgh
MS + 4.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	47.22±1.1gh	16.67±0.5gh
MS + 0.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	83.33±1.0ab	40.48±1.5abc
MS + 1.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	94.44±0.5a	47.62±1.1a
MS + 2.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	66.67±1.0cde	33.33±0.5bcd
MS + 3.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	63.88±0.5cde	30.95±0.5cde
MS + 4.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	61.11±0.5def	28.57±1.0def

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#### 136 3.2 Optimal media for shoot regeneration

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138 After two weeks of explants culture, shoot bud formation started from the calli. Cotyledon  
139 explants showed shoot regeneration frequency in all the combinations, but in case of  
140 hypocotyl explants some combinations did not produce any shoot (Table 2). The highest  
141 (63.88%) and the lowest (13.88%) shoot formation frequency were obtained by using 5 days  
142 old cotyledon explants in MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA and MS + 4.0 mg L<sup>-1</sup> BAP +  
143 0.1 mg L<sup>-1</sup> NAA combinations respectively. On the other hand hypocotyl explants showed  
144 the highest (19.04%) shoot regeneration frequency in MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup>  
145 NAA combination and the lowest (2.38%) in MS + 1.0 mg L<sup>-1</sup> BAP + 0.1 mg L<sup>-1</sup> NAA, MS +  
146 2.0 mg L<sup>-1</sup> BAP + 0.1 mg L<sup>-1</sup> NAA and MS + 2.0 mg L<sup>-1</sup> BAP + 0.2 mg L<sup>-1</sup> NAA combinations.  
147 It was observed that when BAP concentration increased up to 1.0 mg L<sup>-1</sup> along with same  
148 NAA concentration showed the highest shoot regeneration frequency and further increase of  
149 BAP (2.0, 3.0 and 4.0 mg L<sup>-1</sup>) concentration decreased the shoot regeneration frequency.  
150 From the above results, it is determined that the use of cotyledon explants showed more  
151 shoot regeneration frequency than the use of hypocotyl explants cultured on MS medium  
152 supplemented with 15 combinations of BAP and NAA. This agrees with previously reported  
153 result that is frequency of shoot formation from cotyledon explants was generally higher than  
154 the frequency of hypocotyls explants [33]. The maximum frequency (63.88%) of shoot  
155 regeneration of *B. campestris* from cotyledons cultured on MS medium supplemented with  
156 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA was somewhat different from the previously reported result  
157 of maximum shoot regeneration frequency of *B. campestris* from cotyledons cultured on MS  
158 medium supplemented with 2.0 mg L<sup>-1</sup> BAP, 0.5 mg L<sup>-1</sup> and 3 mg L<sup>-1</sup> AgNO<sub>3</sub> [25] and MS  
159 medium added with 3.0 mg L<sup>-1</sup> BAP, 0.1 mg L<sup>-1</sup> NAA and 5.0 mg L<sup>-1</sup> AgNO<sub>3</sub> [34]. A maximum  
160 of 46.66% shoot regeneration frequency of *B. campestris* cv. Tory-7 was reported previously  
161 on 3.0 mg L<sup>-1</sup> BAP + 0.2 mg L<sup>-1</sup> NAA combination [35].

162 **Table 2. Frequency of shoot regeneration from 5 days old cotyledon and hypocotyl**  
163 **explants of *B. campestris* cv. BARI sarisha-12 on MS media supplemented with**  
164 **various concentrations of BAP and NAA. Data consist of three replicates, each**  
165 **comprising 12 explants. The mean values were compared by DMRT. Mean ± SD**  
166 **followed by same letters are not significantly different at P = 0.05.**

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Treatment	Shoot Regeneration frequency (%)	
	Cotyledon	Hypocotyl
MS + 0.5 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	30.56±1.1defg	2.38±0.5ef
MS + 1.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	41.67±1.0bcd	7.14±0.8cde
MS + 2.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	25.00±1.0efgh	2.38±0.5ef
MS + 3.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	22.22±0.5fgh	00±0.0f
MS + 4.0 mg L <sup>-1</sup> BAP + 0.1 mg L <sup>-1</sup> NAA	13.89±0.5h	00±0.0f
MS + 0.5 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	38.89±0.5bcde	9.52±0.5cd
MS + 1.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	52.78±1.5ab	16.66±0.5ab
MS + 2.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	27.78±0.5defgh	2.38±0.5ef
MS + 3.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	25.00±1.0efgh	00±0.0f
MS + 4.0 mg L <sup>-1</sup> BAP + 0.2 mg L <sup>-1</sup> NAA	19.44±1.5gh	00±0.0f
MS + 0.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	47.22±1.5bc	11.9±0.5bc
MS + 1.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	63.89±1.1a	19.04±1.0a
MS + 2.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	36.11±0.5cdef	4.76±0.5def
MS + 3.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	30.56±0.5defg	00±0.0f
MS + 4.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> NAA	25.00±1.0efgh	00±0.0f

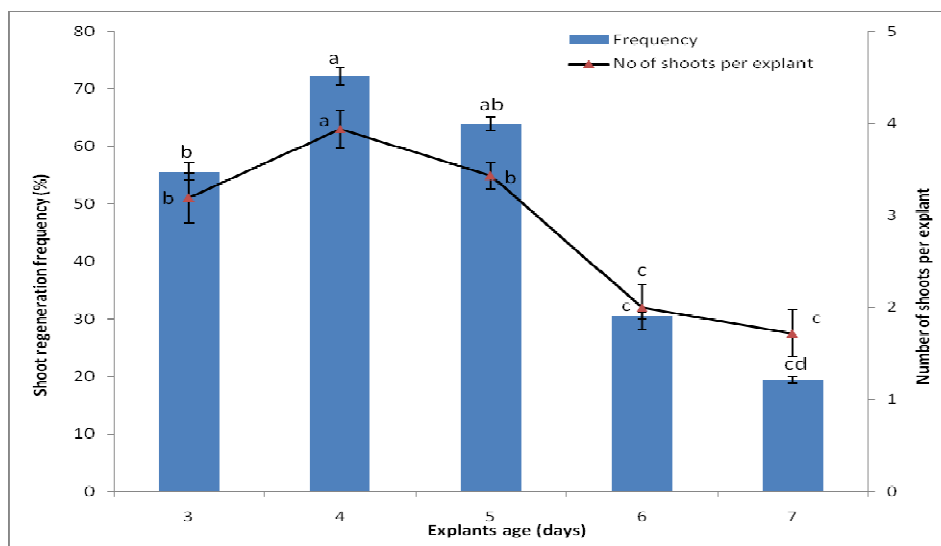
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### 170 3.3 Effect of explant age

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172 In order to investigate the effect of age of explants, cotyledon explants of different ages (3 to  
 173 7 days) were cultured on shoot regeneration media (MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA)  
 174 followed by callus initiation media (MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA). Explants from 2  
 175 days old seedling were too small and were not used in this experiment. Cotyledon explants  
 176 of 4 days old seedlings showed the highest (72.22%) shoot regeneration frequency and  
 177 explants of 7 days old seedlings showed the lowest (19.44%) shoot regeneration frequency  
 178 after two weeks of explant incubation. However, the shoot regeneration frequency of 3 days  
 179 (55.56%) and 5 days (63.89%) old seedling showed no significant difference, but a steady  
 180 decrease in shoot regeneration frequency was observed in the explants derived from 4 days  
 181 to 7 days old seedlings. The result indicates that the frequency of shoot regeneration is  
 182 affected by seedling age and maximum number of shoot is produced from 4 days old  
 183 seedling explants (Fig. 2). This observation is compliant with the previous works of *B. juncea*  
 184 [14] and *B. napus* [36].



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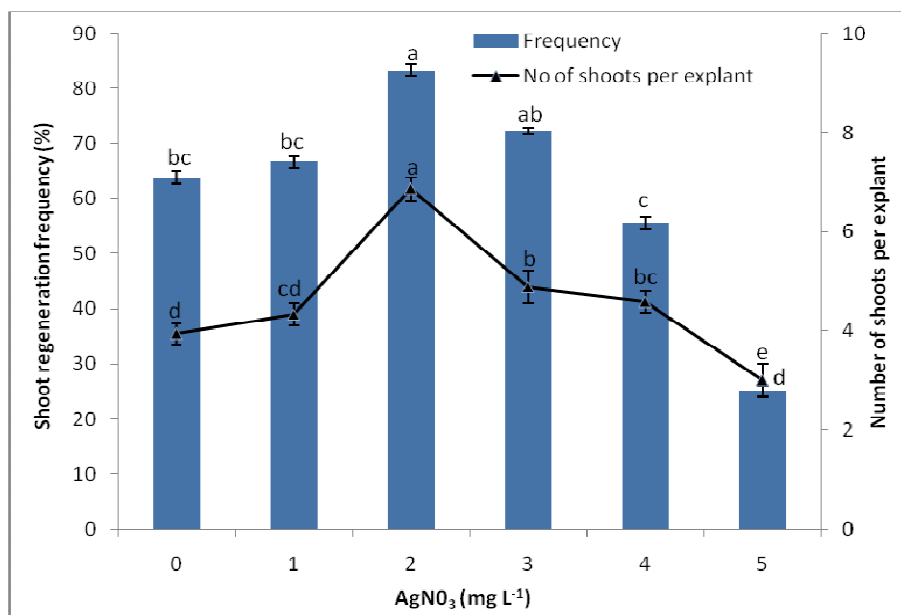
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**Fig. 2.** Effect of explant age on shoot regeneration from cotyledon explants of *B. campestris* cv. BARI sarisha-12. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means. Values with different letters are significantly different at  $P \leq 0.05$  (DMRT).

### 3.4 Influence of $\text{AgNO}_3$

To investigate the effect of  $\text{AgNO}_3$  on shoot regeneration and number of shoots per explant, 4 days old cotyledon explants of *B. campestris* cv. BARI sarisha-12 were cultured on shoot regeneration media ( $\text{MS} + 1.0 \text{ mg L}^{-1} \text{ BAP} + 0.5 \text{ mg L}^{-1} \text{ NAA}$ ) supplemented with different concentration of  $\text{AgNO}_3$  ( $1.0, 2.0, 3.0, 4.0$  and  $5.0 \text{ mg L}^{-1}$ ) (Fig. 3). The highest (83.33%) shoot regeneration frequency and the highest number of shoots per explant (6.86) was observed in shoot regeneration media supplemented with  $2.0 \text{ mg L}^{-1} \text{ AgNO}_3$  and the lowest (25.0%) shoot regeneration frequency and the lowest number of shoots per explant (3.23) was observed in shoot regeneration medium supplemented with  $5.0 \text{ mg L}^{-1} \text{ AgNO}_3$ . The shoot regeneration frequency and number of shoots per explant producing capacity enhanced with the increase of  $\text{AgNO}_3$  concentration up to  $2 \text{ mg L}^{-1}$  but further increase of  $\text{AgNO}_3$  concentration decreased the regeneration frequency and shoot producing capacity. The shoot regeneration frequency and number of shoots per explant is markedly enhanced with the addition of ethylene biosynthesis inhibitor  $\text{AgNO}_3$ . It is observed that the level of enhancements of shoot regeneration and number of shoots per explant depends on the level of concentrations of  $\text{AgNO}_3$ . By adding  $\text{AgNO}_3$  maximum 80% of shoot regeneration frequency was reported previously in case of *B. campestris* cv. Tory-7 [34]. The positive effect of  $\text{AgNO}_3$  was consistent with the previous results from the cotyledon explants of *B. rapa* spp. *oleifera* [37], *B. campestris* spp. *pekinensis* [38, 39] and hypocotyls of *B. juncea* [40].



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213 **Fig. 3.** Effect of AgNO<sub>3</sub> concentrations on shoot regeneration from 4 days old  
 214 cotyledon explants of *B. campestris* cv. BARI sarisha-12. Data consist of three  
 215 replications and 12 explants were used for each replication. Bars represent SD of the  
 216 means. Values with different letters are significantly different at P ≤ 0.05 (DMRT).  
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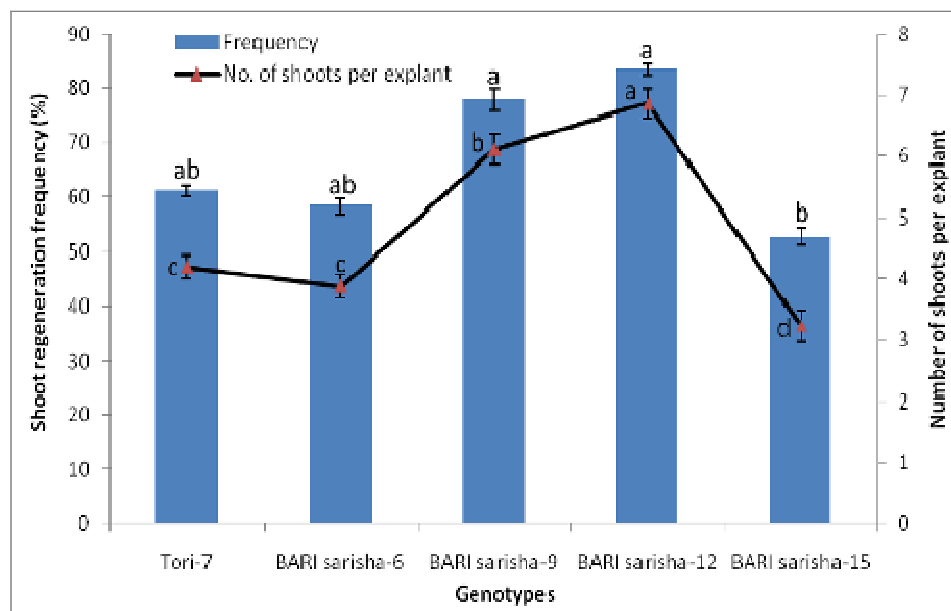
### 218 3.5 Genotypic variation

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220 Cotyledon explants from 4 days old seedlings of five *B. campestris* genotype namely Tori-7,  
 221 BARI sarisha-6, BARI sarisha-9, BARI sarisha-12 and BARI sarisha-15 were cultured on  
 222 shoot regeneration medium (MS + 1.0 mg L<sup>-1</sup> BAP + 0.5 mg L<sup>-1</sup> NAA) in addition with 2 mg L<sup>-1</sup>  
 223 AgNO<sub>3</sub> to determine their shoot regeneration ability and number of shoots per explants.  
 224 Shoot regeneration frequency is 83.33%, 77.78%, 66.67%, 61.11% and 52.78% in BARI  
 225 sarisha-12, BARI sarisha-9, Tori-7, BARI sarisha-6 and BARI sarisha-15, respectively (Fig.  
 226 6). The number of shoots per explant is 6.85, 6.11, 4.2, 3.88 and 3.23 in BARI sarisha-12,  
 227 BARI sarisha-9, Tori-7, BARI sarisha-6 and BARI sarisha-15, respectively (Fig. 4).

228 Result indicated that shoot regeneration frequency and number of shoots per explants were  
 229 greatly influenced by the genotypic variation. From the above result, it is found that BARI  
 230 sarisha-12 showed the highest (83.33%) shoot regeneration frequency and maximum  
 231 number of shoots per explant. On the other hand BARI sarisha-15 showed the lowest  
 232 (52.78%) shoot regeneration frequency and least number of shoots per explant (Fig. 4).





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234 **Fig. 4.** Influence of genotypes on shoot regeneration from 4 days old cotyledon  
 235 explants of *B. campestris*. Data consist of three replications and 12 explants were  
 236 used for each replication. Bars represent SD of means. Values with different letters  
 237 are significantly different at  $P \leq 0.05$  (DMRT).  
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### 239 3.6 Initiation of roots

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241 Root formation frequency varies with the different concentrations of NAA. The highest  
 242 (100%) root formation frequency was observed in MS medium supplemented with  $0.2 \text{ mg L}^{-1}$   
 243 NAA and the lowest (47.22%) were observed in MS medium and MS +  $0.5 \text{ mg L}^{-1}$  NAA  
 244 combination (Table 3). Plantlets produced well developed root system within 10 to 12 days.

245 **Table 3.** Influence of NAA concentrations on rooting of regenerated shoots from  
 246 cotyledon explants of *B. campestris* cv. BAPRI sarisha-12. Data consist of three  
 247 replicates, each comprising 12 plants. The mean values were compared by DMRT.  
 248 Mean  $\pm$  SD followed by same letters are not significantly different at  $P = 0.05$ .

Treatment	Root formation frequency (%)
MS	47.22 $\pm$ 2.0b
MS + $0.1 \text{ mg L}^{-1}$ NAA	91.67 $\pm$ 1.0a
MS + $0.2 \text{ mg L}^{-1}$ NAA	100 $\pm$ 0.0a
MS + $0.5 \text{ mg L}^{-1}$ NAA	47.22 $\pm$ 2.0b

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## 251 4. CONCLUSION

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253 It is apparent from these results that, cotyledon explants showed the higher callus and shoot  
 254 regeneration frequency compared with hypocotyl explants of *B. campestris*. Age of explants  
 255 and  $\text{AgNO}_3$  have great influence on shoot regeneration and multiplication of *B. campestris*.  
 256 MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BAP,  $0.5 \text{ mg L}^{-1}$  NAA and  $2.0 \text{ mg L}^{-1}$   $\text{AgNO}_3$  is  
 257 more efficient medium for multiple shoot regeneration by using 4 days old cotyledon explants  
 258 and it may be utilized in *in vitro* improvement program of *B. campestris*.  
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