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### Exploring Somaclonal Variation in Potato Breeding: Mechanisms and Applications for Enhancing Crop Yields

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#### Abstract

This study focused on enhancing crop yields, particularly in densely populated regions such as Bangladesh. Two widely grown potato cultivars, Diamant and Cardinal, were selected for enhancement through the *in vitro* culture of dedifferentiated tissue. The core objectives of the research encompassed callus induction, plant regeneration from callus to produce somaclonal variants, screening of somaclonal variants based on morphological characteristics, and field evaluations to assess growth and yield performance.

Key findings highlighted the success of callus induction, with an impressive 98% yield for Diamant and 86% for Cardinal. This achievement was realized through the utilization of a semi-solid MS medium supplemented with 2.5 mg/l 2,4-D, where inter-nodal explants displayed superior responses. The optimal medium for

shoot regeneration was identified as MS with 2.0 mg/l KIN and 0.5 mg/l NAA, resulting in notable shoot regeneration rates of 86% for Diamant and 78% for Cardinal. In the field, Somaclones exhibited remarkable advantages. They displayed increased stem height, reaching 56.4 cm (Diamant) and 50.0 cm (Cardinal) after 70 days of planting, surpassing the heights of 49.6 cm (Diamant) and 44.2 cm (Cardinal) observed in parental clones. Furthermore, mini-tubers harvested from Somaclones were significantly heavier, averaging 361.0 g (Diamant) and 300.0 g (Cardinal), while parental clones produced mini-tubers with an average weight of 169.6 g (Diamant) and 159.3 g (Cardinal). Impressively, Somaclones also displayed a higher number of tubers per plant, producing 33.6 (Diamant) and 30.2 (Cardinal), compared to parental clones, which yielded 18.6 (Diamant) and 14.9 (Cardinal) tubers per plant.

**Keywords:** Callus, Genetic Diversity, *In vitro* Culture, Meristem Culture, Mini-Tuber, Somaclonal Variation

#### 1. Introduction

Potato (*Solanum tuberosum* L.) holds a significant position among annual crops and belongs to the Solanaceae family. Its origin can be traced back to the Andes, near the Peru-Bolivia border in South America <sup>[1, 2]</sup>. The role it plays in global food supply is crucial, ranking just below wheat, rice, and maize in terms of human consumption. This versatile crop is extensively cultivated across the world and provides sustenance to over a billion people in various countries <sup>[3]</sup>. Notably, potato consumption has been on the rise, doubling every 10 to 15 years <sup>[4]</sup>.

Bangladesh, one of the most densely populated countries globally, faces a distinct demographic challenge. Recent data from 2023 reveals that approximately 1600 individuals inhabit every square kilometer of the country <sup>[5]</sup>. With a total population of around 170 million and an annual growth rate of 1.2 percent, projections indicate that Bangladesh's population will reach 205 million by 2050 <sup>[5]</sup>. This demographic shift, coupled with urbanization, housing development, and industrial expansion, is progressively diminishing the available land for crop production.

Potato stands out as the highest yielding crop among major crops cultivated in Bangladesh. It surpasses rice by a factor of 4.28, wheat by 6.95, and maize by 2.93. Given its high nutritional value and energy production per unit area, potato cultivation plays a pivotal role in addressing global food crises. However, it's essential to note that only 0.89% of Bangladesh's arable land is currently allocated to potato cultivation, contributing approximately 7.6% to the total cultivated food crops <sup>[6]</sup>. Despite its potential, potato production in Bangladesh falls short, with an average yield of 19.03 tons per hectare, considerably lower than yields in countries like France and Germany, where the average yield reaches 47.94 and 47.41 tons per hectare, respectively. Remarkably, South Korea has witnessed a remarkable increase in potato yield, advancing from 20.0 to 35.0 tons per hectare, primarily through the cultivation of improved potato varieties <sup>[7]</sup>.

To tackle the challenges of potato breeding, genetic engineering and *in vitro* screening offer promising solutions [8, 9]. However, concerns related to the health and environmental impacts of genetically modified foods have elevated the significance of *in vitro* screening as a primary biotechnological tool in potato breeding [10]. This method largely relies on genetic variations that emerge during *in vitro* conditions, known as somaclonal variations [11]. These variations can be attributed to factors such as point mutations, numerical and structural chromosomal changes, as well as epigenetic modifications, including DNA hypo- and hypermethylation [12]. Nonetheless, there is a need for further research to fully understand the molecular mechanisms underlying somaclonal variation [13].

Somaclonal variation provides a pathway to develop improved crop varieties. By generating a diverse range of somaclones and selecting those with desirable traits, plant breeders can accelerate the development of new cultivars [14]. This approach has been successfully applied in crops like potato and banana to create varieties with enhanced disease resistance, yield, and quality. Importantly, somaclonal variation can lead to the development of plants better adapted to environmental stress conditions [11], offering a valuable strategy in the face of climate change.

One remarkable achievement in somaclonal variation involved an old potato variety. One hundred somaclones were produced from leaf protoplasts of Russet Burbank, and significant and stable variations were observed in growth habit, compactness, maturity date, tuber uniformity, tuber skin color, and photoperiodic requirements [15]. Notably, the increased tuber uniformity and early tuberization onset represented agronomic improvements over the parent variety.

## 2. Materials and Methods

Primary explants from potato varieties Diamant and Cardinal, including tubers and branches of field-grown plants, were collected from the Bangladesh Agricultural Research Institute (BARI) in Gazipur. Laboratory experiments were conducted at the Tissue Culture Laboratory within the Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka. Field experiments took place in the fields located in Pancahgarah and Thakurgawn district of Bangladesh.

### 2.1 Preparing Parental Clone (Control)

The process involved the culture of meristem in MS media supplemented with different auxins and cytokinins to induce shoot formation. Subsequently, these newly formed shoots were subcultured in every 28 days. These plants were then subjected to a hardening process using a potting mixture composed of soil, sand, and compost in a 1:1:1 (v/v) ratio. After successful acclimatization, the plants were transplanted into the screen house and utilized as the parental clone (control).

### 2.2 Induction of Callus and Regeneration of Shoot

For callus induction and shoot regeneration, leaves and internodes of *in vitro* virus-free plants were used as the primary explants. The growth medium for all experiments was Murashige and Skoog (MS) medium, supplemented with various concentrations and combinations of auxin and cytokinin.

All cultures were maintained in a culture room with a 16/8 light/dark photoperiod and a temperature of 25°C. After 4-6 weeks, calli were initiated, and approximately 8-10 mm<sup>2</sup> segments of callus were sub-cultured on the same medium at intervals of 2-3 weeks.

### 2.3 Screening of Somaclonal Variants

In this study, *in vitro*-raised potato plants derived from callus were carefully examined under controlled culture conditions to identify somaclonal variants based on distinctive morphological traits. These variants were subsequently labeled as Somaclonal Variants line 'SD1-SD10' for Diamant and 'SC1-SC10' for Cardinal. To ensure the robustness of all regenerants, they were subjected to a hardening process using a potting mixture composed of soil, sand, and compost in a 1:1:1 (v/v) ratio. Following this hardening phase, the acclimatized plants were successfully transplanted into the field. Somaclonal variants are easily identifiable based on specific morphological attributes, such as plant height, leaf morphology, and the presence of abnormal pigmentation [16]. For example, a somaclonal variant of sweet cherry (*Prunus avium*) was characterized by evaluating parameters such as plant vigor, leaf morphology, stomatal density, photosynthetic activity, floral bud formation, and the size, shape, and color of the fruit [17].

### 2.4 Field Assessment of Somaclones for Growth and Yield and Comparison with Parental Clones (Control)

The isolated somaclonal variants, after hardened state, were introduced into the screen house to assess various morphological characteristics. These included plant height, the number of branches, the number of tubers per plant, mean diameter of mini-tuber and the total tuber weight per plant, all in their first generation in screen house following the *in vitro* phase. A comparative analysis was performed between the somaclonal variants and the normal regenerants or control plants.

### 2.5 Data Collection

Data for shoot tip culture, callus formation, and plant regeneration from callus were collected and recorded after 28 days of culture. The results represent the means of three consecutive experiments. For the field evaluation of somaclones and controls, data from ten plants in each of five beds were collected based on plant morphology, plant height, number of branches, and plant vigor. Each data point presented is the mean of 50 plants. Data for shoot length and the number of branches were collected after 70 days of planting, while other measurements were taken after 90 days of planting.

### 2.6 Data Analysis

The data obtained from this study underwent rigorous statistical analysis. One-way analysis of variance (ANOVA) was employed to assess the variation between different treatments. Significance between any two means was determined at a chosen probability level of  $p = 0.05$ , using the Duncan's Multiple Range Test (DMRT). The statistical software SPSS version 15.0 was utilized for these analyses.

## 3. Results and Discussion

### 3.1 Callus Response

A notable 98% of Diamant explants and 86% of Cardinal explants displayed a robust and substantial callus formation within a span of 14-18 days. This is found when the explants were cultured in a semi-solid MS medium supplemented with 2.5 mg/l 2,4-D. Comparing the two types of explants, inter-node explants yielded superior results, with a 98% success rate for Diamant and an 86% success rate for Cardinal. Leaf explants exhibited a slightly lower but still significant rate, with an 80% success rate for Diamant and a 70% success rate for Cardinal, both cultured in an MS medium supplemented with 2.5 mg/l 2,4-D (Table 1, 2).

**Table 1:** Effect of different concentration of auxin and cytokinin in MS medium for response of callus from inter-node and leaf explants of Diamant. Data were recorded up to 28 days of culture

Explant source	Growth Regulators mg/l	Days to callus initiation	Percent (%) of explants formed callus	Callus texture	Callus color	Degree of callus formation
Inter-node Diamant	<b>2-4,D</b>					
	2.5	14	98	Compact	LG	+++
	3.0	20	90	Compact	G	+++
	<b>BA + NAA</b>					
	1.0 + 1.0	16	92	Compact	G	+++
	1.5 + 1.5	20	80	Compact	B	+++
	<b>KIN + NAA</b>					
	1.0 + 1.0	20	46	Friable	G	+++
	1.0 + 1.5	16	45	Friable	LG	++
	<b>2-4,D + BAB</b>					
2.5 + 0.5	20	55	Friable	G	+++	
3.0 + 0.5	25	45	Friable	LG	++	
Leaves Diamant	<b>2-4,D</b>					
	2.5	22	70	Compact	G	+++
	3.0	20	80	Compact	LG	+++
	<b>BA + NAA g/l</b>					
	1.0 + 1.0	20	72	Compact	G	+++
	1.5 + 1.5	20	75	Compact	B	++
	<b>KIN + NAA</b>					
	1.0 + 1.0	20	36	Friable	G	+++
	1.0 + 1.5	20	35	Friable	LG	++
	<b>2-4,D + BAB</b>					
2.5 + 0.5	20	45	Friable	B	+	
2.5 + 1.0	26	40	Friable	B	++	

**B-Brown, LG-Light green, G-Green; ++: Trace callus,+++: Moderate callus,++++: Massive callus**

**Table 2:** Effect of different concentration of auxin and cytokinin in MS medium for response of callus from inter-node and leaf explants of Cardinal. Data were recorded up to 28 days of culture

Explant source	Growth Regulators mg/l	Days to callus initiation	Percent(%) of explants formed callus	Callus texture	Callus color	Degree of callus formation
Inter-node Cardinal	<b>2-4,D</b>					
	2.5	18	86	Compact	LG	++
	3.0	20	78	Compact	G	+++
	<b>BA + NAA</b>					
	1.0 + 1.0	20	75	Compact	G	+++
	1.5 + 1.5	18	65	Compact	B	++
	<b>KIN + NAA</b>					
	1.0 + 0.5	20	40	Friable	G	+++
	1.0 + 1.5	16	38	Friable	LG	++
	<b>2-4,D + BAB</b>					
2.5 + 0.5	24	45	Friable	G	+++	
3.0 + 0.5	26	40	Friable	LG	++	
Leaves Cardinal	<b>2-4,D</b>					
	2.5	25	65	Compact	G	+++
	3.0	22	70	Compact	LG	++
	<b>BA + NAA</b>					
	1.0 + 1.0	20	75	Compact	G	+++
	1.0 + 1.5	26	60	Compact	LG	++
	<b>KIN + NAA</b>					
	2.5 + 0.5	24	45	Friable	G	+++
	3.0 + 0.5	26	40	Friable	LG	++
	<b>2-4,D + BAB</b>					
2.5 + 0.5	20	40	Friable	B	+	
3.0 + 0.5	25	30	Friable	LG	++	

**B-Brown, LG-Light green, G-Green; ++: Trace callus,+++: Moderate callus,++++: Massive callus**

The induction of callus involves a dramatic transformation in the appearance and metabolic activity of cells [18]. This process results in the disorganization of cultured cells and is believed to stem from the disruption of intercellular physical and chemical communication [19]. Somaclonal variation represents a valuable source of genetic diversity for crop improvement, potentially offering traits like disease resistance, improved quality, and increased yield [14, 20]. Numerous researchers have been working to optimize the concentrations of growth regulators for potato

regeneration, leading to significant progress in potato callus induction and plant regeneration [21-25].

Auxin 2, 4-D, either alone or in combination with cytokinins, has been widely employed to enhance callus induction and maintenance [26]. 2, 4-D is found suitable for callus induction from internode and leaf explants of four potato cultivars, including Diamant, among all concentrations and combinations, 2,4-D at 3.0 mg/l was the most effective auxin concentration for callus induction across all cultivars [24]. Internode segments exhibited a

greater potential for callus induction and plant regeneration than leaf explants. The most favorable callusing response for both explant types was observed when the MS medium was supplemented with 2, 4-D<sup>[24]</sup>.

**3.2 Shoot Regeneration and Multiplication from Callus**

The optimal medium for shoot regeneration was found to be one supplemented with 2.0 mg/l KIN (Kinitin) and 0.5 mg/l NAA (Naphthalene Acetic Acid). Under these conditions, Diamant displayed an impressive 85% shoot regeneration from callus, yielding an average of 6.2 shoots per callus. Cardinal also exhibited substantial regeneration, with a rate of 78% and an average of 5.5 shoots per callus. These shoots reached lengths of

8.4 cm in Diamant and 8.6 cm in Cardinal, each carrying an average of 13.4 and 13.0 leaves, respectively (Table 3, Fig 1, 2). The necessity of cytokinins for shoot initiation has been well-documented in the literature<sup>[27, 28]</sup>. In this study, shoot regeneration was observed within two weeks from calli cultured on an MS medium containing 0.1-1.0 mg/l IAA (indole-3-acetic acid) and 2-10 mg/l BAP (benzylaminopurine)<sup>[29]</sup>. Plantlets were successfully regenerated from stem and shoot tip-derived callus by utilizing kinitin and IAA in an MS medium<sup>[30]</sup>. Regeneration of plantlet from shoot segments was achieved for four potato cultivars, with the highest number of plantlet formations recorded on an MS medium supplemented with kinitin and NAA<sup>[31]</sup>.

**Table 3:** Effect of auxin and cytokinin on shoot regeneration & multiplication from callus of Diamant and Cardinal. Data were recorded up to 28 days of culture

Cultivar	Growth Regulators mg/l	Days to shoot initiation	Percent (%) of callus with shoot	No. of shoot / callus	Shoot length (cm)	No. of leaves /shoot
Diamant	<b>BAP</b>					
	2.5	35	45	2.2±0.27 <sup>a</sup>	2.8±0.11 <sup>a</sup>	5.0±0.31 <sup>a</sup>
	3.0	25	50	3.2±0.37 <sup>b</sup>	3.4±0.12 <sup>a</sup>	7.0±0.31 <sup>b</sup>
	<b>KIN</b>					
	3.0	30	58	3.4±0.24 <sup>b</sup>	3.5±0.11 <sup>a</sup>	6.8±0.24 <sup>a</sup>
	4.0	25	66	3.8±0.37 <sup>b</sup>	4.0±0.12 <sup>b</sup>	7.5±0.31 <sup>b</sup>
	<b>BAP + NAA</b>					
	2.0 + 0.5	25	70	5.4±0.40 <sup>d</sup>	7.2±0.09 <sup>e</sup>	12.0±0.71 <sup>e</sup>
	3.0 + 1.0	45	45	2.0±0.31 <sup>a</sup>	3.0±0.23 <sup>a</sup>	6.2±0.37 <sup>a</sup>
	<b>KIN + NAA</b>					
	2.0 + 0.5	20	85	6.2±0.31 <sup>e</sup>	8.4±0.07 <sup>f</sup>	13.4±0.68 <sup>e</sup>
	2.0 + 1.0	28	60	4.6±0.50 <sup>c</sup>	7.5±0.16 <sup>e</sup>	10.8±0.58 <sup>c</sup>
	<b>BAP + IAA</b>					
	2.0 + 1.0	30	45	3.4±0.24 <sup>b</sup>	4.5±0.12 <sup>b</sup>	6.0±0.51 <sup>a</sup>
	2.5 + 0.5	30	50	3.8±0.40 <sup>b</sup>	5.2±0.09 <sup>c</sup>	7.2±0.71 <sup>b</sup>
<b>KIN + IAA</b>						
2.5 + 0.5	22	60	4.0±0.50 <sup>c</sup>	6.4±0.16 <sup>d</sup>	8.4±0.58 <sup>c</sup>	
2.5 + 1.0	25	50	3.6±0.31 <sup>b</sup>	5.7±0.07 <sup>c</sup>	7.8±0.68 <sup>b</sup>	
Cardinal	<b>BAP</b>					
	2.5	38	48	2.0±0.27 <sup>a</sup>	2.5±0.11 <sup>a</sup>	5.0±0.31 <sup>a</sup>
	3.0	28	50	3.0±0.37 <sup>b</sup>	3.2±0.12 <sup>a</sup>	6.6±0.31 <sup>a</sup>
	<b>KIN</b>					
	3.0	37	60	3.2±0.24 <sup>b</sup>	3.3±0.11 <sup>a</sup>	6.2±0.24 <sup>a</sup>
	4.0	28	62	3.5±0.37 <sup>b</sup>	4.0±0.12 <sup>b</sup>	7.0±0.31 <sup>b</sup>
	<b>BAP + NAA</b>					
	1.0 + 1.5	26	68	4.6±0.40 <sup>c</sup>	7.0±0.09 <sup>d</sup>	11.0±0.71 <sup>d</sup>
	2.0 + 1.0	35	65	4.2±0.24 <sup>c</sup>	6.4±0.12 <sup>d</sup>	9.0±0.51 <sup>c</sup>
	<b>KIN + NAA</b>					
	2.5 + 0.5	22	78	5.5±0.31 <sup>d</sup>	8.6±0.07 <sup>f</sup>	12.6±0.68 <sup>e</sup>
	3.0 + 1.0	30	70	4.2±0.50 <sup>c</sup>	7.5±0.16 <sup>e</sup>	11.1±0.58 <sup>d</sup>
	<b>BAP + IAA</b>					
	2.0 + 1.0	32	48	4.0±0.24 <sup>c</sup>	5.3±0.12 <sup>c</sup>	7.5±0.51 <sup>b</sup>
	2.5 + 0.5	28	55	4.0±0.40 <sup>c</sup>	7.0±0.09 <sup>d</sup>	8.4±0.71 <sup>c</sup>
<b>KIN + IAA</b>						
2.5 + 0.5	30	60	4.2±0.50 <sup>c</sup>	7.5±0.16 <sup>e</sup>	10.4±0.58 <sup>d</sup>	
2.5 + 1.0	30	55	4.0±0.31 <sup>c</sup>	7.0±0.07 <sup>d</sup>	9.4±0.68 <sup>c</sup>	

Mean ± SE (standard error) values with similar letter in the same column denotes no significant differences at p > 0.05



**Fig 1:** Initiation of plantlets from de-differentiated tissues



**Fig 2:** Multiplication callus regenerated plantlets

### 3.3 Field Assessment of *In vitro* Raised Parental Clones (control) and Somaclones for Growth and Yield

In the case of plant height, somaclones exhibited an average height

of 56.4 cm for Diamant and 50.0 cm for Cardinal, while parental clones (control) reached heights of 49.6 cm for Diamant and 44.2 cm for Cardinal after 60 days of planting (Table 4, 5 and Fig 3, 4).

**Table 4:** Various parameters of meristem-derived plants of parental clone (control) of cultivars Diamant and Cardinal. Data for shoot length and the number of branches were recorded after 70 days of planting, while other measurements were taken after 90 days of planting

Control Lines	Stem height (cm)	No. of Branches per plant	No. of mini-tuber per plant	Mean Diameter of mini-tuber (mm)	Mean Weight of mini-tuber per plant (g)
<b>Cultivar Diamant</b>					
D <sub>1</sub>	42.5 <sup>b</sup>	1.7 <sup>a</sup>	15.6±1.45 <sup>b</sup>	30.0±0.33 <sup>a</sup>	146.6±1.20 <sup>b</sup>
D <sub>2</sub>	45.6 <sup>c</sup>	2.1 <sup>b</sup>	12.0±1.15 <sup>a</sup>	35.0±0.33 <sup>b</sup>	145.0±1.56 <sup>b</sup>
D <sub>3</sub>	40.2 <sup>a</sup>	1.6 <sup>a</sup>	13.0±0.57 <sup>a</sup>	38.0±0.13 <sup>b</sup>	156.1±1.16 <sup>c</sup>
D <sub>4</sub>	45.6 <sup>c</sup>	1.9 <sup>b</sup>	15.3±0.88 <sup>b</sup>	33.0±0.57 <sup>a</sup>	135.2±1.29 <sup>a</sup>
D <sub>5</sub>	41.5 <sup>b</sup>	1.8 <sup>a</sup>	18.0±0.57 <sup>d</sup>	39.3±0.57 <sup>c</sup>	160.3±0.88 <sup>c</sup>
D <sub>6</sub>	49.6 <sup>d</sup>	2.3 <sup>c</sup>	18.5±0.57 <sup>d</sup>	40.1±0.57 <sup>c</sup>	169.6±0.88 <sup>c</sup>
D <sub>7</sub>	43.5 <sup>b</sup>	2.0 <sup>b</sup>	18.2±0.53 <sup>d</sup>	40.0±0.18 <sup>c</sup>	164.8±1.91 <sup>d</sup>
D <sub>8</sub>	38.3 <sup>a</sup>	2.0 <sup>b</sup>	16.3±0.88 <sup>c</sup>	38.0±0.57 <sup>b</sup>	148.2±0.41 <sup>b</sup>
D <sub>9</sub>	44.4 <sup>b</sup>	2.1 <sup>b</sup>	17.0±0.57 <sup>c</sup>	30.0±0.33 <sup>a</sup>	140.7±1.17 <sup>a</sup>
D <sub>10</sub>	47.2 <sup>c</sup>	2.1 <sup>b</sup>	18.0±1.54 <sup>d</sup>	39.0±0.57 <sup>c</sup>	165.8±0.44 <sup>d</sup>
<b>Cultivar Cardinal</b>					
C <sub>1</sub>	40.5 <sup>c</sup>	1.1 <sup>b</sup>	10.0±0.57 <sup>b</sup>	36.0±0.33 <sup>b</sup>	142.3±0.88 <sup>c</sup>
C <sub>2</sub>	38.4 <sup>b</sup>	1.0 <sup>a</sup>	9.3±0.33 <sup>a</sup>	20.0±0.10 <sup>a</sup>	131.0±0.57 <sup>b</sup>
C <sub>3</sub>	44.1 <sup>d</sup>	2.0 <sup>c</sup>	14.9±0.21 <sup>d</sup>	45.4±0.33 <sup>d</sup>	159.2±0.88 <sup>d</sup>
C <sub>4</sub>	43.4 <sup>c</sup>	2.0 <sup>c</sup>	12.1±0.57 <sup>c</sup>	43.0±0.15 <sup>d</sup>	154.1±1.51 <sup>d</sup>
C <sub>5</sub>	42.5 <sup>c</sup>	2.0 <sup>c</sup>	11.0±0.57 <sup>b</sup>	40.0±0.33 <sup>c</sup>	144.8±0.72 <sup>c</sup>
C <sub>6</sub>	42.2 <sup>c</sup>	1.9 <sup>c</sup>	11.4±0.88 <sup>b</sup>	38.0±0.33 <sup>c</sup>	141.6±0.88 <sup>c</sup>
C <sub>7</sub>	36.2 <sup>b</sup>	1.0 <sup>a</sup>	10.6±0.66 <sup>b</sup>	34.0±0.33 <sup>b</sup>	129.0±0.57 <sup>b</sup>
C <sub>8</sub>	32.6 <sup>a</sup>	1.0 <sup>a</sup>	8.0±0.57 <sup>a</sup>	30.0±0.33 <sup>b</sup>	122.6±0.88 <sup>a</sup>
C <sub>9</sub>	28.6 <sup>a</sup>	0.8 <sup>a</sup>	7.6±0.33 <sup>a</sup>	26.0±0.33 <sup>a</sup>	113.0±0.57 <sup>a</sup>
C <sub>10</sub>	41.3 <sup>c</sup>	2.0 <sup>c</sup>	13.0±0.57 <sup>c</sup>	43.0±0.33 <sup>d</sup>	150.6±0.88 <sup>c</sup>

Mean ± SE (standard error) values with similar letter in the same column denotes no significant differences at  $p > 0.05$

**Table 5:** Various parameter of Callus derived plants (Somaclone) of cultivar Diamant and Cardinal. Data for shoot length and the number of branches were recorded after 70 days of planting, while other measurements were taken after 90 days of planting

Somaclone Lines	Stem height (cm)	No. of Branches	No. of mini-tuber per plant	Mean Diameter of mini-tuber (mm)	Mean Weight of mini-tuber per plant (g)
<b>Cultivar Diamant</b>					
SD <sub>1</sub>	52.5 <sup>b</sup>	3.5 <sup>a</sup>	29.0±0.57 <sup>a</sup>	56.0±0.31 <sup>b</sup>	300.0±0.57 <sup>b</sup>
SD <sub>2</sub>	43.4 <sup>a</sup>	3.3 <sup>a</sup>	25.6±0.88 <sup>a</sup>	53.0±0.66 <sup>a</sup>	255.3±0.33 <sup>a</sup>
SD <sub>3</sub>	50.2 <sup>b</sup>	3.7 <sup>b</sup>	31.0±0.58 <sup>b</sup>	53.0±0.33 <sup>a</sup>	314.3±0.33 <sup>c</sup>
SD <sub>4</sub>	50.6 <sup>b</sup>	3.8 <sup>b</sup>	29.3±0.33 <sup>a</sup>	59.0±0.31 <sup>c</sup>	301.1±0.67 <sup>b</sup>
SD <sub>5</sub>	51.5 <sup>b</sup>	4.0 <sup>c</sup>	31.6±0.88 <sup>b</sup>	56.0±0.33 <sup>b</sup>	320.8±0.59 <sup>c</sup>
SD <sub>6</sub>	55.2 <sup>c</sup>	4.2 <sup>c</sup>	32.1±0.58 <sup>b</sup>	60.0±0.33 <sup>c</sup>	325.0±0.57 <sup>c</sup>
SD <sub>7</sub>	47.1 <sup>a</sup>	3.7 <sup>b</sup>	30.3±0.33 <sup>b</sup>	58.0±0.33 <sup>b</sup>	305.0±0.58 <sup>b</sup>
SD <sub>8</sub>	55.3 <sup>c</sup>	4.3 <sup>c</sup>	32.3±0.33 <sup>b</sup>	60.0±0.31 <sup>c</sup>	332.0±0.29 <sup>c</sup>
SD <sub>9</sub>	56.3 <sup>c</sup>	4.5 <sup>d</sup>	33.6±0.57 <sup>c</sup>	63.0±0.31 <sup>d</sup>	361.0±1.21 <sup>d</sup>
SD <sub>10</sub>	49.3 <sup>b</sup>	3.6 <sup>a</sup>	31.0±0.93 <sup>b</sup>	60.0 ±0.45 <sup>c</sup>	331.8±1.21 <sup>c</sup>
<b>Cultivar Cardinal</b>					
SC <sub>1</sub>	40.5 <sup>a</sup>	2.3 <sup>a</sup>	23.4±0.57 <sup>a</sup>	55.0±0.31 <sup>a</sup>	240.0±0.57 <sup>b</sup>
SC <sub>2</sub>	45.4 <sup>b</sup>	2.5 <sup>a</sup>	21.6±0.88 <sup>a</sup>	58.0±0.66 <sup>a</sup>	222.3±0.33 <sup>a</sup>
SC <sub>3</sub>	47.1 <sup>c</sup>	3.1 <sup>b</sup>	26.5±0.58 <sup>b</sup>	56.0±0.33 <sup>a</sup>	278.3±0.33 <sup>d</sup>
SC <sub>4</sub>	41.6 <sup>a</sup>	2.4 <sup>a</sup>	24.3±0.33 <sup>b</sup>	60.0±0.31 <sup>b</sup>	254.1±0.67 <sup>b</sup>
SC <sub>5</sub>	43.5 <sup>b</sup>	3.1 <sup>b</sup>	26.2±0.88 <sup>b</sup>	62.0±0.33 <sup>b</sup>	257.8±0.59 <sup>c</sup>
SC <sub>6</sub>	44.2 <sup>b</sup>	3.3 <sup>b</sup>	27.1±0.58 <sup>c</sup>	61.0±0.33 <sup>b</sup>	270.0±0.57 <sup>c</sup>
SC <sub>7</sub>	49.0 <sup>c</sup>	3.7 <sup>c</sup>	29.1±0.33 <sup>c</sup>	67.0±0.33 <sup>c</sup>	298.0±0.58 <sup>e</sup>
SC <sub>8</sub>	47.3 <sup>c</sup>	3.4 <sup>c</sup>	28.0±0.33 <sup>c</sup>	65.0±0.31 <sup>c</sup>	284.0±0.29 <sup>d</sup>
SC <sub>9</sub>	42.6 <sup>a</sup>	3.0 <sup>b</sup>	25.0±0.67 <sup>b</sup>	63.0±0.21 <sup>b</sup>	260.0±0.57 <sup>c</sup>
SC <sub>10</sub>	50.0 <sup>d</sup>	3.9 <sup>d</sup>	30.2±0.33 <sup>d</sup>	68.0±0.21 <sup>d</sup>	300.0±0.88 <sup>e</sup>

Mean ± SE (standard error) values with similar letter in the same column denotes no significant differences at  $p > 0.05$



**Fig 3:** *In vitro* raised parental clone and somaclone in the screen house after 7 days of transfer



**Fig 4:** Potato plants in screen house after 60 days



**Fig 5:** Harvest of minituber after 90 days (Diamant)



**Fig 6:** Harvest of minituber after 90 days (Cardinal).

The weight of mini-tubers per plant was notably higher in somaclones, with 361.0 gm for Diamant and 300.0 gm for Cardinal, as opposed to 169.6 gm for Diamant and 159.3 gm for Cardinal in parental clones. Similarly, somaclones outperformed parental clones in terms of the number of tubers per plant, recording 33.6 tubers for Diamant and 30.2 tubers for Cardinal, while parental clones produced 18.6 tubers for Diamant and 14.9 tubers for Cardinal. Mini-tubers for cv. Monalisa was successfully generated and compared their yield potential to that of normal seed tubers in a field experiment, achieving mini-tubers ranging from 355 g to 380 g per plant in different varieties [32-34].

#### 4. Conclusion

Potato (*Solanum tuberosum* L.) is a vital non-cereal crop, playing a crucial role in global food security. In densely populated countries like Bangladesh, where arable land is under increasing pressure due to urbanization and industrialization, achieving efficient potato production is essential. Potato exhibits a higher yield potential compared to other major crops in Bangladesh. However, there is room for improvement to meet growing demands. Biotechnology, specifically somaclonal variation through tissue culture, provides a promising avenue for enhancing potato cultivars.

This study focuses on two popular potato cultivars, Diamant and Cardinal, and explores the application of *in vitro* culture of dedifferentiated tissue for somaclonal variation. The research encompasses callus induction, plant regeneration from callus, and field evaluations of regenerated plants. The morphological traits of somaclones, such as plant height, leaf number, branch number, tuber number, and total tuber weight per plant, are evaluated.

The results indicate successful callus induction and shoot regeneration, with notable variation between Diamant and Cardinal. Somaclonal variants exhibit enhanced traits in the first generation compared to parental clones, showing potential for yield improvement. These findings underscore the significance of somaclonal variation in potato breeding programs, providing a valuable tool for crop improvement. The study presents a comprehensive examination of the use of biotechnology to enhance potato cultivars, offering insights into addressing food security challenges, especially in densely populated region.

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