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RESEARCH PAPER

Relationship between Induction of Novel Somaclonal Variants and Types of Organogenesis in Muskmelon (*Cucumis melo* L.)

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العلاقة بين استقراء المتغيرات الجسدية الجديدة و أنواع الأعضاء في الشمام (*Cucumis melo* L.)

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Abstract. A comparative study on induction of somaclonal variation in muskmelon (*Cucumis melo* L.) cv. Birdie regenerants obtained through direct and indirect organogenesis was carried out. Two types of non-meristematic explants (e.g. cotyledon and petiole) were used for this study. A significantly lower ($p<0.05$) frequency of variation was observed in muskmelon somaclones regenerated via direct organogenesis (MS medium with BAP) compared to indirect (MS medium with BAP and 2,4-D). Morphological study revealed that the somaclones regenerated from proximal cotyledon, petiole and distal cotyledon explants through direct organogenesis did not show any variation in elongation medium at the concentrations of BAP 0.1, 0.3 and 0.5 mg^{-1}, respectively. In contrast, higher number of morphologically somaclonal variants was obtained from these explants at the same concentration of BAP obtained through indirect organogenesis. Other concentrations of BAP, on the other hand, added to the elongation medium showed higher percentage of somaclones with different types of novel variations, e.g. early flowering including higher number of flowers, slow growth of shoots with variant shape of leaves having long and thick petioles, and stubby shoot apices including flattened stem. These variations could be the prime genetic materials to develop new varieties of muskmelon, e.g. high yielding variety, early, late variety, dwarf variety, and variety with desirable body configurations. The results suggest that specific concentrations of BAP or combinations of BAP and 2,4-D have a significant ($p<0.05$) influence on the induction of novel somaclonal variations in muskmelon regenerants. Future cytogenetic and molecular studies reveal that the novel genetic variations at the chromosome level in somaclonal variants can exist.

Keywords: Muskmelon, novel somaclones, direct and indirect organogenesis, variety development.

الملخص: دراسة مقارنة حول تحريض التباين الجسدي في الشمام (Cucumis melo L.). تم إجراء تجديدات الطيور التي تم الحصول عليها من خلال تكوين الأعضاء المباشرة وغير المباشرة. تم استخدام نوعين من النباتات المستأصلة غير الإنشائية (مثل الفلقة والسويقات) لهذه الدراسة. لوحظ تواتر أقل بشكل ملحوظ ($p<0.05$) في التغير somaclones الشمام المتجدد عن طريق تكوين الأعضاء المباشر (وسط MS مع BAP) مقارنة بالغير مباشر (وسط MS مع BAP و 2،4-D). كشفت الدراسة المورفولوجية أن somaclones التي تم تجديدها من نباتات الفلقات القريبة ، والنباتات السويقية ، والنباتات البعيدة من خلال تكوين الأعضاء المباشر لم تظهر أي اختلاف في وسط الاستطالة بتركيزات BAP 0.1 و 0.3 و 0.5 ملغم / لتر على التوالي. في المقابل ، تم الحصول على عدد أكبر من المتغيرات الجسدية النسيلة شكليًا من هذه النباتات المستأصلة بنفس تركيز BAP الذي تم الحصول عليه من خلال تكوين الأعضاء غير المباشر. من ناحية أخرى، أظهرت تركيزات أخرى من BAP المضافة إلى وسط الاستطالة نسبة مئوية أعلى من somaclones مع أنواع مختلفة من الاختلافات الجديدة، على سبيل المثال الإزهار المبكر يشمل عددًا أكبر من الأزهار ، نمو بطيء للبراعم ذات أشكال مختلفة من الأوراق ذات أعناق طويلة وسميكة ، ومقرمشات قصيرة صلبة بما في ذلك الساق المسطحة. يمكن أن تكون هذه الاختلافات هي المواد الجينية الأساسية لتطوير أنواع جديدة من الشمام ، على سبيل المثال تنوع عالي الغلة ، تنوع مبكر ، متأخر ، تنوع قزم ، ومتنوع مع تكوينات الجسم المرغوبة. تشير النتائج إلى أن تركيزات معينة من BAP أو مجموعات من BAP و 2،4-D لها تأثير كبير ($p<0.05$) على تحريض الاختلافات الجسدية الجديدة في تجديدات الشمام. تكشف الدراسات الوراثية الخلوية والجزيئية المستقبلية أن الاختلافات الجينية الجديدة على مستوى الكروموسوم في المتغيرات الجسدية يمكن أن توجد.

الكلمات المفتاحية: الشمام، somaclones ، تكوين الأعضاء المباشر وغير المباشر، تنمية الأصناف.

Introduction

Muskmelon (*Cucumis melo* L.) species is a valued agricultural crop and widely cultivated in Asia, America and European countries (Al Mawaali et al., 2017). However, it is highly prone to a wide range of diseases, which can result reduced yield and quality (Jelaska, 1986). Therefore, availability of useful alternative agronomically important traits will be more useful for speedy development of desired variety as compared to conventional breeding for muskmelon improvement (Pijnacker and Ramulu, 1990, Mohiuddin et. al., 1998). The *in vitro*-generated variation and induction of somaclonal variation can improve the genetic diversity and may also amplify the pre-existing genetic heterogeneity in somatic cells, thus facilitating the overall breeding and improvement of the economically im-

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portant plants (Patnaik et al., 1999). *In vitro* technique can also be used to induce economically useful agronomic characteristics into crop to increase productivity without loss of available desired genetic characters (Mohiuddin, 1998). The ability to produce morphologically normal plants at high frequency with little or no somaclonal variation as well as induction of high frequency of variations in somaclones is a prerequisite to the application of tissue culture techniques in crop improvement (Mohiuddin et al., 2000, & 2003, Ren et al., 2012).

Application of *in vitro* tissue culture techniques to crop improvement has been focused on selection for herbicide resistance, stress tolerance, amino acid over production, rice fortification having Fe and Zn, disease resistance, etc. from somaclonal variants (Larkin and Scowcroft, 1981; Evenor et al., 1994; Al-Noor et al., 2019). It has been known over the five decades that cultured plant cell and tissue undergo various morphological and genetic changes especially in chromosome numbers and ploidy level during *in vitro* culture (Murashige and Nakano, 1966, Ren et al., 2013). This change is also known as somaclonal variation displayed among *in vitro* tissue culture-derived plants and it has been described for several plant species (Larkin and Scowcroft, 1981; Orton 1984; Bajaj, 1990; Mofidabadi et al., 2001; Shasthree et al., 2009; Saraswat and Kumar, 2019).

Considerable work in muskmelon tissue culture has been conducted (Moreno et al., 1985; Trulson and Shahin, 1986; Niedz et al., 1989; Oridate et al., 1992; Gray et al., 1993; Mohiuddin et al., 1998, and Ren et al., 2013) but to our best knowledge negligible report has been evaluated yet on relationship in production of morphologically normal somaclones (true-to-type) at high frequency through direct organogenesis as well as induction of novel somaclonal variants in muskmelon plants via indirect organogenesis. This present study was aimed to investigate the extent of true-to-type somaclone production (no variation) and induction of novel somaclonal variants as observed phenotypically in muskmelon regenerants and obtained through direct and indirect organogenesis.

Materials and Methods

Plant Materials

This study focused on the Birdie cultivar of muskmelon, which is the most widely used in Malaysia as a fruit crop. Testas were removed manually from mature seeds of Birdie (Sakata Seed Corp., Japan) and sterilized by 20% Clorox (containing 5.25% sodium hypochlorite) with one drop of 'Tween 20' for 15 min followed by 3X wash with sterile distilled water. The seeds were then aseptically germinated on filter paper soaked with MS (Murashige and Skoog, 1962) liquid medium in Petri dishes having 3% sucrose. After eight days cotyledons were separated from germinated seedlings and cut into four pieces by a transverse cut followed by a longitudinal cut. The proximal cotyledons (attached to hypocotyls) and distal cotyledons were used for this study. The second-top-leaf petiole was used as explant, obtained from 21-day-old muskmelon seedlings germinated in MS solid medium in Magenta boxes having 2% phytagel (Sigma, USA). Each petiole was bisected into equal pieces (4-5 mm long) and it was used in this study.

Somaclone Induction and Elongation Medium

Both cotyledon and petiole explants were cultured aseptically on somaclone induction medium (SIM) consisting of MS nutrients, MS vitamins, 3% sucrose, 2 g^{-1} phytagel, and BAP at the concentration of 1.0 and 2.0 mg^{-1} either alone (direct organogenesis) or in combination with 2,4-D at 0.1 and 0.3 mg^{-1} (indirect organogenesis). Ten explants of each type were cultured on SIM containing each concentrations of BAP alone or in combinations with 0.1 and 0.3 mg^{-1} 2,4-D. This was repeated 5 (five) times. Subsequently, 15-day-old somaclones as obtained from direct and indirect organogenesis and these were then cultured onto somaclone elongation medium (SEM) containing MS medium supplemented with BAP at 0.07, 0.1, 0.3, 0.5 and 0.7 mg^{-1}.

Root Induction Medium and Culture Conditions

The elongated somaclones derived from proximal cotyledons, distal cotyledons and petioles were rooted in MS medium containing NAA at the concentrations of 0.01, 0.05 and 0.03 mg^{-1}, respectively (Mohiuddin, 1998). All the media combinations were adjusted to pH 5.7 before autoclaving at 121°C for 15 minutes at 1.05 kg/cm^2 pressure (15-20 psi). Cultures were incubated in the growth chamber at 26±1°C under 16/8 h light (39.3 µmol m^{-2} s^{-1})/dark regime.

Evaluations of Somaclonal Variants

The extents of variations were evaluated morphologically on the somaclones after elongation in SEM for four weeks at each concentration of BAP as mentioned earlier. For the identification of morphologically true-to-type somaclones (no variation) and variants (having variation) obtained from each explant of muskmelon at each formulation of hormone(s) were closely examined. Variations in somaclones were scored of the following quantitative traits: (i) early flowering including higher number of flowers, (ii) slow growth of shoots with deformed shape of leaves having long and thick petiole, and (iii) stubby shoot apices with flattened stem. On the other hand, normal somaclones were scored of the following quantitative traits: (i) normal organ development, (ii) rapid growth, (iii) normal flower formation and fruit development, (iv) good root induction ability and (v) ability to acclimatize to the ambient environmental conditions.

Statistical Analysis

The data were statistically analysed by analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also carried out to show the significance of relationship among the percentage result (mean) of somaclones either morphologically normal or variant obtained through the direct or indirect organogenesis.

Results

Huge numbers of somaclone primordia were initiated from muskmelon explants cultured on somaclone induction medium (Figure 1a). Somaclones induced from proximal and distal cotyledons and petiole explants of muskmelon via direct organogenesis, elongated faster in elongation medium compared to the somaclones as obtained from identical explants via indirect organogenesis. Moreover, proximal cotyledon-derived somaclones elongated earlier than somaclones derived from either distal cotyledon or petiole explants. The distal cotyledon derived somaclones obtained from both direct and indirect organogenesis, however, elongated slower as compared to other two explant-derived somaclones.

The proportion of morphologically normal (true-to-type) and variant somaclones induced from different explants varied at identical concentration of BAP in elongation medium (Tables 1 and 2). Similarly, the proportion of normal and variant somaclones induced through either direct or indirect organogenesis from different explants also varied to identical BAP concentration (Tables 1 and 2).

BAP at the concentration of 0.1, 0.3 and 0.5 mg^{-l} significantly ($p<0.05$) induced the highest rates (100%) of morphologically normal somaclones (Figure 1b) through direct organogenesis from proximal cotyledon, petiole and distal cotyledon, respectively (Table 1). Other concentrations of BAP also induced normal somaclones but at lower rates. SEM having 0.007 and 0.7 mg^{-l} BAP induced the lowest rates of normal somaclones from all explants (Table 1). On the other hand, the rates

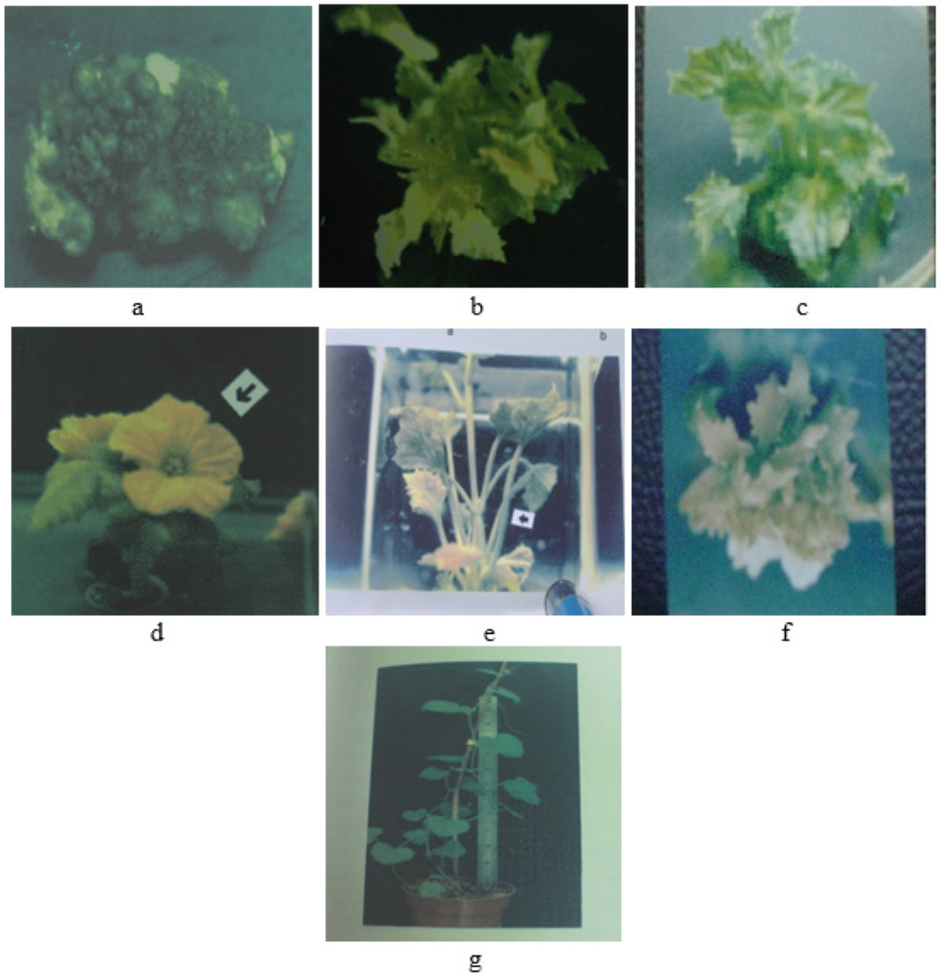

Figure 1. Somaclone and its variation. a) Somaclone primordia initiated from proximal cotyledon, b) Shoots elongated in shoot elongation medium, c) Morphologically normal somaclones, d) Early flowering obtained in somaclones, e) Slow growth of shoots with distorted leaves having long and thick petioles, f) Stubby shoot apices and g) Successfully acclimatized somaclones.

Table 1. Variations on production of morphologically normal somaclones of muskmelon obtained through direct and indirect organogenesis

BAP (mg/l)	Production of precent (%) morphological normal somaclones					
	Direct organogenesis			Indirect organogenesis		
	Proximal cotyledon	Distal cotyledon	Petiole	Proximal cotyledon	Distal cotyledon	Petiole
0.07	93±2.7[a]	73±5.2[b]	85±4.7[ab]	74±1.6 [a]	80±8.1[a]	67±6.6[a]
0.1	100±0.0[a]	79±4.0[b]	91±2.3[a]	71±4.3 [a]	63±5.6[a]	64±1.7[a]
0.3	84±3.4[b]	86±2.1[a]	100±00[a]	62±3.7 [a]	75±3.9[a]	72±2.9[a]
0.5	82±3.0[b]	100±0.0[a]	86±2.8[ab]	68±0.8 [a]	76±4.3[a]	62±6.5[a]
0.7	71±3.4[c]	80±6.3[b]	80±5.9[b]	62±6.5 [a]	74±4.5[a]	57±4.9[a]

Note: Percentage results are means of five replications±Standard Error. Means with the same letters (superscript) within same columns are not significantly different ($p<0.05$)

of morphologically normal somaclones obtained via indirect organogenesis were generally lower as compared to the direct organogenesis (Table 1). No significant difference was observed among the rates of normal somaclones induced through indirect organogenesis from proximal cotyledon, distal cotyledon and petiole explants at the different concentrations of BAP (Table 1).

From the result in this study, it was found that the direct organogenesis at specific concentrations of BAP 0.1, 0.3 and 0.5 mg^{-1} did not produce any somaclonal variants morphologically in proximal cotyledon (Figure 1c), petiole and distal cotyledon of muskmelon, respectively (Table 2). However, other concentrations of BAP induced somaclonal variants from all three explants of muskmelon. MS medium containing 0.7 mg^{-1} BAP induced a significantly higher ($p<0.05$) rate of somaclonal variants from proximal cotyledon (29%) and petiole explants (20%), while MS medium having BAP 0.07 mg^{-1} induced the highest rate of somaclonal variants from distal cotyledon explants (27%) through direct organogenesis (Table 2). The rates of morphologically variant somaclones, on the other hand, obtained through indirect organogenesis were higher compared to that of direct organogenesis (Table 2). About 2 to 4 folds higher somaclonal variants were found in indirect organogenesis as compared to direct organogenesis (Table 2). No significant effect was observed among the different rates of somaclonal variants induced from explants at different concentrations of BAP through indirect organogenesis (Table 2).

The morphologically normal somaclones obtained from the three different explants via direct organogenesis were longer height at different concentrations of BAP than those obtained through indirect organogenesis (Table 3). BAP at 0.1, 0.3 and 0.5 mg^{-1} produced normal somaclones from proximal cotyledon, distal cotyledon and petiole through direct organogenesis, which attained lengths of 9.2 cm, 7.9 cm and 9.1 cm, respectively. However, at these BAP concentrations, normal somaclones were 6.4, 5.1 and 4.7 cm in length from respective explants obtained through indirect organogenesis which is considerable lower (Table 3). Furthermore, lower height was observed in the somaclonal variants obtained from all three explants through direct and indirect organogenesis (Table 4). On average, lowest height attained from

Table 2. Variations on production of novel somaclonal variants of muskmelon attained through direct and indirect organogenesis

BAP (mg/l)	Production of precent (%) of somaclonal variants					
	Direct organogenesis			Indirect organogenesis		
	Proximal cotyledon	Distal cotyledon	Petiole	Proximal cotyledon	Distal cotyledon	Petiole
0.07	7±4.7 [b]	27±3.3 [a]	15±5.4 [b]	26±1.0 [a]	20±8.4 [a]	33±4.2 [a]
0.1	0.0±0.0 [c]	21±2.8 [a]	9±3.8 [b]	29±2.8 [a]	37±3.4 [a]	36±1.0 [a]
0.3	16±2.6 [a]	14±3.6 [a]	0.0±0.0 [c]	38±2.2 [a]	25±2.4 [a]	28±2.0 [a]
0.5	18±2.3 [a]	0.0±0.0 [b]	14±2.1 [b]	32±0.5 [a]	24±3.0 [a]	38±3.9 [a]
0.7	29±2.2 [a]	20±6.4 [a]	20±6.5 [a]	38±3.9 [a]	26±2.9 [a]	43±2.9 [a]

Note: Percentage results are means of five replications±Standard Error. Means with the same letters (superscript) within columns are not significantly different ($p<0.05$).

Table 3. Variations on Average Height of Normal Somaclones obtained through Direct and Indirect Organogenesis

BAP (mg/l)	Average height of morphological normal somaclones (in cm)					
	Direct organogenesis			Indirect organogenesis		
	Proximal cotyledon	Distal cotyledon	Petiole	Proximal cotyledon	Distal cotyledon	Petiole
0.07	8.6±0.1	7.2±0.3	8.7±0.2	5.8±0.2	4.8±0.1	4.6±0.1
0.1	9.2±0.1	6.6±0.3	8.8±0.2	6.4±0.3	4.9±0.1	4.8±0.1
0.3	8.9±0.2	7.9±0.2	7.8±0.2	6.8±0.2	5.1±0.1	4.9±0.1
0.5	8.7±0.1	7.8±0.2	9.1±0.1	6.2±0.2	5.6±0.1	4.7±0.1
0.7	6.6±0.3	7.6±0.2	7.2±0.4	6.4±0.2	5.3±0.1	4.7±0.1

Note: Average results are means of five replications±Standard Error.

somaclonal variants regenerated from explants through indirect organogenesis in comparison to direct (Table 4).

Early flowering (EF) was the most common novel variants observed in muskmelon somaclones (Figure 1d) with higher rates attained frequently from all explants through direct and indirect organogenesis (Tables 5 and 6). Maximum percent of EF (e.g. 14% and 19%) was obtained at 0.7 mg^{-1} BAP from proximal cotyledon explants through direct and indirect organogenesis, respectively. On the other hand, regenerated novel variants (e.g. slow growth, SG) of shoots with distorted leaves having long and thick petioles (Fig. 1e) and stubby shoot apices (SSA) with flatted stem (Figure 1f) was found comparatively lower in number than EF via both direct and indirect organogenesis (Tables 5 and 6).

The rate of somaclonal variants obtained from proximal cotyledon, distal cotyledon and petiole explants ranged from 4 to 14% of EF, 2 to 9% of SG with variant shape of leaves and 2 to 6% of SSA with flattened stem via direct organogenesis (Table 5). However, these novel variants ranges increased when plants were regenerated via indirect organogenesis (Table 6).

No variations were observed in the somaclones obtained through direct organogenesis from proximal cotyledon, petiole and distal cotyledon explants at BAP 0.1, 0.3 and 0.5 mg^{-1}, respectively (Table 5). However, proximal cotyledon at BAP 0.1 mg^{-1} induced 14%, 11% and 4% of EF, SG and SSA novel variants, respectively through indirect organogenesis (Table 6). Similarly, petiole explants also induced above mentioned novel variants (e.g. 13%, 9% and 6%) in same order at 0.3 mg^{-1} BAP, respectively via indirect organogenesis. Consequently, distal cotyledons at the concentration of 0.5 mg^{-1} BAP induced 12% of EF, and 6% of each SG and SSA novel variants via indirect organogenesis (Table 6). The other concentrations of BAP, on the other hand, showed mostly a higher percentage (%) of novel somaclonal variants as obtained through indirect organogenesis (Table 6).

Although different types of novel somaclonal variants were obtained via direct organogenesis from all explants but no SSA variants was attained in the medium containing 0.07 mg^{-1} of BAP from proximal cotyledon explants via direct organogenesis (Table 5), whereas same explant at same concentration of BAP produced 5% SSA novel variant via indirect organogenesis (Table 6). Moreover, BAP at 0.1 and 0.5 mg^{-1} also did not produce same (SSA) novel variant from petiole explants of muskmelon through direct organogenesis while the same BAP concentrations

Table 4. Variations on average height of novel somaclonal variants obtained through direct and indirect organogenesis

BAP (mg/l)	Average height of somaclonal variants (in cm)					
	Direct organogenesis			Indirect organogenesis		
	Proximal cotyledon	Distal cotyledon	Petiole	Proximal cotyledon	Distal cotyledon	Petiole
0.07	7.8±0.5	4.8±0.5	6.6±0.3	5.4±0.2	4.3±0.1	4.4±0.1
0.1	0.0±0.0	4.8±0.3	7.7±0.5	4.9±0.1	4.5±0.2	4.3±0.1
0.3	6.9±0.2	6.2±0.4	0.0±0.0	4.8±0.2	4.7±0.2	4.5±0.2
0.5	7.1±0.1	0.0±0.0	6.8±0.5	5.0±0.2	4.8±0.2	4.0±0.1
0.7	5.9±0.4	6.7±0.6	4.5±0.3	5.1±0.2	4.7±0.2	4.5±0.3

Note: Average results are means of five replications±Standard Error

Table 5. Effect of direct organogenesis on production of novel somaclonal variants in muskmelon cv. Birdie

BAP (mg/l)	Percentage of novel somaclonal variants								
	Proximal cotyledon			Distal cotyledon			Petiole		
	ef	sg	ssa	ef	sg	ssa	ef	sg	ssa
0.07	4 (9.5±0.1)	3 (5.6±0.0)	0	13 (5.4±0.1)	9 (3.9±0.1)	5 (5.2±0.0)	8 (7.8±0.1)	4 (4.0±0.1)	3 (6.9±0.0)
0.1	0	0	0	11 (5.3±0.1)	6 (3.9±0.1)	4 (4.8±0.1)	7 (8.7±0.1)	2 (4.0±0.0)	0
0.3	9 (7.9±0.1)	5 (4.9±0.0)	2 (7.2±0.0)	7 (7.6±0.1)	5 (4.0±0.1)	2 (6.9±0.0)	0	0	0
0.5	10 (8.1±0.1)	5 (5.0±0.1)	3 (7.0±0.0)	0	0	0	9 (8.4±0.1)	5 (3.9±0.0)	0
0.7	14 (6.8±0.1)	9 (4.9±0.1)	6 (5.3±0.1)	12 (8.0±0.1)	6 (3.8±0.1)	2 (7.8±0.0)	11 (4.9±0.1)	6 (3.7±0.1)	3 (4.8±0.0)

Note: ef: early flowering including higher number of flowers, sg: slow growth of shoots with variant shape of leaves having long and thick petiole, ssa: stubby shoot apices and flattened stem. The numbers in the parentheses are average heights of novel variants in cm ± Standard Error

induced SSA novel variants in 7% and 8% of shoots obtained via indirect organogenesis (Tables 5 and 6).

The mean height (in parentheses) of novel somaclonal variants obtained from different explants of muskmelon at all concentrations of BAP through direct organogenesis was greater (Table 5) than the height (in parentheses) of novel somaclonal variants obtained through indirect organogenesis (Table 6). The ranges height of novel somaclones initiated through direct organogenesis from proximal cotyledon was 4.9 - 9.5 cm, from distal cotyledon was 3.8 - 8.0 cm and from petiole was 3.7 - 8.7 cm in height (Table 5). On the other hand, the height ranges were lower when somaclonal variants regenerated through indirect organogenesis (Table 6) as compared to direct organogenesis (Table 5). Average height of novel somaclonal variants found in plants regenerated from proximal cotyledon through direct organogenesis is relatively higher as compared to distal cotyledons and petiole. Average lowest height was found in plants regenerated from distal cotyledon as compared to two other explants (Table 5). A similar trend was also observed in height of novel somaclonal variants as initiated from all explants through indirect organogenesis (Table 6). The somaclones as well as novel variants induced roots vigorously in MS medium containing NAA at the concentrations of 0.01 and 0.03 mg^{-1} and successfully acclimatized to the ambient humidity level in soil (Figure 1g).

Discussion

There is no doubt that genetic variation especially morphological variations of plant cells cultured *in vitro* conditions is a general phenomenon (Skirvin, 1978; Constantin, 1981; Ren et al., 2013; Al-Noor et al., 2019) and has now been known for over 50 years. Larkin and Scowcroft (1981) reported that *in vitro* plant cell culture

Table 6. Effect of indirect organogenesis on production of novel somaclonal variants in muskmelon cv. Birdie

BAP (mg/l)	Percentage of novel somaclonal variants								
	Proximal cotyledon			Distal cotyledon			Petiole		
	ef	sg	ssa	ef	sg	ssa	ef	sg	ssa
0.07	16 (5.8±0.3)	5 (4.7±0.2)	5 (4.6±0.2)	7 (4.6±0.2)	7 (3.3±0.5)	6 (5.0±0.2)	17 (5.0±0.2)	8 (3.6±0.1)	8 (4.5±0.1)
0.1	14 (5.2±0.2)	11 (4.4±0.1)	4 (5.2±0.1)	16 (5.0±0.4)	11 (3.7±0.0)	10 (4.7±0.4)	18 (4.9±0.1)	11 (3.4±0.2)	7 (4.2±0.5)
0.3	16 (5.5±0.0)	11 (3.9±0.2)	11 (5.3±0.5)	13 (5.2±0.1)	8 (3.9±0.4)	4 (4.6±0.1)	13 (5.1±0.5)	9 (3.7±0.0)	6 (4.5±0.1)
0.5	15 (5.6±0.3)	10 (4.1±0.1)	7 (4.9±0.1)	12 (5.9±0.2)	6 (3.4±0.2)	6 (5.2±0.1)	18 (4.3±0.1)	12 (3.3±0.3)	8 (4.5±0.3)
0.7	16 (5.5±0.1)	12 (4.4±0.5)	10 (5.2±0.4)	11 (5.2±0.5)	8 (3.6±0.1)	7 (5.0±0.3)	19 (5.1±0.1)	14 (3.8±0.1)	10 (4.4±0.4)

Note: ef: early flowering including higher number of flowers, sg: slow growth of shoots with variant shape of leaves having long and thick petiole, ssa: stubby shoot apices and flattened stem. The numbers in the parentheses are average heights of novel variants in cm ± Standard Error.

itself generated genetic variability, was so called somaclonal variation, in the regenerants. During *in vitro* regeneration studies, a number of somaclones was found in R0, R1 and R2 generations in medicinally important cucurbit, *Citrullus Ccolosynthis* (L.) Schrad (Shasthree et al., 2009). Our experiments conducted on muskmelon through direct and indirect organogenesis confirmed this conspicuous phenomenon.

Interestingly, it was observed that greater number of morphologically normal somaclones obtained via direct organogenesis produced lower rate of plants with variations compared to indirect organogenesis. This may be due to the minimal production of callus by the cytokinin-type-hormone, BAP, added in the direct organogenesis protocol. High frequency multiple shoots were formed on MS nutrient medium containing cytokinins through direct organogenesis without intervening callus phase in *Passiflora foetida* L. (Anand et al., 2012). Plant regeneration from explants via an intermediate callus stage (indirect organogenesis) was often associated with more variations than regenerants induced via direct organogenesis whereby little or no callus was produced (Harini and Sita, 1993).

The addition of auxin (2,4-D) to BAP in SIM resulted in lower rates of induction of normal shoots suggesting that the presence of 2,4-D in the medium may inhibit the production of phenotypically normal somaclones, which resulted increase in the production of somaclonal variants. On the other hand, BAP at concentrations of 0.1, 0.3 and 0.5 mg^{-1} did not produce any somaclonal variants obtained from proximal cotyledon, petiole and distal cotyledon explants through direct organogenesis, while the reverse trend was observed in indirect organogenesis. It can be suggested that muskmelon shoots with normal traits can be obtained at certain concentration of BAP through direct organogenesis. It should be noted that the production of morphologically normal shoots is an important factor to ensure success of genetic manipulation (gene transformation) experiments of cucurbits e.g. muskmelon for variety development (Mohiuddin et al., 2000). There are some more reports found available on variety developed through direct organogenesis in tomato (Ewa et al., 2000) and garlic (Taşkın et al., 2013) even production of true-to-type *in vitro*-propagated *Aloe vera L.* plants (Khatun et al., 2018) and virus free plant variety (Ahmed et al., 2019).

Various types of novel variants such as early flowering, slow growth with long and thick petioles, and stubby shoot apices were observed among the shoots of muskmelon derived from different explants. Shasthree et al. (2009) also observed a number of variations in habit, leaf and tendrillar character, fruit number, sizes, colours, and seed coat colours. Similar variation was observed in gametoclonal variation in *Populus euphratica* (Mofidabadi et al., 2001) as well as somaclonal variation of cucumber (Mohiuddin et al., 2003).

Our study confirmed that the range of morphogenic changes in somaclones was significantly higher when somaclones were obtained through indirect organogenesis. This technique could be utilized for the production of somaclonal variants like early variety, late variety, early flowering and/ high yielding variety, dwarf variety needs less nutrients, variety with desirable body configurations of muskmelon, etc. There are several published reports indicating above mentioned achievements obtained from sugarcane early variety development (Sreenivasan and Jalaja, 1983), early flowering in both woody bamboo (Yuan et. al., 2017) and in *Swertia chirayita*, an endangered medicinal herb (Sharma et. al., 2014). Other achievements similar to our findings were also obtained from dwarf variety generally needs less nutrients or other purpose like ornamental plant production (Leva and Petruccelli, 2011), production of high yielding variety observed in fruit crops (Harsimrat and Manjot, 2020), and variety with desirable body configurations to produce new genotypes for breeding purposes (Vitamvas et. al., 2019). Finally, we could say that this study shows that the production of normal somaclones or novel variant somaclones is therefore greatly influenced by the growth regulator selected in *in vitro* method.

Conclusion

Greater number of novel somaclonal variants were obtained significantly from muskmelon explants through indirect organogenesis as compared to direct organogenesis. These variants could be the leading genetic materials to develop new varieties of muskmelon with especial agronomically important characteristics for future variety development program.

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Abbreviation

BAP	6-benzylaminopurine
2,4-D	2,4-dichlorophenoxyacetic acid
NAA	Naphthalene acetic acid

References

Ahmed MS, Dilnawaz AG, Jacqueline B, Satomi H, Manuel Z, Aslam J, Muhammad ZI, Shahid IA. (2019). Somaclonal variation for red rot and sugarcane mosaic virus resistance and candidate genes integrity assessment in somaclones of selected sugarcane varieties (*Saccharum officinarum* L.). Pakistan Journal Agricultural Science 56(1): 15-27.

Al Mawaali QS, Al-Sadi A, Al-Said FA, Rahman MS, Al-Zakwani I, Ali A, Al-Yahyia M, Deadman MI. (2017). Effect of grafting on resistance to vine decline disease, yield and fruit quality in muskmelon cv. Sawadi. Journal of Agricultural and Marine Sciences 23(1): 2-10.

Al-Noor MM, Quadir QF, Naher J, Rashid H-or, Chakrobarty T, Jewel ZA, Razia S. Nath UK. (2019). Inducing variability in rice for enriched iron and zinc content through *in vitro* culture. Plant Tissue Culture and Biotechnology 29(2): 161-174.

Anand SP, Jayakumar E, Jeyachandran R, Nandagobalan V, Doss A. (2012). Direct organogenesis of *Passiflora foetida* L. through nodal explants. Plant Tissue Culture and Biotechnology 22(1): 87-91.

Bajaj YPS. (1990). Somaclonal variation–origin, introduction, cryopreservation, and implications in plant breeding. In: Biotechnology in agriculture and forestry. Vol. II. Springer-Verlag, Berlin, Heidelberg. p.p 3-48.

Constantin MJ. (1981). Chromosome instability in cell and tissue cultures and regenerated plants. Environmental and Experimental Botany 21: 359-368.

Evenor D, Pressman E, Ben-Yephet Y, Rappaport L. (1994). Somaclonal variation in celery and selection by coculturing toward resistance to *Septoria apiicola*. Plant Cell Tissue and Organ Culture 39: 203-210.

Ewa H-F, Maria L, Anna P, Kazimierz M. (2000). Selection for virus resistance in tomato exposed to tissue culture procedures. Acta Physiologiae Plantarum 22: 317–324.

Gray DJ, BcColley DW, Compton ME. (1993). High frequency somatic embryogenesis from quiescent seed cotyledons of *Cucumis melo* cultivars. American Society Horticultural Science 118(3): 425-432.

Harini I, Sita GL. (1993). Direct somatic embryogenesis and plant regeneration from immature embryos of chilli (*Capsicum annuum* L.). Plant Science 89: 107-112.

Harsimrat KB, Manjot K. (2020). Role of plant growth regulators in improving fruit set, quality and yield of fruit crops: a review. Horticultural Science and Biotechnology 95(2): 137-146.

Jelaska S. 1986. Cucurbits. In: Biotechnology in Agriculture and Foresty. Vol. 2. Crops I. Bajaj YPS, ed. Springer-Verlag, Berlin Heidelberg. p. 371-386.

Khatun MM, Tanzena T, Sabina Y, Salimullah M, Iftekhar A. (2018). Evaluation of genetic fidelity of *in vitro*-propagated *Aloe vera* plants using DNA-based markers. Science Asia 44: 87-91.

Larkin PJ, Scowcroft WR. (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics 60:197-214.

Leva AR, Petruccelli R. (2011). Dwarf olive trees for ornamental use: a morphological evaluation. The Journal of Horticultural Science and Biotechnology 86(3): 217-220.

Mofidabadi AJ, Jorabchi A, Shahrzad S, Mahmodi F. (2001). New genotypes development of *Populus euphratica* Oliv. using gametoclonal variation. Silvae Genetica 50: 275-279.

Mohiuddin AKM. (1998). Improvement in organogenesis and the development of a transformation procedure for cucumber and muskmelon. Ph.D. Thesis, University Putra Malaysia.

Mohiuddin AKM, Chowdhury MKU, Abdullah ZC, Harikrishna K, Napis S. (1998). Studies on the improvement of *in vitro* shoot regeneration of muskmelon (*Cucumis melo* L.) cv. Birdie. Asia Pacific Journal of Molecular Biology and Biotechnology 6(1): 69-74.

Mohiuddin AKM, Harikrishna K, Chowdhury MKU, Abdullah ZC, Napis S. (2000). Influence of acetosyringone on transformation of cucumber mediated by *Agrobacterium tumeficiens*. Plant Tissue Culture 10(2): 167-174.

Mohiuddin AKM, Napis S, Abdullah ZC, Chowdhury MKU, Harikrishna K. (2003). Enhancement of cucumber somaclonal production by plant growth regulator. Bangladesh Journal Plant Breeding Genetics 16(2): 9-16.

Moreno V, Garcia-Aogo M, Granell I, Garcia-Sogo B. (1985). Plant regeneration from calli of melon (*Cucumis melo* L. cv. 'Amarillo Oro'). Plant Cell Tissue and Organ Culture 5: 139-146.

Moreno V, Zubeldia I, Garcia-Sogo B, Nuez F, Roig LA. (1996). Somatic embryogenesis in protoplast-derived cells of *Cucumis melo* L. In: Horn W, Jenson CJ, Odenbach W, Schieder O, editor. Genetic manipulation in plant breeding. Walter de Gruyter and Co., Berlin, New York. pp. 491-493.

Murashige T, Nakano R. (1966). Tissue Culture as a potential tool in obtaining polyploidy plants. Journal of Heredity 57: 115-118.

Murashige T, Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15: 473-487.

Niedz RP, Smith SS, Dunbar KB, Stephens CT, Murakish HH. (1989). Factors influencing shoot regeneration from cotyledonery explants of *Cucumis melo*. Plant Cell Tissue and Organ Culture 24: 27-30.

Oridate T, Atsumi H, Ito S, Araki H. (1992). Genetic difference in somatic embryogeesis from seeds of (*Cucumis melo* L.). Plant Cell Tissue and Organ Culture 18: 313-319.

Orton TJ. (1984). Somaclonal Variation: Theoretical and Practical Consideration. In: Gene Manipulation in plant improvement, Gustafson JP, ed., Plenum Press, New York. pp. 427-468.

Patnaik J, Sahoo S, Debata BK. (1999). Somaclonal variation in cell suspension culture-derived regenerants of *Cymbopogon martini* (Roxb.) Wats var. motia. Plant Breeding 118: 351-354.

Pijnacker LP, Ramulu KS. (1990). Somaclonal variation in potato: a karyotypic evaluation. Acta Botanica Neerlandica 39(2): 163-169.

Ren Y, Haejeen B, Ian SC, Jean G, Bhimanagouda SP, Kevin MC. (2012). *Agrobacterium*-mediated transformation and shoot regeneration in elite breeding lines of western shipper cantaloupe and honeydew melon (*Cucumis melo* L.). Plant Cell Tissue and Organ Culture 108:147-158.

Ren Y, Haejeen B, Jean G, Keerti SR, Bhimanagouda SP, Kevin MC. (2013). Shoot regeneration and ploidy variation in tissue culture of honeydew melon (*Cucumis melo* L. *inodorus*). In Vitro Cellular and Developmental Biology-Plant. 49: 223-229.

Saraswat R, Kumar M. (2019). Plant regeneration in Buckwheat (*Fagopyrum esculentum* Moench.) via somatic embryogenesis and induction of meristemoids in abnormal embryos. Plant Tissue Culture and Biotechnology 29(1): 33-47.

Sharma V, Barkha K, Nidhi S, Anoop KD, Vikash SJ. (2014). *In vitro* flower induction from shoots regenerated from cultured auxillary buds of endangered medical herb *Swertia chirayita* H. Karst. Biotechnology Research International 2014: 1-5

Shasthree T, Savitha R, Mallaiah B. (2009). Somaclonal variations in an endangered and medicinally important cucurbit, *Citrullus Ccolosynthis* (L.) Schrad. Biotechnology 3(4): 17-23.

Skirvin RM. (1978). Natural and induced variation in tissue culture. Euphytica 27: 241–266.

Sreenivasan TV, Jalaja NC. (1983). Sugarcane varietal improvement through tissue culture. In: Plant cell culture in crop improvement. Basic Life Sciences, Vol. 22. Sen SK, Giles KL eds. Springer, Boston, MA. p. 371-376.

Taşkın H, Gökhan B, Mehmet K, Saadet B. (2013). Use of tissue culture techniques for producing virus-free plant in garlic and their identification through real-time PCR. The Scientific World Journal 2013: 1-5.

Trulson AJ, Shahin EA. (1986). *In vitro* plant regeneration in the genus *Cucumis*. Plant Science 47: 35-43.

Vitamvas J, Viehmannova I, Petra HC, Mrhalova H, Eliasova K . (2019). Assessment of somaclonal variation in indirect morphogenesis-derived plants of *Arracacia xanthorrhiza*. Pesquisa Agropecuária Brasileira 54: 1-9.

Yuan JL, Yue JJ, Xiao-Ping GU, Choun-Sea L. (2017). Flowering of woody bamboo in tissue culture systems. Frontiers Plant Science 8: 1-8 (Article 1589).