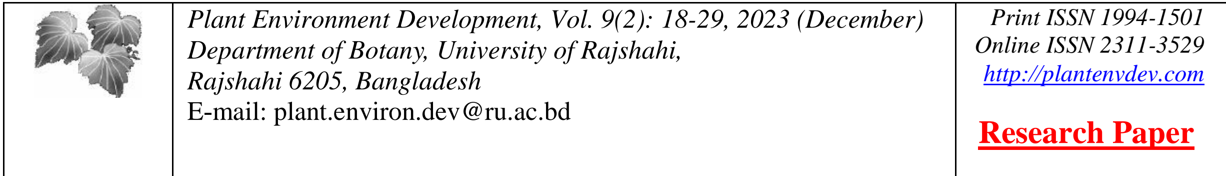
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Research Paper

Development of new melon (*Cucumis melo* L.) varieties through *In vitro* induction of somaclonal variation

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Abstract

Melon is one of the most popular high value fruit for its fragrance, taste, nutrition and economic importance all over the world. To develop suitable somaclones of melon varieties, callus induction and subsequently plant regeneration technique was employed using *In vitro* grown leaf explants. Firstly, seeds of two popular melon of Bangladesh (Boro athal, Chinal) cultivars were surface sterilized and cultured on semisolid MS medium supplemented with different concentrations of GA_3. Seed germination was the highest in MS medium supplemented with 0.2 mg/L GA_3 for Boro athal cultivars. High shoot proliferation was noticed in MS medium containing 2.0 mg/L BA. Among the different concentrations and combinations of plant growth regulators used in this investigation, IAA (2.0 mg/L) + BA (0.5 mg/L) was found the best media formulation for callus induction, where 90% of explants were induced for callus formation, and the calli were greenish in colour and compact in texture. The highest number of greenish shoot primordial was also occurred in this media formulation when the calli were kept in light for a prolonged time, where the no. of shoots per callus 5.31± 0.09 and the average length of shoot was 5.11±0.33 cm. Media containing no plant growth regulators (MS0) didn't show any shoot regeneration from the callus clump. The genetic diversity of regenerated shoots was determined by RAPD technique, and the banding patterns were different from the mother plants, and even among the regenerated plants indicating the successful development of new somaclones. Then, the shoots were cultured in MS0 or in different concentration of auxins (NAA and IBA) for root induction. 100% of *In vitro* grown cultured shoots were induced to develop roots in MS0 media, whereas in 1.0 mg/1 NAA or IBA, the highest 70% of that was found to develop roots.

Key words: Plant Growth Regulators (PGRs), Callus, Plant Regeneration, Somaclone, Random Amplified Polymorphic DNA (RAPD)

Introduction

Melon (*Cucumis melo* L.) is one of the most important crops within the Cucurbitaceae family, presenting a high variability in fruit traits among different cultivars, ranging from non-sweet fruits that are harvested before maturity and consumed as vegetables, to sweet fruits with high sugar concentrations that are eaten in salads or as dessert. It is widely cultivated worldwide and is welcomed by people all over the world because of its unique flavor and nutritional value

(Manchali et al., 2021). Melon fruits display a broad range of phenotypic variation. A huge variability exists for characteristics associated with fruit quality, such as flesh color, sugar content and aroma (Burger et al., 2006). Leida et al. (2015) reported the variability of a set of genes involved in ripening behavior and sugar accumulation in melon fruit, providing a framework for studying the putative role of those genes in the diversification of the species, and associating allelic variants with the phenotypic differences within the germplasm.

Genetic improvement of *C. melon* can be achieved by tissue culture, because the genetic variation of this crop is limited by sexual compatibility with other Cucurbitaceae. In addition, tissue culture of cucumis can be useful to achieve variability through somaclonal variation (Bezirganoglu, 2017). Callus growth is triggered by plant hormones. In plant tissue culture medium, specific auxin to cytokinin ratios result in a disorganized growing and dividing mass of callus cells. Successful regeneration from melon was reported for the first time by Moreno et al. (1985). Further attempts have been directed towards defining the factors that influence the regeneration ability, including: genotype, explant type, nutrition medium composition, culture condition, etc. (Kintzios & Taravira, 1997; Nunez-Palenius et al., 2008). The role of the genotype as a main factor determines regeneration capacity of melon is proved by many authors (Monforte et al., 2003; Nunez-Palenius et al., 2008). Plant regeneration in melon has been achieved with different explant types: hypocotyls, cotyledons, leaves, cotyledonary nodes, petioles roots, etc. (Kintzios et al., 2002; Choi et al., 2012; Sebastiani & Ficcadenti, 2016). There are data in the literature on the variation in regeneration depending on the position, proximal or distal, and size of the explant (Mendi et al., 2010; Bezirganoglu, 2017). Nevertheless, melon regeneration is still not a routine procedure and hormonal requirements in *In vitro* cultivation are poorly studied. Ones of the most commonly used plant growth regulators for induction of regenerative processes are Benzyladenine (BA) and Kinetin, used alone or in combination with Indole-3-acetic acid (IAA) (Keng & Hoong, 2005; Souza et al., 2006). Investigations of many authors established that some additional factors as light, temperature, humidity and gelling agent also influence the regeneration (Curuk et al., 2003).

In Bangladesh most the melon varieties have low sugar accumulation traits, thus people are uninterested to consume these fruits though it has huge nutritional and medicinal value. For better production of melon in our country, every year huge amount of hybrid seeds are imported from abroad. But the commercial growers face several problems such as high market value of F1 hybrid seeds, low seed germination rate and disease susceptibility. Thus, the present research was conducted to develop new melon varieties, and to increase diversity of melon using *In vitro* induction of somaclonal variation, which would be suitable for cultivation in different agro-ecological zones in Bangladesh.

Materials and Methods

Plant materials

Seeds of melon (*Cucumis melo* L. cv. Boro athal and Chinal) were germinated in culture vessels under aseptic conditions. Next, *In vitro* produced melon plants' leaves were utilized as explants in a variety of callus formation and regeneration procedures. The seeds were collected from local market of Bangladesh.

Fig.1 Seeds of melon (*Cucumis melo* L. cv. Boro athal and Chinal).

Seed germination

Dried seeds of *Cucumis melo* L. cv. Boro athal and Chinal were washed thoroughly under running tap water for 30 min to remove dried mucilagenous substances and dust etc., followed by treatment with 10% teepol (liquid detergent) for 10 min. Then the seeds were surface sterilized in 70% ethanol for 1 min, and finally with 0.1% $HgCl_2$ for 4-5 min, and washed 4-5 times with sterile double distilled water under laminar airflow hood. Then the seeds were placed on MS medium either growth regulator free or on medium supplemented with different concentration of GA_3. Then the germinated seedlings were multiplied in MS medium supplemented with different concentrations of BA and KIN.

Callus induction

Leaves from plants grown *In vitro* were utilized as explants to create callus, and the explants were cultivated in MS (Murashige and Skoog, 1962) media treated with varying amounts and combinations of BA, IAA, and NAA. After that, they were kept in the dark to develop callus. The leaf segments were cut into 10-15 mm and were placed 2-3 pieces in each culture vessel for inducing callus. Each culture vessel contains same type of explants. After 21-29 days, explants induced to callus, which were compact, soft, greenish, off-white and brown in colour. Following the induction of callus from the explants, the calli were moved into the new medium to continue growing and being maintained.

Shoot regeneration

By transplanting certain calli in MS semisolid medium supplied with IAA and BA in different concentrations and combinations, shoots were regenerated. This culture was grown for 16/8 hours under light-dark settings at 25±1 °C with white light (light fluctuated between 2000–3000 lux).

Genetic diversity study of regenerated plants (Somaclones)

DNA isolation, detection and quantification

Employing a modified cetyl trimethyl amonium bromide (CTAB) technique (Doyle, 1991), total genomic DNA was extracted from each sample. A 2 ml Eppendorf tube holding 200–300 mg of the finely ground material was placed in a liquid nitrogen vessel after the sample was crushed using a mortar and pestle. In the meantime, 850 µl homogenization buffer was incorporated, which comprised 2-Mercaptoethanol ($HOCH_2CH_2SH$) (Sigma-Aldrich®, St. Louis, MO, USA), 100 mM Tris-HCl (PH 8.0), 2% w/v CTAB, 20 mM EDTA, 1.42 M NaCl, and 2% w/v PVP-40. The RNA contamination was also to be eradicated using 4 µl of RNase A. After that, the mixture was carefully vortexed and incubated for 30 to 45 minutes at 65 °C (tubes were inverted every 15 minutes). After that, the tubes got filled with the same quantity (850 µl) of extraction solution encompassing chloroform : isoamyl alcohol (24:1) (from Fisher

Scientific International Inc., Hampton, NH, USA, and Amresco LLC, Solon, OH, USA, respectively), and it was gently shaken for 10 minutes to mix it. The mixes were then centrifuged for 10 minutes at 13,000 g. After pipetting 500 µl of the top aqueous layer into a fresh 1.5 ml Eppendorf tube, 1 ml of 70% cold ethanol—double the sample volume—was added. After that, the mixture was gradually inverted many times to cause DNA to precipitate. After two hours keeping at 4 °C for a maximum yield of DNA, the mixtures were centrifuged at 13,000 g for fifteen minutes at room temperature. After separating the supernatant, the pellet was allowed to air dry for 20 minutes. Ultimately, the pellet was diluted in 50–100 µl deionized water and kept cold for additional research. DNA was detected on a 1% (w/v) agarose gel stained with a diamond solution (Promega) after 30 minutes of 120 volts in 1X TBE buffer. Using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA), the absorbance at 260 nm ($A260_{nm}$) and 280 nm ($A280_{nm}$) were measured in order to evaluate the content and purity of DNA. For further examination, samples with an $A260_{nm}/A280_{nm}$ ratio of 1.7–1.9 were chosen and kept at -20 ºC.

RAPD Analysis

Using PCR-based RAPD analysis, the genetic resilience of the regenerated plants was evaluated. Five decamer RAPD primers in all were chosen based on the quantity and quality of produced fragments (**Table 1**).

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Table 1 List of RAPD primer for PCR amplification

RAPD Primer	Sequence(5´-3´)
Random primer 1(RP_1)	5´-GACTTGCACAC-3´
Random primer 2 (RP_2)	5´-ACCGCCTGCT-3´
Random primer 3 (RP_3)	5´-AGGCCGGTCA-3´
Random primer 4 (RP_4)	5´-TGATCCCTGG-3´
Random primer 5 (RP_5)	5´-GTTTCGCTCC-3´

The RAPD reactions were performed in a volume of 20 µl consisting 10 µl GoTaq Ready mix (Promega, USA), 1 µl primer, 8 µl sterile distilled H_2O and 1 µl template DNA. Cycling conditions were as follows: 5 min at 95 °C, 35 cycles of 1 min at 95 °C, 1 min at 29 °C, 1 min at 72 °C, and 5 min at 72 °C. After PCR, the products were stained by visualized through agarose gel electrophoresis (2%) after staining by Diamond^{TM} Nucleic Acid dye (Promega, USA). The image was taken with the Gel Documentation Systems (ProteinSimple, USA). 100 bp DNA ladder was used.

Rooting

The regenerated shoot apex were isolated from one another and aseptically removed from the culture vessels when they had grown to a length of 4-5 cm with six fully formed leaves. These shoots were micro-cut by breaking off the basal leaves, and each micro-cut was cultured in a tube with 10–12 milliliters of rooting medium containing various auxin combinations or MS0.

Data analysis

For each treatment, mean values with standard deviation (SD) were calculated using excel. The RAPD banding pattern

was determined using visual intensity detecting Image J software.

Result and Discussion

One of the most significant species in the Cucurbitaceae family is melon *(Cucumis melo* L.). This is because of the wide range of fruit characteristics that it possesses, including color, shape, size, flavor, and texture (Paris et al., 2012). Numerous variants have been generated by traditional breeding techniques. On the other hand, there is a rising need for new high-yielding cultivars that combine disease resistance with fruit quality, and can be grown in a variety of climates. In Bangladesh, most the melon varieties have low sugar accumulation traits. Thus, people are uninterested to consume these fruits though it has huge nutritional and medicinal value. Using tissue culture techniques such as somaclonal variation opens up new avenues for developing genetic diversity with better traits.

Germination of Seeds and Shoot Multiplication

Seed germination rate along with the length of germinated seedlings were markedly affected by the supplement of GA_3. Both the highest germination rate (85.20 ±0.25%) and the longest seedlings (5.25 ±0.35cm) were observed at 0.2 mg/l GA_3 containing MS medium in Boro athal cultivar (**Table 2**; Figure 2A). Many reports suggest the effect of GA3 on the germination of seeds, such as according to Diaz and Mar (1971), GA_3 is known to stimulate the germination of seeds when dormancy is imposed by different mechanisms like incomplete embryo development, mechanically resistant seed coats, presence of inhibitors and factors relating to physiological competence of the embryo axis etc. In another report, Nerson (2007) described that the germination ability of cucurbit seeds is related both to external and internal factors, which was also found during our investigation. Shoot tips from well developed seedlings were cultured on MS (Murashige and Skoog, 1962) medium supplemented with cytokinin (BA, KIN) in different concentrations. Although in all medium efficient shoot multiplication from the shoot tip explants was noticed, both the highest shoot multiplication (8.83±0.32) and length of shoot (9.10±0.77) was induced in MS medium supplemented with 2.0 mg/l BA after 30 days of culture (**Table 3**).

Table 2 *In vitro* seed germination performance under different treatments. Data were recorded after incubation and each treatment consists of 6 seeds and repeated thrice.

Treatments (mg/L)	Cultivars	Time taken to Germination (in days)	% of seed Germination (Mean±SD)	Length of germinated Seedlings (in cm) (Mean±SD)
MS0	Boro athal	7	68.25 ± 0.44	4.60 ± 0.22
	Chinal	8	65.14 ± 0.44	3.90 ± 0.33
GA_3 0.2	Boro athal	4	85.20 ± 0.25	5.25 ± 0.35
	Chinal	6	80.32 ± 0.41	4.95 ± 0.66
0.5	Boro athal	8	68.80 ± 0.48	4.52 ± 0.44
	Chinal	9	55.28 ± 0.33	4.35 ± 0.33
1.0	Boro athal	9	45.31 ± 0.22	3.16 ± 0.33
	Chinal	10	36.24 ± 0.35	3.05 ± 0.77

Table 3 Effect of different concentrations of cytokinin (BA and KIN) singly in MS medium on shoot multiplication from *In vitro* grown shoot tip explants of two muskmelon cultivars. Each treatment consisted of 10 explants. Data were recorded after 4 weeks of culture.

Treatments (mg/L)	Cultivars	No. of micro-shoots/explant (Mean± SE)	Length of the longest shoot (incm) (Mean± SE)
BA 0.5	Boro athal	5.58±0.15	6.25±0.35
	Chinal	4.15±0.44	5.15±0.22
2.0	Boro athal	8.83±0.32	9.10±0.77
	Chinal	6.33±0.25	8.65±0.47
KIN 0.5	Boro athal	4.62±0.66	5.15±0.44
	Chinal	3.25±0.16	4.61±0.25
2.0	Boro athal	5.24±0.22	6.86±0.22
	Chinal	4.33±0.55	5.15±0.32

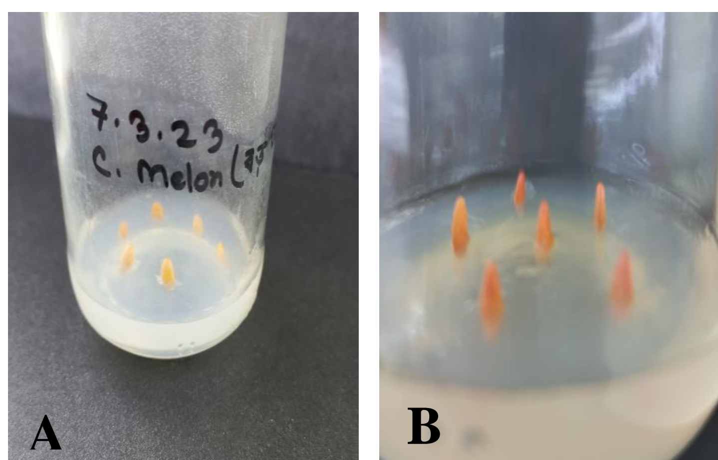

Fig. 2 Inoculation of melon seeds after surface sterilization. A. Boro athal, B. Chinal

Callus Induction

Leaf segments of *In vitro* grown plants were cultured separately on to agar-gelled MS nutrient medium supplemented with different concentrations of IAA alone, and in combinations of IAA+BA and BA+NAA or without any plant growth regulators (MS0). All the cultures were incubated in dark in growth cabinet at 25 °C for 4 weeks. The explants from *In vitro* grown plants were induced to develop callus within 10-15 days of culture. Different parameters were taken into consideration for finding out the most effective culture media formulation for the development of callus with high potential for plant regeneration. In terms of the percent of explants were induced to develop callus and the degree of callus development, 2.0 mg/L IAA with 0.5 mg/L BA was found the most effective medium formulation among the different PGR formulations in Boro athal cultivar (**Table 4; Fig. 3C**). Although there are several studies with varying results about the effect of plant growth regulators, a report was also found with the results in agreement to this investigation (Narayan, 2015).

Table 4. Effect of IAA, IAA+BA (mg/L) and NAA + BA (mg/L) in MS medium for callus induction from leaf explants of two melon varieties (Boro athal and Chinal). Data were recorded after 4 weeks of culture.

Growth Regulators mg/L	Cultivar	No. of explants	% response	Degree of callus formation	Color	Texture
MS0	Boro athal	10	0	-	-	-
	Chinal	10	0	-	-	-
IAA 0.5	Boro athal	10	40	+	Off white	Friable
	Chinal	10	30	+	Off white	Soft
1.0	Boro athal	10	80	+++	Off white	Compact
	Chinal	10	50	++	Off white	Soft
2.0	Boro athal	10	90	+++	Off white	Compact
	Chinal	10	70	++	Off white	Soft
3.0	Boro athal	10	60	++	Off white	Compact
	Chinal	10	50	++	Off white	Soft
4.0	Boro athal	10	40	+	Off white	Compact
	Chinal	10	30	+	Off white	Soft
IAA +BA 0.5 +0.5	Boro athal	10	40	+	Greenish	Compact
	Chinal	10	30	++	Off white	Compact
1.0+.5	Boro athal	10	80	+++	Greenish	Compact
	Chinal	10	50	++	Off white	Compact
2.0+0.5	**Boro athal**	**10**	**100**	**+++**	**Greenish**	**Compact**
	Chinal	10	70	++	Off white	Compact
3.0+0.5	Boro athal	10	60	++	Greenish	Compact
	Chinal	10	50	++	Off white	Compact
4.0+0.5	Boro athal	10	20	+	Greenish	Compact
	Chinal	10	10	+	Off white	Compact
NAA +BA 0.5 +0.5	Boro athal	10	10	+	Brown	Compact
	Chinal	10	10	+	Brown	Compact
1.0+.5	Boro athal	10	30	+	Brown	Compact
	Chinal	10	20	+	Brown	Compact
2.0+0.5	Boro athal	10	60	++	Brown	Compact
	Chinal	10	40	+	Brown	Compact
3.0+0.5	Boro athal	10	30	+	Brown	Compact
	Chinal	10	20	+	Brown	Compact
4.0+0.5	Boro athal	10	20	+	Brown	Compact
	Chinal	10	20	+	Brown	Compact

High callus: +++, Moderate callus: ++, Poor callus: +, No response: -

The greenish calli of Boro athal variety which was developed in combination of IAA and BA were subcultured in the same composition and kept in light for shoot regeneration. Like callus response, maximum no. of shoot primordia differentiation occurred in 2.0 mg/L IAA and 0.5 mg/L BA after subcultures (**Table 5**, **Fig. 3 C, D**) which was in agreement with the previous report of Narayan (2015). Cell clumps constituting green spots showed marked differences as compared to other cells with respect to size, adherence and pigmentation. These cells were compactly arranged, dark green in colour and showed noticeable protuberances which gradually turned into shoot primordia. Generally, shoot initiation was observed after four weeks of incubation on the subculture medium of same hormonal composition. A group of cells constituting green spots formed shoot-like dark green and compact structures that further grew in size, also observed by Pal et al. 2007 in *Cucurbita pepo*.

Table 5 Effect of MS0 and IAA+BA in MS medium on shoot regeneration of calli derived from leaf explants of Boro athali melon variety. Data were recorded after 4 weeks of culture.

Plant Growth Regulators mg/L	Cultivar	No. of explants	% response	No. of shoots per callus	Length of shoots (cm)
MS0	Boro athal	10	-	-	-
IAA +BA 0.5 +0.5	Boro athal	10	40	2.12±0.15	1.22±0.17
1.0+.5	Boro athal	10	80	3.25±0.16	2.20±0.66
2.0+0.5	**Boro athal**	**10**	**100**	**5.31±0.09**	**5.11±0.33**
3.0+0.5	Boro athal	10	60	4.14±0.55	3.55±0.38
4.0+0.5	Boro athal	10	20	2.26±0.23	2.49±0.27

Genetic diversity study

In the present investigation, four regenerated plants from *In vitro* grown leaf derived calli of Boro athal variety using IAA+BA were multiplied, and were then assessed by 5 RAPD primers to check the genetic variability from mother plants. Among them 3 showed informative and reproducible results. DNA was isolated from leaves of four newly regenerated plants for RAPD analysis. RAPD banding patterns were different from the mother source (control), and even among the regenerated plants studied for RAPD analysis (**Fig. 4**). Molecular characterization indicated the successful development of somaclonal variant through callus induction and plant regeneration using different plant growth regulators.

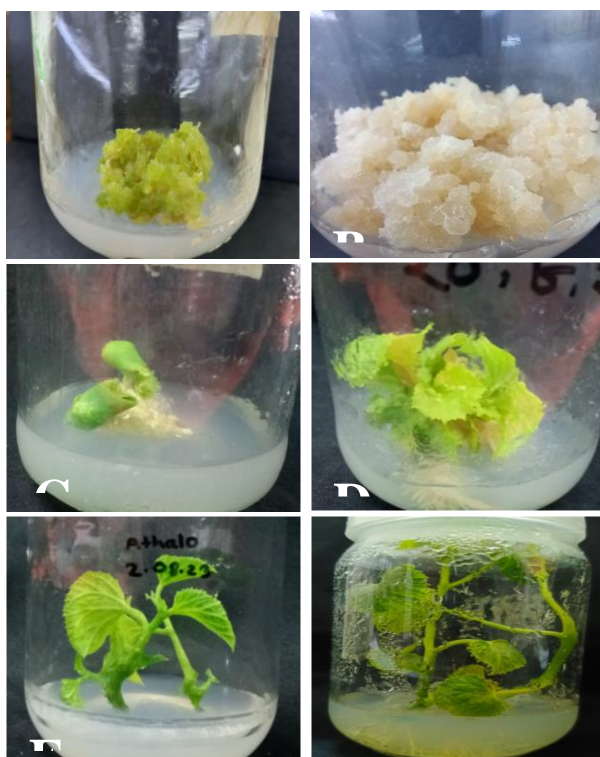

Fig. 3 Callus Induction, regeneration, multiplication and rooting. A. Callus induction from Boro athal variety (at 2.0 mg/L IAA +0.5 mg/L BA). B. Callus Induction from Chinal variety (at 2.0 mg/L IAA +0.5 mg/L BA). C, D: Shoot regeneration from Boro athal at 2.0 mg/L IAA +0.5 mg/L BA E. Multiplication of regenerated plants, and F. Rooting of regenerated plants in MS0**.**

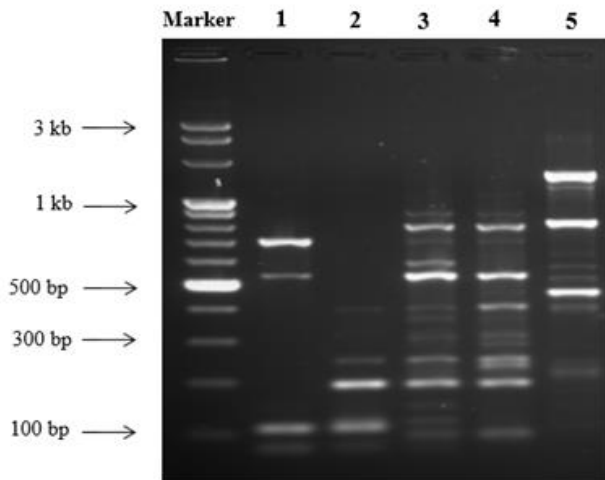

Fig. 4 RAPD profile of mother plant and calli derived regenerated plants (RAPD primer # 2). Lane 1- mother plant; lane 1-4 regenerated plants. Marker size: 100 bp.

Rooting of Regenerated Plants

After confirming by molecular characterization, regenerated shoots were then transferred individually in full strength MS semi-solid media with different PGR formulations (NAA and IBA) or without PGR to induce sufficient roots. Micro shoots (2-4 cm) were considered to transfer onto rooting media. Data were collected on the percentage of shoots induced root development and the number of roots/shoot after 4 weeks of culture. The results are shown in (**Table 6).** Root formation from *In vitro* regenerated shoots in all kinds of rooting experiments investigated but the results were different. It was observed that, when regenerated micro shoots were cultured onto MS media without plant growth regulators (MS0), then 100% of shoots developed roots. No shoot developed root with callus in MSO rooting media. The highest mean number of roots/shoot in MSO media was 9.5. *In vitro* regenerated micro shoots were excised and cultured individually onto MS media supplemented with 0.5, 1.0 and 2.0 mg/L NAA and IBA, separately. Among these six different formulations of NAA and IBA investigated, in both cases highest 70% of cultured shoots developed root. Root formation along with callus proliferation was also noticed in these and other NAA supplemented rooting media. These findings are in agreement with strawberry (Karim et al., 2015).

Table 6 Effect of different rooting media formulation on root induction from *In vitro* regenerated shoots. In each treatment, at least 10 shoots were rescued and cultured. Data were recorded after 4 weeks of culture.

PGR supplements in callus induction medium	% of shoots induced root development	No. of roots/shoot
MS0 (Control)	100	9.5±0.2
NAA		
0.5	60	5.2±0.25
1.0	70	6.8±0.62
2.0	50	3.7±0.53
IBA		
0.5	60	3.4±0.35
1.0	70	4.5±0.5
2.0	40	3.6±0.55

Conclusion

In this study, we followed *In vitro* induction for somaclonal variation method in which initially callus was formed and then subsequently shoot regeneration was performed. The described protocol is simple in handing which can be used effectively for the varietal improvement of other commercially and agronomically important crops too.

Author contribution

The experiment was designed and investigated by RK and TA. RK and SA have written the main manuscript. AR and MN helped to analyze the data, and to edit and revise the manuscript. All the authors read the final version of the manuscript, and there is no conflict of interests.

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References

Bezirganoglu, I. (2017). Tissue culture of *Cucumis melo*. Agricultural Research and Technology: Open Access Journal, 6: 1-2.

Burger, Y., Sa'ar, U., Paris, H. S., Lewinsohn, E., Katzir, N., Tadmor, Y., and Schaffer, A. A. (2006). Genetic variability for valuable fruit quality traits in *Cucumis melo*. Israel Journal of Plant Sciences, 54(3): 233-242.

Choi, J. Y., Shin, J. S., Chung, Y. S., and Hyung, N. I. (2012). An efficient selection and regeneration protocol for Agrobacterium-mediated transformation of oriental melon (*Cucumis melo* L. var. makuwa). Plant Cell, Tissue and Organ Culture (PCTOC), 110: 133-140.

Curuk, S., Ananthakrishnan, G., Singer, S., Xia, X., Elman, C., Nestel, D., and Gaba, V. (2003). Regeneration *in vitro* from the hypocotyl of *Cucumis* species produces almost exclusively diploid shoots, and does not require light. Horticultural Science, 38(1): 105-109.

Diaz, D. H., & Martin, G. C. (1972). Peach seed dormancy in relation to endogenous inhibitors and applied growth substances1. Journal of the American Society for Horticultural Science, 97(5): 651-654.

Doyle, J. (1991). DNA protocols for plants. In *Molecular techniques in taxonomy*. Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 283-293

Karim, R., Ahmed, F., Krishna Roy, U., Ara, T., Islam, R., & Hossain, M. (2018). Varietal improvement of strawberry (*Fragaria × ananassa* Duch.) through somaclonal variation using *In vitro* techniques.

Journal of Agricultural Science and Technology, 17(4): 977-986.

Keng, C. L., & Hoong, L. K. (2005). *In vitro* plantlets regeneration from nodal segments of musk melon (*Cucumis melo* L.). Biotechnology, 4(4): 354-357.

Kintzios, S. E., & Taravira, N. (1997). Effect of genotype and light intensity on somatic embryogenesis and plant regeneration in melon (*Cucumis melo* L.). Plant Breeding, 116(4): 359-362.

Leida, C., Moser, C., Esteras, C., Sulpice, R., Lunn, J. E., de Langen, F., ... & Picó, B. (2015). Variability of candidate genes, genetic structure and association with sugar accumulation and climacteric behavior in a broad germplasm collection of melon (*Cucumis melo* L.). BMC Genetics, 16: 1-17.

Manchali, S., Chidambara Murthy, K. N., Vishnuvardana, & Patil, B. S. (2021). Nutritional composition and health benefits of various botanical types of melon (*Cucumis melo* L.). Plants, 10(9): 1755.

Mendi, Y. Y., Eldoğan, S., Gutakev, R., İpek, M., Çürük, P., & Cetiner, S. (2010). Regeneration and histological analysis of snake melon (*Cucumis melo* var. flexuosus (L.) Naudin) by direct organogenesis. Turkish Journal of Agriculture and Forestry, 34(4), 309-317.

Monforte, A. J., Garcia-Mas, J., & Arus, P. (2003). Genetic variability in melon based on microsatellite variation. Plant Breeding, 122(2), 153-157.

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

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15(3).

Narayan, J.P. (2015). *In vitro* culture for quick callus induction and organogenesis in *Cucumis melo* L. var. agrestis Naudin. Proceedings of RIE Science Education Meet, Ajmer, India, 1-6.

Nerson, H. (2007). Seed production and germinability of cucurbit crops. Seed Science and Biotechnology, 1(1): 1-10.

Nunez-Palenius, H. G., Gomez-Lim, M., Ochoa-Alejo, N., Grumet, R., Lester, G., & Cantliffe, D. J. (2008). Melon fruits: genetic diversity, physiology, and biotechnology features. Critical Reviews in Biotechnology, 28(1): 13-55.

Pal, S. P., Alam, I., Anisuzzaman, M., Sarker, K. K., Sharmin, S. A., & ALAM, M. F. (2007). Indirect organogenesis in summer squash (Cucurbita pepo L.). Turkish Journal of Agriculture and Forestry, 31(1): 63-70.

Paris, H. S., Amar, Z., & Lev, E. (2012). Medieval emergence of sweet melons, *Cucumis melo* (Cucurbitaceae). Annals of Botany, 110(1): 23-33.

Patnaik, J., Sahoo, S., & Debata, B. K. (1999). Somaclonal variation in cell suspension culture-derived regenerants of *Cymbopogon martinii* (Roxb.) Wats var. motia. Plant Breeding, 118(4): 351-354.

Sebastiani, M. S., & Ficcadenti, N. (2016). *In vitro* plant regeneration from cotyledonary explants of *Cucumis melo* L. var. cantalupensis and genetic stability evaluation using RAPD analysis. Plant Cell, Tissue and Organ Culture (PCTOC), 124: 69-79.

Souza, F. V. D., Garcia-Sogo, B., Souza, A. D. S., San-Juán, A. P., & Moreno, V. (2006). Morphogenetic response of cotyledon and leaf explants of melon (*Cucumis melo* L.) cv. Amarillo Oro Brazilian Archives of Biology and Technology, 49: 21-27.