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(Original Article)

Establishment of Efficient *In vitro* Culture Protocol and Screening of Soma clonal Variation Among Regenerated Plants in Cumin (*Cuminum cyminum* L.)

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Abstract

Genetic diversity among in vitro plant regeneration in cumin has been examined by molecular markers analysis (ISSR and SRAP markers). Two landraces of cumin named Egyptian landraces (EGY genotype) and Indian landraces (IND genotype) were used as donor parents, three types of explants (hypocotyl, cotyledon and root) and four MS tissue culture media with different concentrations of growth regulators (auxin and cytokinin) were used to study the impact of genotype, type of explants and growth regulators on callus formation and plant regeneration in cumin. Significant differences among two cumin landraces were observed for regeneration rate and number of shoots per explant. These differences were depending on genotype, explant type and concentration of growth regulators. The best regeneration medium (MS with 0.5 mg/L 2,4-D) used for establishment of regenerated plants. Donor parent, Egyptian landraces (EGY) and Indian landraces (IND) and its regenerated plants on regeneration medium were selected and subjected to somaclonal variation analysis using molecular markers. ISSR (inter- simple-sequence-repeat) and SRAP (sequence-related-amplified-polymorphism) markers were used to detect the genetic variations between two cumin landraces. Subsequently, primers which exhibited high polymorphism between donor parents were used to analysis of somaclonal variation between each donor parent and its regenerated plants (somaclones), as well as among somaclones. These markers revealed polymorphism showing clear different DNA fragment patterns in all somaclones, which were eminent in their differences from parents.

Keywords: *Somaclonal variation, Cumin, In vitro culture. ISSR, SRAP*

Introduction

Cumin (*Cuminum cyminum* L.) diploid (2n = 14) (Lodha and Mawar 2014). Cumin is one of the most important medicinal herbs in the world which belongs to the Apiaceae (Baranski, 2008; Dubey *et al*., 2017).

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Genetic variations in crop plants are one of the most important sources to obtain elite genotypes which could be used in breeding programs. The major drawbacks of the cumin plants are low genetic diversity and narrow genetic base, which cannot be developed by conventional breeding programs. Therefore, genetic improvement through transgenic plant production via tissue culture approach offers a great progression in obtaining superior plants which are stress tolerant (Aruna and Sivaramakrishnan, 1996)

Somaclonal variations which occur among regenerated plants from in vitro culture has been considered as one of the most important sources of genetic variants in crop plants (Anil *et al.,* 2018). Thus, the establishment of simple and efficient regeneration method is an essential requirement of taking advantage of cell and tissue culture for genetic improvement.

Several studies have been conducted on callus induction and shoot regeneration of cumin so far (Tawfik and Noga, 2002; Ebrahimie *et al*. 2007, Soorni1 and Kahrizi, 2015), but study on different explant such hypocotyl, cotyledon and root, has not been reported up to now, or is very limited.

Molecular markers like Inter-Simple Sequence Repeats (ISSR) and Sequenced Related Amplified Polymorphism (SRAP) have been suggested to be useful for confirmation of genetic fidelity in micro-propagated plants (Martínez-Estrada *et al.,* 2017; El-Shahed *et al.,* 2017; Abd El-Fattah *et al.,* 2019). These two techniques have the advantages of giving reproducible results, low cost and primers can be designed without any prior knowledge of sequences (Pradeep *et al.,* 2002). The objective of this study aims to establishment of proficient tissue culture techniques in cumin and detect Somaclonal variation in regenerated cumin plants as revealed by the analysis of molecular markers.

Materials and Methods

This work was carried out at the Tissue culture and Bio-technology Laboratory, Genetics Department, Faculty of Agriculture, Assiut University during 2020-2022.

Table 1. The composition of different media used in this study

Medium code	Composition
M1	MS +0.5 mg/12.4-D
M2	MS + 2 mg/1 BA+ 1 mg/1 IAA
M3	MS + 1 mg/1 BA+ 0.5 mg/1 IAA
M4	MS + 0.5 mg/1 BA+ 0.1 mg/1 IAA

Plant Materials

Two cumin (*Cuminum cyminum* L.) landraces were used in the present investigation, namely; Egyptian landraces (EGY genotype) and Indian landraces (IND genotype).

The impact of cumin genotype, type kind of explants, and growth regulators on callus formation, regeneration rate, and number of shoots per explants was studied. The experiment was designated based on the following factors: two cumin

landraces (EGY genotype and IND genotype), three types of explants (hypocotyls, cotyledons and roots) and different growth regulators concentrations as shown in Table (1) and designated in randomized complete block design (RCBD) with three replicates.

Sterilization of seeds

Mature cumin seeds were immersed in sodium dodecyl sulfate (SDS) 2% for 15 minutes first, then soaked for 2 hours under running tap water. The seeds were surface sterilized with 35% Clorex for 15 minutes under aseptic conditions of laminar flow hood, and finally rinsed with double distilled water three times.

Germination

Aseptic seeds placed on placed on MS (Murashige and Skoog, 1962). The germination medium consists of (quarter strength of MS supplemented with 8gm/L agar and 20gm/L sucrose). The pH was adjusted to 5.9. The medium was sterilized in an autoclave at 1.2 Kg/ cm2 (121ºC) for 20 minutes. About 20 ml of the medium was dispensed into glass vials (150 ml). Five seeds were planted per vial, then vials were incubated in the dark at 22 ºC for 13 days for the Indian genotype and 15 days for the Egyptian genotype (initiation of germination) and then were transferred to illumination condition at 24-25 ºC for 2 days.

Preparation of explant and Callus induction

Cotyledon, hypocotyl and root explants were excised from cumin seedling (15-17-day-old seedlings) and they were placed on MS media containing 36gm/L sucrose, supplemented with 2,4-D alone or various combinations of 6-Benzyladenine (BA) and indole-3- acetic acid (IAA) (Table 1). This experiment was conducted in three replications per treatment and each replicate had 18 explants. The cultures were incubated at 23±1ºC in total darkness for four weeks, the number and percentage of developed calli were recorded after one-month culture.

Shoot regeneration

Embryo genic calli were sub-cultured into fresh mediums in illumination condition for shoot regeneration. First, calli developed from hypocotyl, cotyledon and root explant on M1 and M2 medium were sub-cultured into M3 for a month, then they were sub-cultured into M4. Second, calli developed from hypocotyl, cotyledon and root explant on M3 and M4 were transferred to fresh medium M4 for two months, with resub-culture done every month on the same media. Regenerated micro-shoots (2-3 cm long) were removed and transferred to rooting medium (MS medium + 0.5 mg/L IAA).

Statistical Analysis

Tissue culture traits was evaluated as the following parameters: Callus formation (C %), calculated as percentage of explants produced callus, Regeneration rate (Reg %), calculated as percentage of explant product shoot and Number of shoots per explant (No. shoots/exp). Analysis of variance according to

(Gomez and Gomez 1984) was performed. The means were compared by LSD test at 5% probability level.

Somaclonal Variation

In this part of the present investigation, molecular analysis was carried out to study and determine somaclonal variations among in vitro cumin plants regenerated.

Molecular studies

DNA was extracted from fresh leaves of the two cumin landraces and its regenerated plants (60 regenerated plants for each cumin genotypes) using the CTAB method (Murray and Thompson 1980).

Molecular-marker analyses

Ten ISSR primers and 10 SRAP primer combinations, were used in this study to amplify the template DNA. Amplification reactions and amplification conditions were carried out according to Abd El-Fatah (2018).

Data scoring, marker parameters and statistical analysis were calculated according to Ghislain *et al*. (1999), Powell *et al*. (1996), and Prevost and Wilkinson (1999). A dendrogram was constructed according to) (Dice, 1945) using NTSYS-pc 2.20 software (Rohlf, 2000).

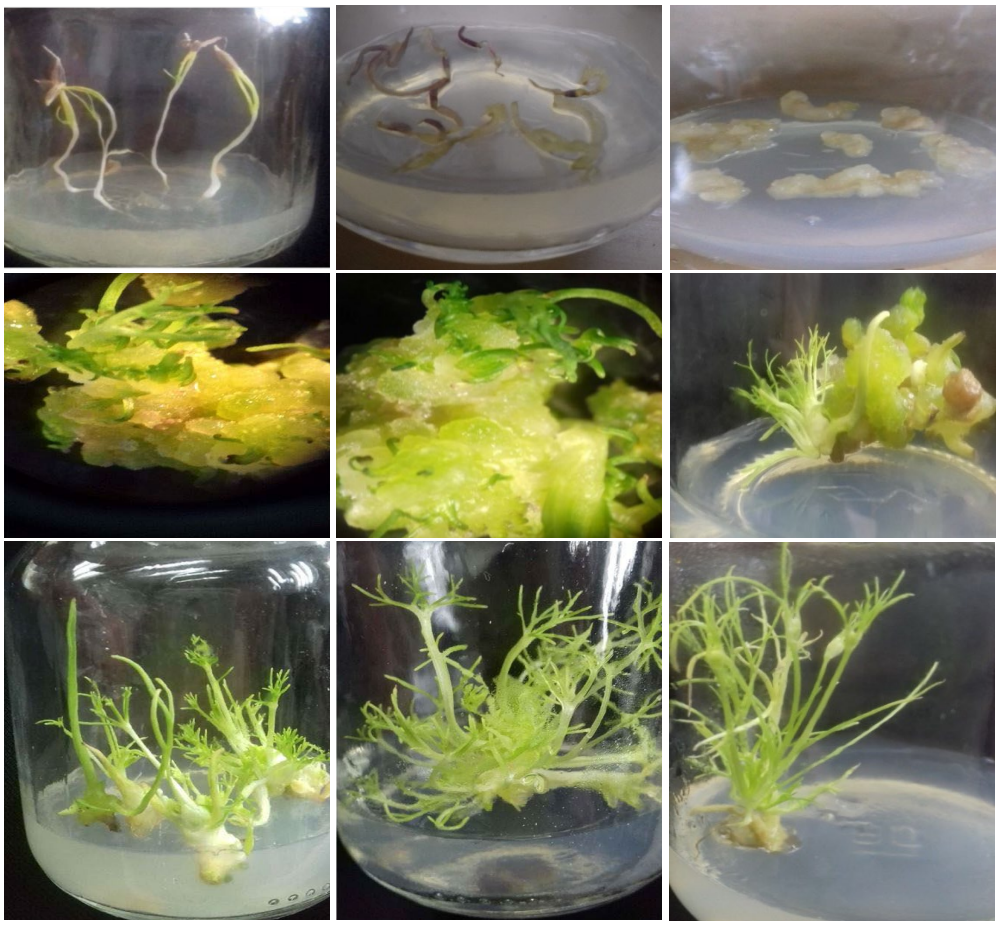

Fig 1. Stages of Plant regeneration from cumin tissue culture

Results

Tissue culture response

Generally, the results revealed that embryogenic callus with globular structures was developed from two cumin landraces and three types of cultured explants (cotyledon, hypocotyl and root) on MS medium supplemented with different growth regulators as shown in Fig. (1).

Callus formation (C %)

The results revealed that callus was developed from the cotyledon, hypocotyl and root of two cumin landraces on all tested growth regulators (Table 2).

Analysis of variance results (Table 3) showed that there were no significant differences among genotypes and growth regulator concentrations for callus formation percentage but there are significant differences ($p<0.01$) among explant types for callus formation percentage. So that the cotyledon and hypocotyl explant showed high percentage of callus formation (100% for each) than root explant (25.46%).

The results also revealed significant interaction between the type of explant, genotypes and growth regulators treatment. These results suggested that the explant performed differently from one genotype to another also from one growth regulator treatment to another (Table 2).

Table 2. Mean of cumin in vitro culture traits

		C %						RGE %						No. shoots/exp					
Parent	**Explant**	**M1**	**M2**	**M3**	**M4**	**AVR**	**Mean**	**M1**	**M2**	**M3**	**M4**	**AVR**	**Mean**	**M1**	**M2**	**M3**	**M4**	**AVR**	**Mean**
G1	**H**	100.00	100.00	100.00	100.00	100.00		16.67	12.96	18.52	22.22	17.59		14.33	74.00	41.67	116.67	61.67	
	C	100.00	100.00	100.00	100.00	100.00	73.77	51.85	9.26	9.26	11.11	20.37	16.05	216.67	5.67	2.67	196.67	105.42	59.36
	R	9.26	40.74	11.11	24.08	21.30		11.11	9.26	11.11	9.26	10.19		25.33	2.33	13.33	3.00	11.00	
G1	**H**	100.00	100.00	100.00	100.00	100.00		22.22	18.52	22.22	16.67	19.91		20.00	73.33	76.33	73.33	60.75	
	C	100.00	100.00	100.00	100.00	100.00	76.54	64.82	14.82	9.26	9.26	24.54	19.14	226.67	7.33	2.67	86.67	80.83	51.72
	R	25.92	12.96	51.85	27.78	29.63		14.82	14.82	12.96	9.26	12.96		25.00	10.33	17.00	2.00	13.58	
AVR	**H**	100.00	100.00	100.00	100.00	100.00		19.45	15.74	20.37	19.45	18.75		17.17	73.67	59.00	95.00	61.21	
	C	100.00	100.00	100.00	100.00	100.00		58.34	12.04	9.26	10.19	22.45		221.67	6.50	2.67	141.67	93.13	
	R	17.59	26.85	31.48	25.93	25.46		12.97	12.04	12.04	9.26	11.58		25.17	6.33	15.17	2.50	12.29	
Mean		72.53	75.62	77.16	75.31	75.15		30.25	13.27	13.89	12.96	17.59		88.00	28.83	25.61	79.72	55.54	
LSD 0.05	GENO	2.925						1.4584						4.7644					
	EXP	3.5825						1.7862						5.8352					
	TRT	4.1367						2.0625						6.7379					

G1 (Egyptian Landraces), G2 (Indian Landraces), H (Hypocotyl Explant), C (Cotyledon Explant), R (Root Explant), M1 (MS + 0.5mg/L 2,4-D), M2 (MS + 2mg/L BA+ 1mg/L IAA), M3 (MS + 1mg/L BA + 0.5mg/L IAA), M4 (MS + 0.5mg/L BA + 0.1mg/L IAA), (C %) Callus formation, (Reg %) Regeneration rate, (No. shoots/exp) Number of shoots per explant. AVR (average)

Generally, the results revealed that shoots were regenerated from the explants of all genotypes on all growth regulator treatments (Table 2). However, the rates of regeneration were varied depending upon the genotype, type of explant and concentration of the growth hormones.

The regeneration rate ranged from 16.05% in EGY genotype to 19.14% in IND genotype These differences between the genotypes were highly-significant (Table 3). Results showed that the cotyledon explants exceeded the hypocotyl and root explants in shoot formation (Table 3). These differences were highly significant (Table 3).

Table 3. Analysis of variance for the percentage of explant produced callus (C%), regeneration rate (Reg%) and number of shoots per explant (No. shoots/exp)

Source	DF	C %	REG %	No. shoots/exp
REP	2	21.2	101.53	104.6
GENO	1	138.9	171.53**	1050.3**
EXP	2	44446.7**	734.24**	39782.2**
CONCT	3	66.9	1283.88**	19484.5**
GENO*EXP	2	138.9	5.59	1310.4**
GENO*CONCT	3	407.5**	85.74**	3917.3**
EXP*CONCT	6	66.9	1099.01**	28370.6**
GENO*EXP*CONCT	6	407.5***	15.85	1277.9**
Error	46	38.0	9.45	100.8**
CV		8.2	17.47	18.08

Overall explants and genotypes, the M1 medium revealed the highest % of shoot formation (30.25%), while the M4 was the lowest one (12.96%) These differences between the medium were highly significant (Table 3). This indicating that the regeneration rate depends on the genotype, explant and growth hormones treatment.

Number of shoots per explant (No. shoots/exp)

Results in Table (2) revealed that the mean numbers of shoot per explant ranged from 51.72 in IND genotype to 59.36% in EGY genotype (Table 2). These differences between the genotypes were highly significant (Table 3).

Results in Table (3) also revealed that the mean numbers of shoot per explant ranged from 17.17 (on M1) to 95.0 (on M4) of the cultured hypocotyls, from 2.67(on M3) to 221.67 (on M1) of the cultured cotyledons and from 2.5 (on M4) to 25.17 (on M1) of the cultured roots. These differences between the type of explants were highly significant and suggested the cotyledon explants were exceeded the hypocotyl and root explants in shoot regeneration.

Overall explants and genotypes, the M1 and M4 media revealed the highest number of shoots/exp (88.0 and 79.2 shoots/exp, respectively) while the M2 and M3 showed the lowest mean values (28.83 and 25.61, respectively) (Table 2). These differences between the medium were highly significant (Table 3). This indicating that the regeneration rate depends on the genotype, explant and concentration of growth hormones. The different types of interactions were also significant suggesting that No. of shoots/explant depends upon the genotype, the explant, the concentration of growth hormones as well as the interaction between them (Table 3).

Genetic diversity between two cumin landraces

Ten ISSR and 10 SRAP marker systems being employed to assess the genetic variations between the two cumin landraces were quite informative and were able to generate adequate polymorphism. In total, six ISSR primer, and six SRAP primer combinations gave reproducible and polymorphic bands between the two cumin landraces. The twelve primers generated a total of 90 DNA bands, with an average of 7.5 bands per primer. Out of 90 DNA bands, 26 bands were polymorphic (28.89% polymorphism).

Somaclonal variation

In the present investigation, ISSR and SRAP molecular markers analysis were used to detect somaclonal variations in cumin plants regenerated from the two cumin landraces (donor parents).

Firstly, the two primers (844B ISSR and SRAP-3) which gave the highest polymorphism between donor parents were used to detect somoclonal variations among 60 regenerated plants for each donor parent. The results revealed that 45 (75%) regenerated plant for each donor parent were identical with its parent at DNA level in these genome sequences. While 15 (25%) regenerated plants were variation from their parents at DNA levels in these genome sequences.

Subsequently, six ISSR primers and six SRAP primer combinations which gave reproducible and polymorphic bands among the two landraces (donor parent) were used to examine the genetic diversity among the regenerated plants (15 somaclones for each donor parent) and their donor parents as well as among somaclones.

Table 4. Genetic marker information generated from cumin donor parents and its somaclones using ISSR and SRAP marker systems

	T.B	N.P.B	P.P.B	PIC.	MI.	RP.
SRAP-1	13	7	53.85	0.23	1.60	4.56
SRAP-2	11	11	100.00	0.28	3.08	4.31
SRAP-3	12	11	91.67	0.32	3.50	5.38
SRAP-4	11	11	100.00	0.42	4.60	7.31
SRAP-5	6	5	83.33	0.32	1.61	2.81
SRAP-6	14	9	64.29	0.27	2.43	6.44
ISSR-1	9	7	77.78	0.30	2.09	4.44
ISSR-2	9	3	33.33	0.14	0.43	2.00
ISSR-3	11	8	72.73	0.25	1.98	4.31
ISSR4	6	4	66.67	0.22	0.88	2.00
ISSR-5	8	4	50.00	0.23	0.93	2.94
ISSR-6	8	7	87.50	0.28	1.95	3.38
Total	118	87	--	--	--	--
Average	9.83	7.25	73.73	0.27	2.10	4.16

The two molecular marker systems generated a total of 118 bands across 32 somaclones and two donor parents out of them, 87 bands (73.73%) were polymorphic, and 31 bands (26.27%) were common between all genotypes (Table

4). The number of polymorphic bands ranged from 3 (ISSR-2) to 11 (SRAP-2, SRAP-3 and SRAP-4) with an average of 7.25 polymorphic bands per primer. The percentage of polymorphism across all primers ranged from maximum 100% (SRAP-2 and SRAP-4) to minimum 33.33% (ISSR-2). The PIC values ranged from 0.14 (ISSR-2) to 0.32 (SRAP-3) with an average 0.27. The MI The values ranged from 0.43 (ISSR-2) to 4.6 (SRAP-4). The highest RP value 7.31 was recorded for SRAP-4 while the minimum value 2.00 was recorded for ISSR-2 and ISSR-4 (Table 4). Figure 2, for example shows, the genetic variations between the donor parents and its regenerated plants using ISSR and SRAP primers.

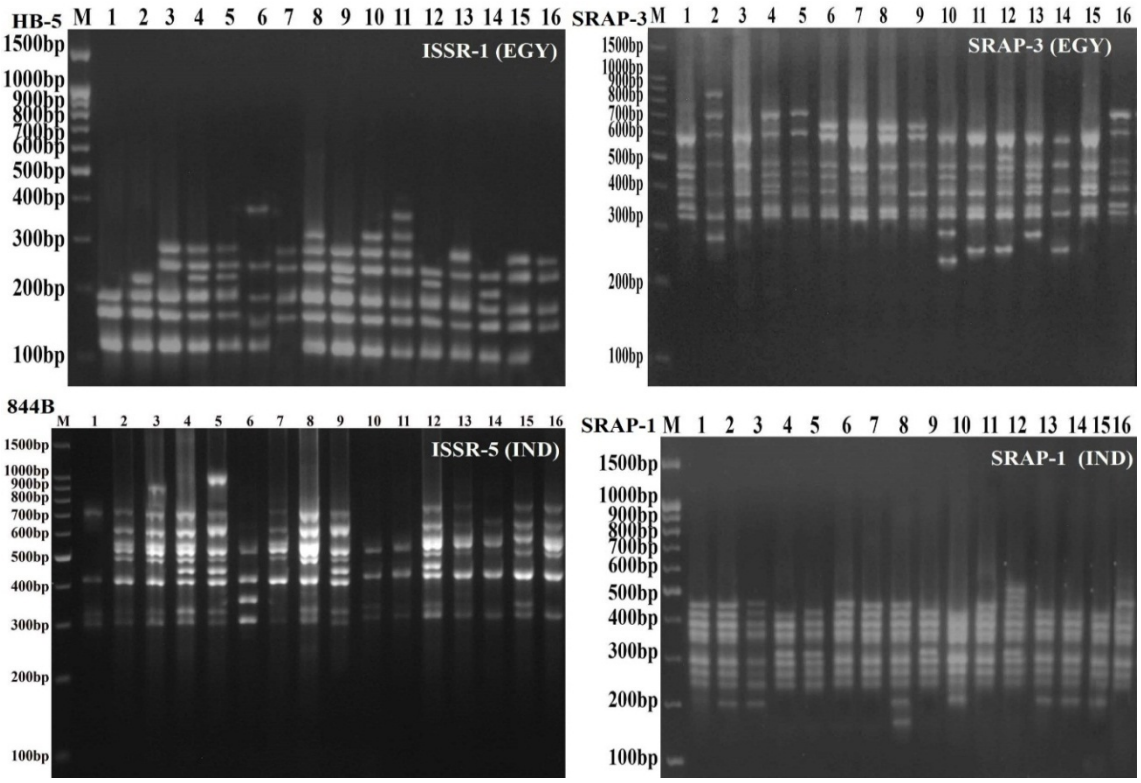

Fig. 2. SSR and SRAP profile of donor parents' plant (EGY and IND genotypes) and its somaclones Genetic variations between each donor parent and its regenerated plants (somaclones)

For Indian cumin landraces (IND donor parent) and its regenerated plants, both ISSR and SRAP markers generated a total of 118 DNA fragments, 76 bands of them (64.41%) were polymorphic. Out of the 76 polymorphic bands, 36 were parental bands which were absence in some somaclones and 40 were novel non-parental bands which were appeared only in some somaclones.

For Egyptian cumin landraces (EGY donor parent) and its regenerated plants, both ISSR and SRAP markers generated a total of 118 DNA fragments, 81 bands of them (68.64%) were polymorphic. Out of the 81 polymorphic bands, 39 were parental bands which were absence in some somaclones and 42 were novel non-parental bands which were appeared only in some somaclones.

Genetic similarity

The genetic similarity among all somaclones and their donor plants ranged from minimum 0.60 between somaclones EGY13 and IND8 to maximum 0.89 between somaclones EGY4 and EGY8 with an average of 0.745. The dendrogram generated based on a combined ISSR and SRAP data set revealed a better representation of the relationship between the tested cumin genotypes than individual markers (Fig. 3). The dendrogram grouped all somaclones and their donor plants into two major groups with five clusters. The first group comprised of cluster-1 which included the Egyptian landrace (donor parent EGY) and its regenerated plants (14 somaclones) as well as somaclones IND1 and somaclones EGY12 which separated in a single branch in the second cluster. This second group include divided into three cluster, cluster 3,4 and 5. This group include the Indian landraces and its regenerated plants except somaclones IND1 which separated in the first group.

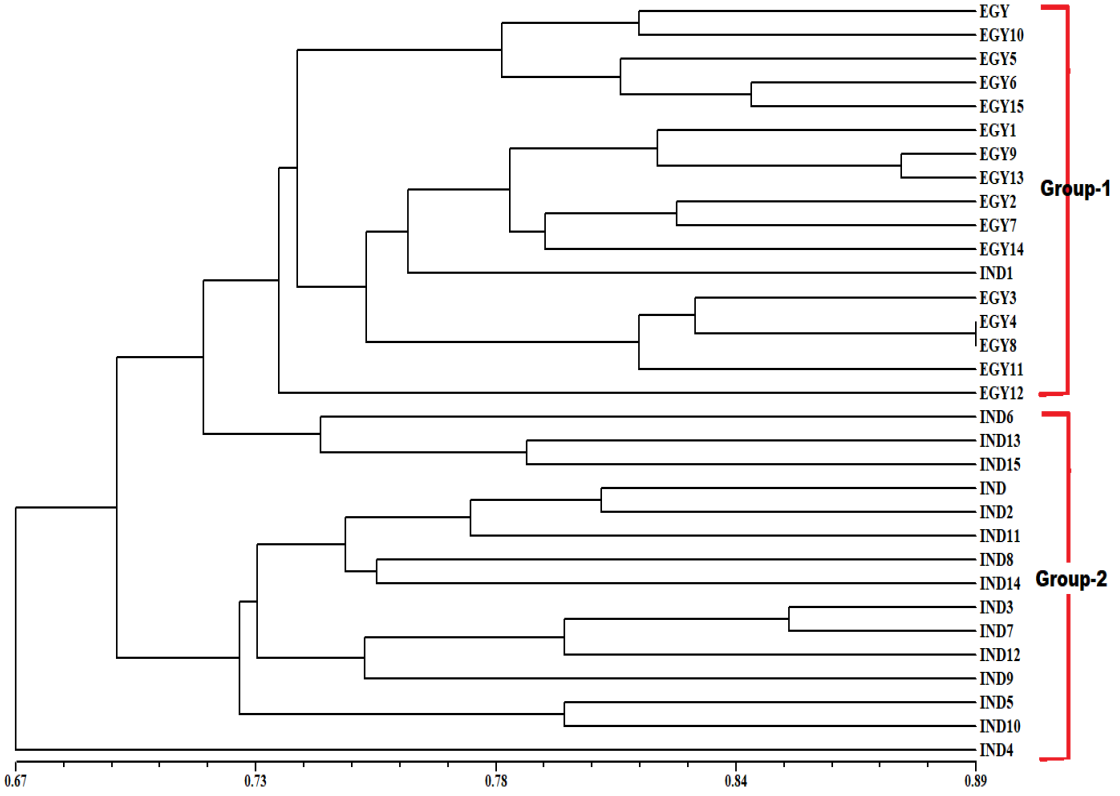

Fig. 3. Endrogram of two cumin landraces (donor parents) and its somaclones developed from ISSR and SRAP data

The genetic similarity between the EGY donor parent and its somaclones was ranged between 0.71 and 0.84 with an average 0.76. The highest genetic similarity was recorded between the donor parent and EGY10 somaclones (0.84), while the lowest value (0.71) was found between the donor parent and EGY3 somaclones which had the highest degree of genetic changes from the donor parent. Similarity index among the somaclones also ranged from 0.68 to 0.93. Close genetic similarity was found between the EGY4 and EGY8 (0.93), while the lowest similarity was recorded between EGY6 and EGY12 (0.68).

The genetic similarity between the IND donor parent and its somaclones was ranged between 0.78 and 0.86 with an average 0.82. The highest genetic similarity was recorded between the donor parent and IND2, IND3 and IND7 somaclones (0.86), while the lowest value (0.78) was found between the donor parent and IND1 somaclones which had the highest degree of genetic changes from the donor parent. Similarity index among the somaclones also ranged from 0.78 to 0.89. Close genetic similarity was found between the IND3 and IND7 (0.89), while the lowest similarity was recorded between IND14 and IND15 (0.78).

Discussion

The present investigation was carried out to establishment efficient protocol tissue culture and analyze the somaclonal variations among plants regenerated from i*n vitro* culture of cumin. Two cumin landraces as donor parents (Egyptian and Indian landraces), three types of explants (cotyledon, hypocotyl and root) and four concentrations of growth regulators were used to establish plant regeneration. Several previous studies on cumin *in vitro* culture referred that, callus induction and plant regeneration are affected by many factors such as genotype, explant type, growth regulators such as (auxins and cytokinin's) and environmental conditions (Safarnejad, 2011; Soorni *et al.,* 2012; Praveen *et al.,* 2011). These results showed that the variations between EGY and IND genotypes were significant for Reg rate and No. shoots/exp while not significant for callus formation. These differences in response to tissue culture from one genotype to another were affected by type of explants and type and concentration of growth regulators in culture media as shown Table (2). The effect of genotype on response to tissue culture in cumin were also observed by (Soorni and Kahrizi, 2015; Kanani *et al.,* 2019) who reported that the in vitro reactions of the genotypes to regeneration ability were differed significantly and dependent on the culture medium. In the present investigation three types of explants, cotyledon, hypocotyl and root were exhibited varied response for tissue culture traits, callus formation and plant regeneration rate. However, the cotyledonary explant exceeded the hypocotyls and root in shoot regeneration on culture media under different concentrations of growth regulators. These results agree with (Jabeen *et al.,* 2005; Ali *et al.,* 2012; Sharma and Srivastava 2014; Soorni *et al.,* 2015; Gerszberg *et al.,* 2016) who found that the cotyledonary explants produced the highest number of shoots. These results revealed that the interactions among three factors genotypes, type of explant and concentration of growth were also significant suggesting that plants regeneration depends upon the genotype, the explant, the concentration of growth regulators as well as the interaction between them. Overall varieties, type of explants and growth regulators, results indicated that cotyledon were the best explant for regeneration in cumin genotypes and the M1 medium (0.5mg/l 2,4-D) was the best for tissue culture response in cumin genotype. Regenerated plants from the two donor parents were subjected to analysis of somaclonal variation through molecular markers analysis. ISSR and SRAP were used to study the genetic variation among the donor parents. Both markers differ in mechanism principle, reproducibility, distribution in the plant genome and amount of polymorphism (Zietkiewicz *et al.,*

1994; Provan *et al.,* 1999; Li and Quiros 2001). Both marker systems were quite informative and were able to generate adequate polymorphism for identification of these genotypes. six ISSR and six SRAP primers generated a total of 90 bands through the two landraces (donor parents). The number of generated DNA fragments (67 bands) using SRAP primers was more than the DNA fragments (51 bands) amplified by ISSR primers although both SRAP and ISSR markers exhibited the same percentage of polymorphism (20%) among donor parents. The two marker systems were successful in characterizing the two cumin landraces by unique positive and/or negative markers. The present finding is consistent with the earlier report of Henareh *et al.* (2016); Kiani and Siahchehreh (2018). In our study, the ISSR and SRAP primers were used to analysis somaclonal among regenerated plants from two donor parent. The results revealed that both marker systems were sufficient to detect polymorphism among each donor parent and its somaclones, as well as among somaclones. The percentage of polymorphism among each donor parent and its somaclones was different from one parent to another.

Our results revealed that the highest polymorphism was found between the donor parent EGY and its somaclones as well as among somaclones themselves while the lowest polymorphism was found between the donor parent IND and its somaclones. These results indicate that the molecular changes which occurred in cumin tissue culture depending on the genotype (Soniya *et al.,* 2001). All polymorphic bands among parent donor and its somaclones were occurred in parental bands which were absence in some somaclones and/or new non-parental bands which were generated only in some somaclones. This result is in line with determination of genetic polymorphism of somaclones in cumin, biochemical markers (Gupta, 2013), RAPD (Bahmankar *et al.,* 2019; Bahraminejad *et al.,* 2012; Soniya *et al.,* 2001), SRAP (Bhatt *et al*., 2017).

The variations observed in the ISSR or SRAP patterns may be due to different causes including loss or gain of a primer annealing, due to point mutations or by the insertion or deletion of sequence or transposable elements (Peschke *et al.,* 1991).

Conclusion

This study has shown that in *in vitro* culture of cumin, cotyledonary explants was better responsive in terms of number of shoots per explant than hypocotyl and root explants and showed that the MS medium with 0.5mg/L 2,4-D was better for shoots regeneration compared with MS basal supplemented with BA in conjunction with IAA from cotyledonary explants. These results suggest that the genotypes, explant and plant growth regulators are important factors affected induction of somaclonal variants in cumin tissue culture. Thus, the results suggest that testing a large number of different genotypes and different combinations of plant growth regulators giving a good chance of obtaining a high percentage of genetic variation among regenerated plants that can be exploited in different breeding programs. The results also refer to the importance of using different

molecular marker systems in early detection of the extent of molecular changes occurring in plants regenerated from tissue cultures.

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توطيد برتوكول فعال لزراعة الأنسجة وفحص الاختلافات الوراثية بين النباتات المتكشفة في الكمون

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الملخص

تم اختبار الاختلافات الوراثة الناتجة عن مزارع الكمون باستخدام التحليل الجزيئي. استخدم في هذا البحث اثنين من السلالات الارضية لنبات الكمون كآباء (سلالة مصرية وسلالة هندية) وثلاثة أنواع من الاجزاء النباتية (الأوراق الفلقية والسويقة تحت الفلقية والجذور) وأربع تركيزات مختلفة من منظمات النمو على بيئة موراشيجي وسكوج لدراسة تأثير التركيب الوراثي والأجزاء النباتية ومنظمات النمو على تكشف الكالوس وتكشف النباتات في الكمون. اظهرت النتائج فروق معنوية جداً في معدل تكشف النباتات وعدد النباتات المتكشفة لكل جزء نباتي، اعتمدت هذه الاختلافات على التركيب الوراثي والأجزاء النباتية المستخدمة ونوع وتركيز منظمات النمو. تم اختيار أفضل بيئة لتكشف النباتات (بيئة MS + 0.5 ملليجرام/لتر 2,4-D) للزراعة عليها. تم اختيار الآباء المعطية (السلالة المصرية والسلالة الهندية) والنباتات المتكشفة عنهما من مزارع الانسجة لدراسة الاختلافات الوراثية باستخدام الواسمات الجزيئية. استخدم الواسم الجزيئي ISSR والواسم الجزيئي SRAP لدراسة الاختلافات الوراثية في الكمون بين الآباء المعطية (السلالة المصرية والسلالة الهندية). الواسمات الجزيئية التي أظهرت معدلات عالية لتعدد الأشكال بين الآباء تم استخدامها لدراسة الاختلافات الوراثية بين الأب المعطي والنباتات المتكشفة منه في السلالتين وكذلك الاختلافات الوراثية بين النباتات المتكشفة من مزارع الانسجة وبعضها. أظهرت هذه الواسمات اختلافات واضحة بين الأباء والنباتات المتكشفة عنها في مزارع الانسجة.