

Somatic Embryogenesis and Genetic Fidelity in Camelina by RAPD Markers and Flow Cytometry

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Abstract

Camelina (*Camelina sativa* L. Crantz) is an oily, medicinal plant that is a member of the Brassicaceae family. It has a lot of important agronomic characteristics, including as good environmental adaptability and tolerance to cold, heat, pests, and diseases. The present investigation aimed to improve somatic embryogenesis, and camelina regeneration, and evaluate genetic stability in the regenerated plantlets using RAPD markers and flow cytometry. Two explants of the hypocotyl and cotyledon were used, together with four different combinations of PGRs made up of NAA, BAP, 2,4-D, and Kin, to optimize somatic embryogenesis and regeneration in Camelina. Because several somatic embryogenesis developmental phases could be observed on embryogenic calluses at the same time, the results suggested that somatic embryogenesis in camelina is a simultaneous occurrence. The greatest rate of somatic embryogenesis and regeneration was seen in the cotyledon explant grown in the MS + 0.3 mgL⁻¹ NAA + 0.7 mgL⁻¹ BAP. The results also revealed that the MS + 0.25 mgL⁻¹ IAA + 0.5 mgL⁻¹ NAA had the best rooting response and a favorable seedling survival rate. In the present work, RAPD markers were utilized for the first time in camelina to confirm the genetic fidelity of in vitro regenerated plants and their donor mother plant. The amplified products showed 64 different, scorable bands, and the regenerated plants were an identical replica of their donor mother plants. The fidelity of the ploidy level was assessed by flow cytometry, and the findings confirmed monomorphic patterns in both the regenerated plants and their donor mother plants. According to the present findings, it can be said generally that somatic embryogenesis may be advantageous for large-scale multiplication, breeding programs, and in vitro conservation in camelina.

Key Message

It has been shown that the type of auxin and cytokinin employed during camelina embryogenesis and regeneration has a significant influence on the procedure. It is better for these operations to use a cotyledon explant. In camelina, somatic embryogenesis occurs concurrently, and the genetic similarity of the donor mother plant and the in vitro regenerated plants was confirmed using RAPD markers and flow cytometry.

Introduction

Camelina (*Camelina sativa* L. Crantz) is an oily, medicinal plant of the Brassicaceae family that exhibits significant agronomic traits like tolerance to cold, heat, pests, and diseases, as well as high environmental adaptability (Yemets et al. 2013; Yuan and Li 2020). Camelina seeds have about 40% oil content. Linolenic acid (omega-3) and linoleic acid (omega-6), the two primary fatty acids found in camelina seed, make up about 40% of its oil content. The researchers also noted that camelina oil is beneficial for reducing blood cholesterol and inflammation since it is high in biological ingredients like tocopherols and phenol (Yuan and Li 2020; Mondor and Hernandez-Alvarez 2022). Camelina has gained

increasing economic attention due to its agronomic benefits as well as its medical, and industrial qualities (Obour K 2015; Yuan and Li 2020). Around 1500 BC, camelina seeds were used for agriculture, according to archaeological studies conducted in European nations. In the 20th century, there has also lately been information about its use in oilseed products (Zubr 1997; Bujnovsky et al. 2020). Camelina is superior to other oilseeds in terms of generation time and program ease. Thus, the quality of seeds and other agronomic traits can be quickly improved. (Yemets et al. 2013; Yuan and Li 2020). One of the goals of the programs is to improve Camelina's fatty acid profile through biotechnology (Frohlich and Rice 2005). Any plant species that will be subjected to genetic engineering must have an efficient, fast, and repeatable in vitro regeneration technique (Pandey et al. 2013; Bahmankar et al. 2017). One of the most crucial methods used in biotechnology research, including the creation of transgenic plants, artificial seeds, micropropagation, and germplasm protection, is somatic embryogenesis. Genetic characteristics, physiological traits, and cell state in plant tissues are all factors that determine somatic embryogenesis in plants (Bahmankar et al. 2017, 2018). The cotyledon explant showed a greater response to regeneration than the explants of the root and hypocotyl, according to one of the earliest tissue culture experiments on camelina, and the optimal hormonal composition had 4.4 M BAP and 0.54 M NAA (Tattersall and Millam 1998). Another study utilizing petiole explants in MS media supplemented with 4 mg/L BAP and 0.1 mg/L NAA observed the greatest rate of regeneration (Yemets et al. 2013). To our knowledge, the somaclonal diversity of regenerated plants has not been taken into consideration, despite the few studies on camelina tissue culture that have been described. Somaclonal variation is a frequent occurrence in plant tissue culture, necessitating a comparison of the regenerated plants' genetic faithfulness to their donor mother plants (Bahmankar et al. 2017). A few of the reported reasons for the phenomenon of somaclonal variety include changes in DNA methylation, cytogenetic diversity, adjustments to the DNA sequence, and epigenetic changes (Bahmankar et al. 2017; Saadat et al. 2023). Molecular markers are primarily a useful method for assessing genetic fidelity at the DNA level, but because these techniques are ineffective at detecting changes in ploidy level and cytogenetic variety, flow cytometric analysis is more effective. Researchers believe that flow cytometry is an effective and quick approach for recognizing changes in the ploidy level of regenerated plants, which will help to analyze the somaclonal variety of various plants (Carlioni et al. 2014; Jena et al. 2018; Abdolinejad et al. 2020). Some researchers have noted somatic variety in tissue culture studies, whereas others are convinced that tissue-cultured plants retain their genetic fidelity (Bakhtiar et al. 2014; Bahmankar et al. 2017). Although there have only been a few studies on camelina tissue culture, to our knowledge, no studies have been published to evaluate its genetic fidelity. The current work aims to evaluate somatic embryogenesis, regeneration, and genetic fidelity in regenerated plants in comparison to their donor mother plants using RAPD markers and flow cytometry studies.

Materials and Methods

Somatic Embryogenesis and Regeneration Optimization

Using 1.5% sodium hypochlorite solution for 8 minutes and 70% ethanol for 2 minutes, camelina seeds were surface sterilized. The prepared seeds were grown in MS media (Duchefa, Haarlem, Netherlands)

(Murashige and Skoog 1962) and kept at $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the growth chamber (Bahmankar et al. 2017). From in vitro seedlings that were 7 days old, cotyledon and hypocotyl explants (0.5 cm in length) were made. Then, they were cultivated in the MS medium, which has 0.8% (w/v) agar and 3.0% (w/v) sucrose (Duchefa), and they were supplemented with 4 distinct PGRs (Duchefa) (Table 1).

Table 1
The various PGR combinations utilized in the current study

Medium Code	NAA (mgL^{-1})	BAP (mgL^{-1})	2,4-D (mgL^{-1})	Kin (mgL^{-1})
A	0.1	0.5	-	-
B	0.3	0.7	-	-
C	-	-	0.1	0.5
D	-	-	0.3	0.7

The cultured explants were moved to the growth chamber, which was set to 24°C and 2°C with 75% relative humidity. For the first two weeks of the culture, the media was kept in the dark. The generated calli were subsequently located in a growth room with a 16-hour photoperiod, a cool white 18-W fluorescent light producing $50\text{ mol}^2\text{ s}^{-1}$ of photosynthetic photon flux density, and a temperature of $24^{\circ}\text{C} \pm 2^{\circ}$. This process was repeated every two weeks. Explant responses were examined in terms of callus formation %, somatic embryo percentage, and regeneration after approximately 12 weeks. Five weeks following the first culture, a callus development % was noted. By the ninth week, somatic embryogenesis indications were visible in some of the studied mediums without transferring the calli to the PGRs-free medium. Different stages of somatic embryogenesis were documented using a stereomicroscope (Olympus Model SZ-PT, Shinjuku, Tokyo, Japan) and a Canon PowerShot SX50 HS camera (Ohta-Ku, Tokyo, Japan). The ratio of somatic embryogenesis-producing explants to all other explants utilized in culture was used to compute the somatic embryogenic percentage. The plant regeneration percentage was calculated after the first culture had been in place for 12 weeks by dividing the number of regenerated explants by the number of embryogenic calli.

Experimental Design and Data Analysis

Three replications (as Petri dishes) of a factorial experiment with a completely randomized design were used to carry out the project. Four different PGR combinations and two hypocotyl and cotyledon explants were among the factors that were evaluated. Each Petri plate had a different medium, and five explants were cultivated in each dish. To determine the statistical significance, related data on the proportion of callus generation was treated to ANOVA and LSD. To account for the lack of a normal distribution for certain features, such as embryogenesis and regeneration%, as well as the fact that the normal distribution had not been established by any of the data processing techniques, the Kruskal-Wallis and Mann-Whitney non-parametric tests were applied, and median clustering was carried out. The data was analyzed using Excel and the SAS 9.2 software programs.

In Vitro Rooting and Acclimatization

The regenerated 3 to 4-cm tall seedlings were removed from the media and put into pots containing half-strength MS medium supplemented with the various PGR concentrations mentioned in Table 2 because the best regenerative response was seen in MS + 0.3 mgL⁻¹ NAA + 0.7 mgL⁻¹ BAP.

Table 2
Different concentrations of PGRs used in
camelina rooting experiment

Medium Code	IAA (mgL ⁻¹)	NAA (mgL ⁻¹)
A	-	-
B	0.25	0
C	0	0.25
D	0.25	0.5

Every three to four days, the experiment was checked, and the days to root production and the rooting % were noted. The mature, rooted seedlings were placed in sterilized garden soil-filled plastic pots after three weeks. The pots were placed in the greenhouse for two weeks with plastic covers over them to keep the plants moist. As the seedlings adjusted, the plastic cover was progressively removed. The proportion of plantlets that survived was noted, and they were kept alive until flowering and seed production.

Designing experiments and data analysis

The experiment used a three-replication, completely random design, and the means were compared using the LSD test ($p \leq 5\%$) and expressed as the mean $\pm$ SE.

Evaluation of Genetic Fidelity

Total genomic DNA was isolated from young, fresh leaves of the regenerated camelina and donor mother plants using a modified version of the CTAB method (Murray and Thompson 1980). The purity and concentration of the extracted genomic DNA were assessed on a 1% agarose gel and spectrophotometer, and the final concentration of the samples was reduced to 50 ng/l. For DNA amplification, a total of 10 RAPD markers were applied (OPU6, OPU12, OPS17, OPJ18, OPJ19, OPJ21, OPC13, OPC8, OPQ6, and OPF5) (Table 5) (Bahmankar et al. 2017). 25 ml of double-distilled water, 12.5 ml of PCR amplification kit (10x), 1 ml of primer (10 pmol), and 2 ml of DNA (50 ng/ml) were used to conduct the PCR reaction. A 5-min initial denaturation stage at 95°C was followed by 30 cycles of 1 min at 95°C, 30 sec at 51–54°C (depending on the primers used), and 2 min at 72°C, as well as an 8–min extension step at 72°C. The amplified products (Gel Logic 212 PRO, Rochester, NY) were separated by electrophoresis on 1% agarose gels, stained with ethidium bromide, and irradiated by UV light for visualization.

Flow cytometric (FCM) analysis

In FCM studies using the propidium iodide (PI) staining technique, *Solanum lycopersicum* cv. Stupicke (2C DNA = 1.96 pg; Dolezel et al. 2007) was utilized as an internal reference standard plant. Sharp razor blades were used to chop 1 cm of *Camelina sativa* (L.) Crantz. and *Solanum lycopersicum* cv. Stupicke leaves in 1 ml of woody plant buffer (WPB) in a petri dish. The nuclei suspension was then passed through a 30 m nylon mesh filter made by Partec (Munster, Germany)(Loureiro et al. 2007). The nuclei suspension was then injected with 50 l of PI and 50 l of RNase. The amount of genomic 2C DNA contained in the nuclei suspension was assessed using BD FACSDiva™ software on a BD FACSCanto™-KE flow cytometer (BD Biosciences, Bedford, MA, USA) equipped with an argon ion laser (488 nm). Partec (Partec, Munster, Germany) FloMax ver. 2.4e was used to generate histograms, which were then analyzed over three replications of the genome size data. The relative fluorescence intensity of tagged nuclei was calculated on a linear scale. The absolute DNA content of a sample was calculated using the values of the G1 peak means (Dolezel et al. 2003; Dolezel et al. 2007), as follows: Sample 2C DNA quantity (pg) and sample 2C DNA content (pg) are equal to sample G1 peak mean/standard G 1 peak mean. The 1Cx-DNA value was calculated using a conversion method suggested by (Dolezel et al. 2003) where 1 pg of DNA equals 978 mega base pairs (Mbp). The typical coefficients of variation (CV%) for FCM measurements were less than 5%.

Results and Discussion

In the current study, the impact of two explants of cotyledon and hypocotyl under various types and concentrations of plant growth regulators was examined in order to optimize somatic embryogenesis and regeneration in camelina. Different investigated treatment combinations produced both non-embryogenic and embryogenic calluses. Embryonic calluses were tangled and dense. Compared to embryonic calli, non-embryonic calli were clumpier, more fragile, and bigger. The majority of embryonic calluses were lemon green, while non-embryonic calluses were grayish-white and pale white (Fig. 1). It is remarkable that calluses simultaneously displayed various somatic embryogenesis stages. According to this phenomenon, camelina somatic embryogenesis occurs simultaneously (Fig. 2). Although the number of produced embryos increased with prolonged incubation, not all of the embryonic calluses were able to create spherical embryos. It should be emphasized that not all calluses have the same capacity for embryogenesis; only spherical calluses with a smooth surface possess this capacity; brittle and rough calluses do not (Quiroz-Figueroa et al. 2002; Bahmankar et al. 2017). The current investigation found that while the main effects were significant the interaction impact of explant and culture medium on callus formation percentage was not significant (Fig. 3). According to the findings of a mean comparison, cotyledon explants responded better than hypocotyl explants in terms of callus creation percentage. The MS medium supplemented with $0.3 \text{ NAA mgL}^{-1} + 0.7 \text{ BAP mgL}^{-1}$ also produced the highest percentage of callus development (Fig. 3).

Embryonic (right) and non-embryonic (left) calli in Camelina

The findings suggested that, for the treatment combinations of hypocotyl explant in MS medium completed with $0.1 \text{ mgL}^{-1} \text{ NAA} + 0.5 \text{ BAP mgL}^{-1}$ and cotyledon explant in MS medium completed with

0.3 NAA mgL⁻¹ + 0.7 mgL⁻¹ BAP, respectively, the minimum and maximum percentages of calli with embryogenesis and regeneration were achieved (Fig. 4). If cotyledon explants were employed, the findings of the slicing interaction demonstrated that PGRs had a considerable impact on somatic embryogenesis in both investigated explants. Accordingly, the MS medium supplemented with 0.3 mgL⁻¹ NAA + 0.7 BAP mgL⁻¹ showed the maximum embryogenesis (Table 3). The findings also demonstrated that somatic embryogenesis could be seen using hypocotyl explants, however its rate was considerably lower than that of cotyledon (Fig. 4). According to the results of the slicing interaction on regeneration, PGRs had a significant impact on regeneration only when employing cotyledon explants, and the highest rate was seen in cotyledon explants in MS media supplemented with 0.3 NAA mgL⁻¹ + 0.7 BAP mgL⁻¹ (Table 3, Fig. 4). Somatic embryogenesis and regeneration were maximized in the cotyledon explant in MS media supplemented with 0.3 NAA mgL⁻¹ + 0.7 BAP mgL⁻¹, which was found to be the most effective treatment combination. Researchers in a different study also highlighted the proper response of cotyledon explants in camelina tissue culture (Tattersall and Millam 1998). In the present study, the embryogenesis and regeneration of the cotyledon explant were superior to those of the hypocotyl.

It is notable that one of the important elements influencing the ability of explants to develop embryos is the quantity of endogenous hormones. Different explants have varying levels and types of endogenous hormones, which affects their capacity to develop into embryos (Gaj 2004).

Table 3
Slicing of the interaction effect of explant on the various levels of PGRs for somatic embryogenesis and plant regeneration in camelina

Source of Variance	Degree of Freedom	Somatic Embryogenesis	Plant Regeneration
Cotyledon	3	0.0001**	0.002*
Hypocotyl	3	0.02*	0.7 ns

ns, *, and ** indicate non-significant and significant at 5% and 1% levels of probability, respectively.

The number of embryos created is minimal in comparison to the number of cells in the cell mass, even when all cells are assumed to have equal potential. Because of this, somatic embryogenesis may only occur in a small portion of the cell mass and is restricted to a certain type of plant tissue (Quiroz-Figueroa et al. 2002; Toonen et al. 2004; Bahmankar et al. 2017). Researchers found that the embryogenesis potential in tissues produced from embryos is high and diminishes towards the root and hypocotyl (Mujib et al. 2016; Bahmankar et al. 2017). It can be concluded that in addition to the type of explant, the type of PGRs has also been effective on embryogenesis and regeneration in camelina because in the current study, embryogenesis and subsequent regeneration in the treatment combination consisting of auxin-type of NAA and cytokinin-type of BAP showed a better response than the treatment combination of auxin-type of 2,4-D and cytokinin-type of Kin. It is interesting that the auxin-type 2,4-D treatment combinations demonstrated a noticeably inferior response in terms of embryogenesis and regeneration (Fig. 4). Researchers have hypothesized that this behavior is caused by 2,4-D, a very stable auxin that has

demonstrated strong resistance to enzymatic degradation and has formed strong associations with plant cells, which are gradually eliminated from callus tissue (Ammirato and Steward 1971; Lad et al. 1997; Bahmankar et al. 2017). Even though explant cells need exogenous auxin to divide into mitosis, further auxin depletion in the callus is necessary for the advancement of embryogenic morphogenesis (Feher 2003; Lim et al. 2009). Because 2,4-D was likely eliminated from callus tissue in the current study faster than auxin-type NAA, somatic embryo growth, and regeneration were higher in the treatment combinations containing NAA auxin (Fig. 4). Other studies have also revealed that the most crucial variables in somatic embryogenesis are the types and amounts of growth regulators as well as the physiological condition of explants (Tawfik and Noga 2002; Nic-Can et al. 2015). The capacity for somatic embryogenesis and their regeneration is generally influenced by variables such as types of explants, type of PGRs, developmental stage of donor plants, and position of cells in cultured plant tissue.

Rooting and Adaptation

The findings showed that IAA (0.25 mgL^{-1}) + NAA (0.5 mgL^{-1}) treatment resulted in faster rooting in seedlings than other treatments, and the highest percentage of rooting was found in this treatment (Table 4). According to the findings, auxin IAA and NAA together have strengthening effects on rooting properties and function as a complement (Fig. 5, Table 4). The auxin-containing media are often where roots form in plants (Reis et al. 2017; Bahmankar et al. 2018).

Limited rooting was also obtained in the current study's auxin-free medium, which may be related to plant genotype and levels of endogenous hormones (Table 4). Researchers in other plant species discovered that using IAA and NAA simultaneously was successful in promoting root induction in regenerated plants (Asadi et al. 2009; Bahmankar et al. 2017). Increased NAA content in culture medium has been demonstrated in several experiments to greatly promote plant rooting (Bahmankar et al. 2017; Emoghene et al. 2018).

Table 4
Comparisons of the means of the effects of various media on the survival and roots of regenerated plantlets in *Camelina*

Medium	Days to Rooting	Rooting %	Ex vitro survival%
PGRs-Free	19.33 ± 0.66^a	0.1 ± 0.0^c	0^c
IAA	10.33 ± 0.33^b	0.33 ± 0.16^{bc}	0.33 ± 0.16^{bc}
NAA	10.33 ± 0.66^b	0.36 ± 0.06^b	0.47 ± 0.07^{ab}
IAA + NAA	7.66 ± 0.33^c	0.7 ± 0.05^a	0.7 ± 0.07^a

According to the DMRT test at $P < 0.05$, the mean values in each column are followed by various letters to show significant differences.

The employed treatments greatly outperformed the control in terms of seedling survival percentage, which was desirable (Fig. 5). One of the problems with plant micropropagation is the survival rate of seedlings when moved to natural environments for adaptation to the new climate in the greenhouse or field. Additionally, important elements for the establishment and survival of regenerated seedlings include the type of plant, the environment, and the potting soil (Tawfik and Noga 2001). In tissue culture experiments, regenerated plants' viability is typically reduced due to a number of factors, including low photosynthetic capacity caused by the presence of sugar in the culture medium, low light, insufficient CO₂, and high relative humidity in the culture medium, all of which negatively impact the plant's photosynthetic efficiency and survival rate (Hazarika 2006).

Genetic Fidelity of Regenerated Plantlets

Somaclonal variation is a genetic and epigenetic phenomenon that affects the nucleus' genome and cytoplasm. It is typically seen in plants that have been cultured in vitro (Bahmankar et al., 2017; Larkin & Scowcroft, 1981; Nwauzoma & Jaja, 2013). Ten RAPD primers were first employed, nine of which produced obvious bands, ranging in size from roughly 200 bp to 1500 bp, to examine the genetic fidelity of in vitro regenerated plants in comparison to their donor mother plants (Table 5).

Table 5
Information regarding the RAPD markers that were examined for determining the genetic fidelity of the regenerated plants.

Primer	Sequence (5'→3')	Annealing Temperature (°C)	Number of Amplified Bands	Number of monomorphic Bands	Number of Polymorphic Bands	Band Size (bp)
OPU6	ACCTTT GCGG	35	6	6	0	200– 1500
OPU12	TCACCAGCCA	35	6	6	0	200– 1500
OPS17	TGGGGACCAC	35	7	7	0	200– 1500
OPJ18	TGGTCGCAGA	35	4	4	0	500– 1100
OPJ19*	GGACACCACT	35	-	-	-	-
OPJ21	ACGAGGGACT	35	6	6	0	200– 1500
OPC13	AAGCCTCGTC	35	11	11	0	200– 1500
OPC8	TGGACCGGTG	35	8	8	0	200– 1500
OPQ6	GAGCGCCTTG	35	9	9	0	200– 1500
OPF5	CCGAATTCC C	35	7	7	0	200– 1500

*: Not repeatable.

Nine carefully chosen primers produced a total of 64 scorable bands, with an average of 7.11 bands being produced by each primer (Table 5, Fig. 6). There were 4 to 11 bands produced by each primer (Table 5). The primers OPJ18 produced a minimum of 4 bands, whereas OPC13 followed by OPQ6, which produced 9 bands, provided a maximum of 11 bands (Table 5, Fig. 6). According to our findings, the donor mother plant and all regenerated plants were monomorphic in all the scorable bands. In comparison to donor mother plants, the monomorphic band pattern produced by RAPD markers demonstrated the genetic integrity in regenerated plants, showing the absence of somaclonal variation.

RAPD markers have also proven that other plants lack somaclonal variation (Borsai et al., 2020; Singh et al., 2016; Thakur et al., 2021). Although somaclonal diversity in plant tissue culture may result in the creation of novel plant cultivars, researchers think that this phenomenon has detrimental consequences on the quick spread of superior plant clones (Bahmankar et al., 2017; Saadat et al., 2023). Flow cytometry was used to assess the somaclonal variation of in vitro regenerated plants in comparison to their donor

mother plants. The camelina was the left-most peak in the flow cytometric histograms, and the *Solanum lycopersicum* cv. Stupicke (2C DNA = 1.96 pg), which served as the internal standard, was the right-most peak (Fig. 7).

The average amount of 2C DNA in the donor mother plants and the regenerated camelina plants was 1.5 ± 0.06 pg and 1.5 ± 0.04 pg, respectively. These values are equivalent to previous research (Martin et al. 2017; Chaudhary et al. 2020). The present study's findings showed that the DNA in regenerated plantlets remained constant during embryogenesis, indicating genetic stability (Fig. 7). Therefore, it can be claimed that the flow cytometry technique confirmed the results that were obtained using RAPD markers. Other researchers have emphasized the ideal effectiveness of flow cytometry by using it in their studies to examine somatic variation and ploidy alterations in regenerated plants compared to mother plants (Jena et al. 2018; Raji et al. 2018; Abdolinejad et al. 2020). According to the study's findings, the donor mother plants and the regenerated plantlets had the same levels of ploidy ($2n = 6x = 40$) and 2C DNA content. The optimized micropropagation protocol appears to ensure in vitro regeneration of plants with constant genome size in camelina for use in its breeding programs because the similarity of the ploidy level of in vitro plants with their donor mother plants was indicative of genetic fidelity in regenerated plants. Molecular markers and flow cytometry were used in this study for the first time to confirm the genetic fidelity of in vitro regenerated plants. According to the current findings, somatic embryogenesis may be advantageous for widespread camelina proliferation and in vitro conservation.

Conclusion

The simultaneous observation of various somatic embryogenesis stages on the embryonic callus suggests that somatic embryogenesis in camelina is a simultaneous event. The interaction between explant and PGRs had a substantial impact on embryogenesis and subsequent regeneration. In camelina, the highest rate of somatic embryogenesis and seedling regeneration was obtained in cotyledon explant cultures in MS medium supplemented with 0.3 mgL^{-1} NAA + 0.7 mgL^{-1} BAP. In MS media supplemented with 0.25 mgL^{-1} IAA + 0.5 mgL^{-1} NAA, the best rooting and viability response of seedlings was seen. Using RAPD markers and flow cytometry, the genetic fidelity of in vitro regenerated plants and their donor mother plant in camelina was verified for the first time in the current study. According to the present findings, somatic embryogenesis may generally be advantageous for large-scale proliferation and, breeding programs and in vitro conservation in camelina.

Abbreviations

2,4-Dichlorophenoxyacetic acid

BAP: 6-Benzylaminopurine

MS: Murashige and Skoog

NAA: α -Naphthaleneacetic acid

PGR: Plant growth regulator

IAA: Indole-3-acetic acid

RAPD: random amplified polymorphic DNA

Declarations

Competing Interests

“The authors have no relevant financial or non-financial interests to disclose.”

Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The authors declare no conflict of interest.

Author Contribution

Moslem Bahmankar carried out the experiment and wrote the manuscript.

Maryam Salehi commented on the manuscript and contributed to interpreting the results.

Hassan Rahn timer was involved in planning and supervising the work.

Sadat Noori helped supervise the project and contributed to interpreting the results.

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Moslem Bahmankar, Hassan Rahn timer, Maryam Salehi and Seyed Ahmad Sadat Noori. The first draft of the manuscript was written by Moslem Bahmankar and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

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Figures



Figure 1

Embryonic (right) and non-embryonic (left) calli in Camelina

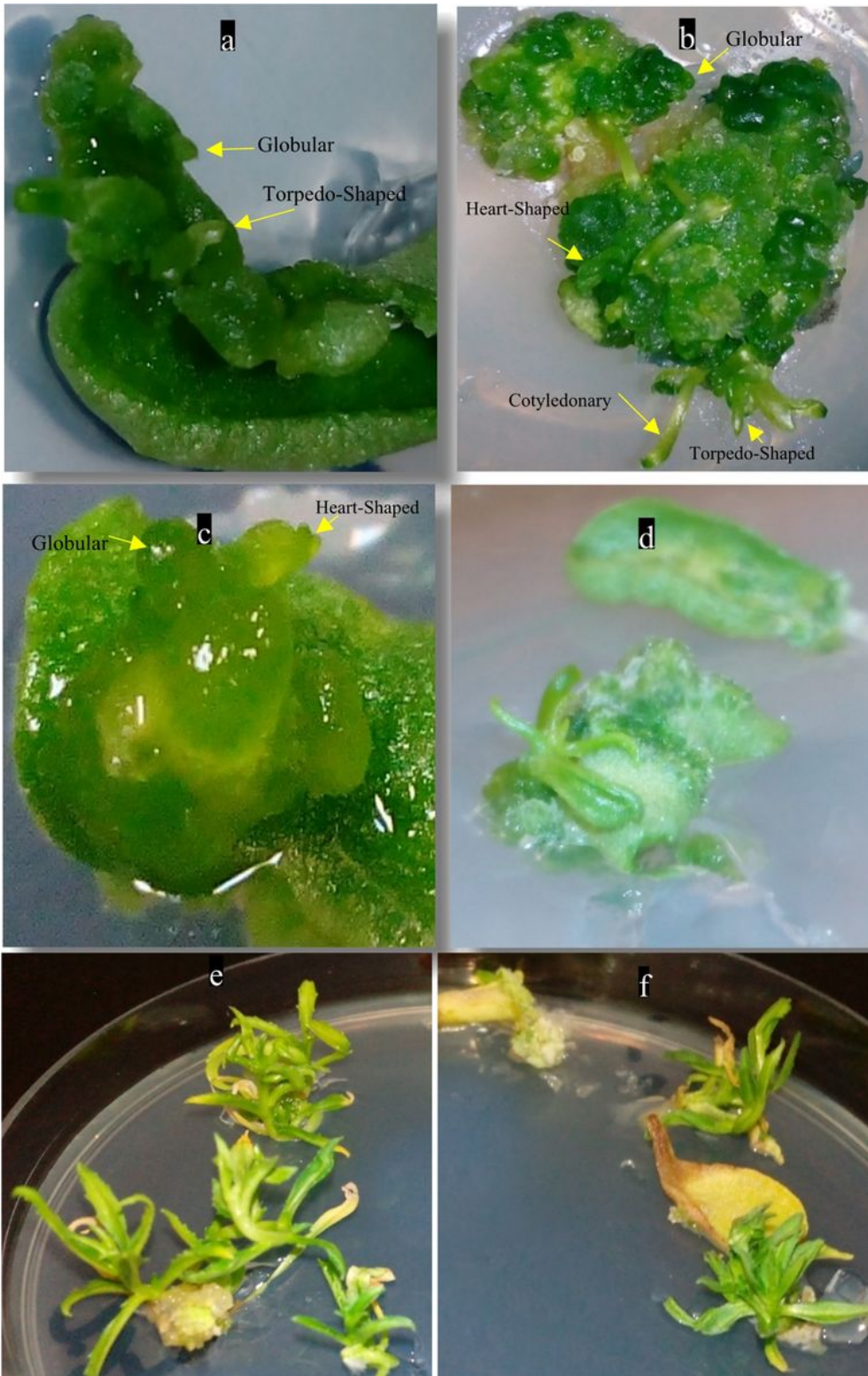


Figure 2

On MS medium, various stages of somatic embryogenesis and plant regeneration were completed along with $0.3 \text{ NAA mgL}^{-1} + 0.7 \text{ BAP mgL}^{-1}$ (a), (b) and (c) simultaneous induction stages of the somatic embryo from cotyledon explants. (d), (e), and (f) Regenerated and elongated shoots from induced somatic embryo.

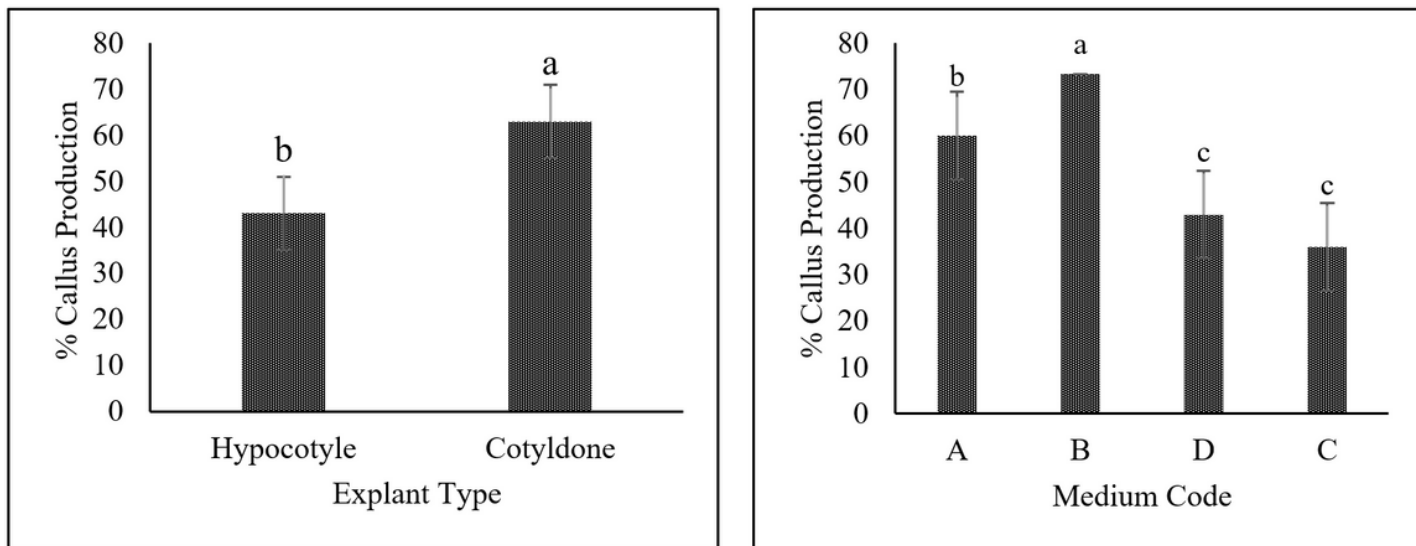


Figure 3

Explant type (left) and culture medium (right) mean comparisons for callus production %. Based on the LSD test at 5%, the mean values in each column are followed by various letters to indicate significant differences.

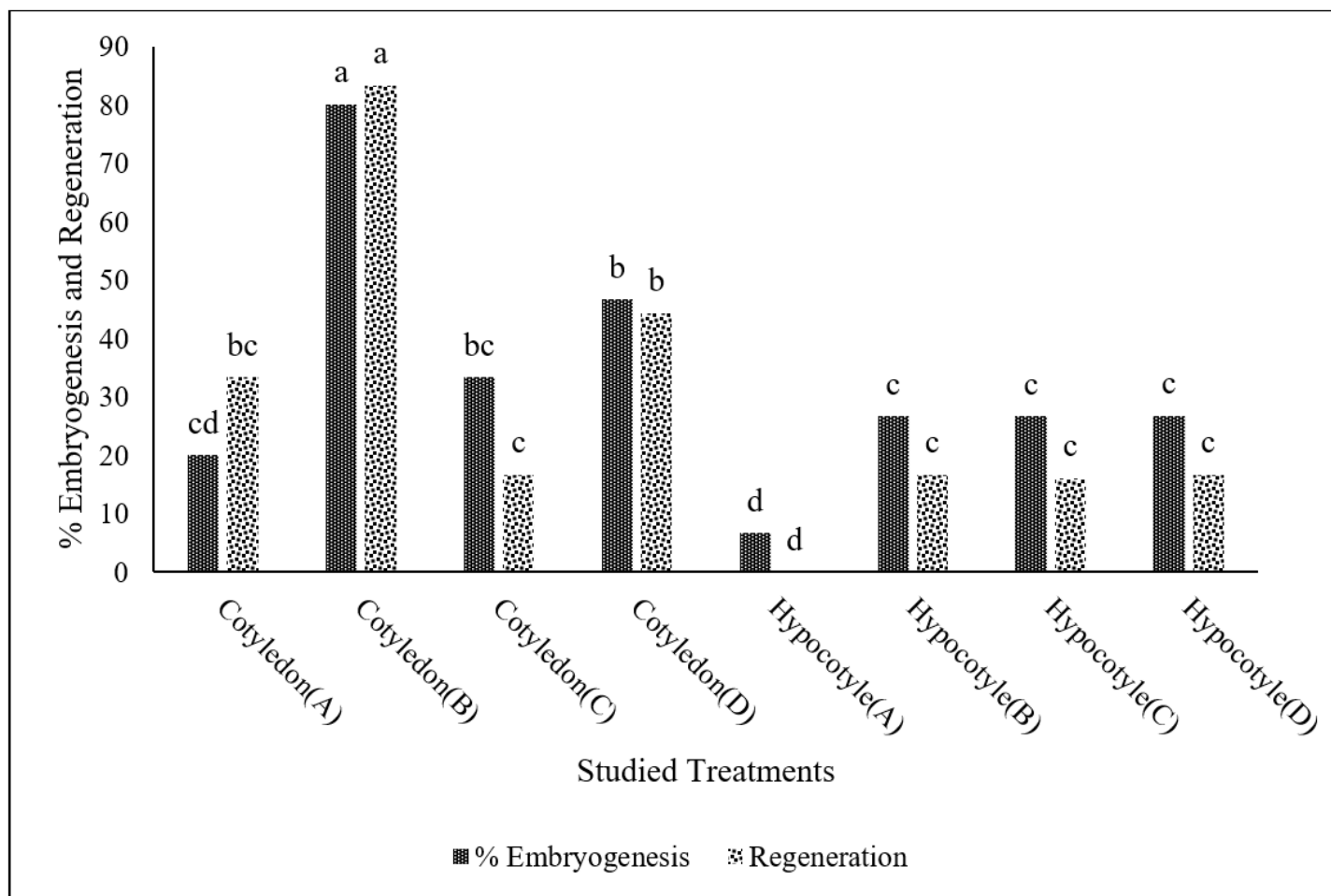


Figure 4

Rank comparisons of plant regeneration and somatic embryogenesis in the investigated treatments. Values in each column followed by the same letters are not significantly different according to the Kruskal-Wallis nonparametric test.



Figure 5

Rooting and development of plantlets in camelina (*Camelina sativa* L. Crantz). (a) Rooted camelina planted, (b) Pot covered by a plastic cover (c) Developed camelina plantlets and (d) mature camelina

plant.

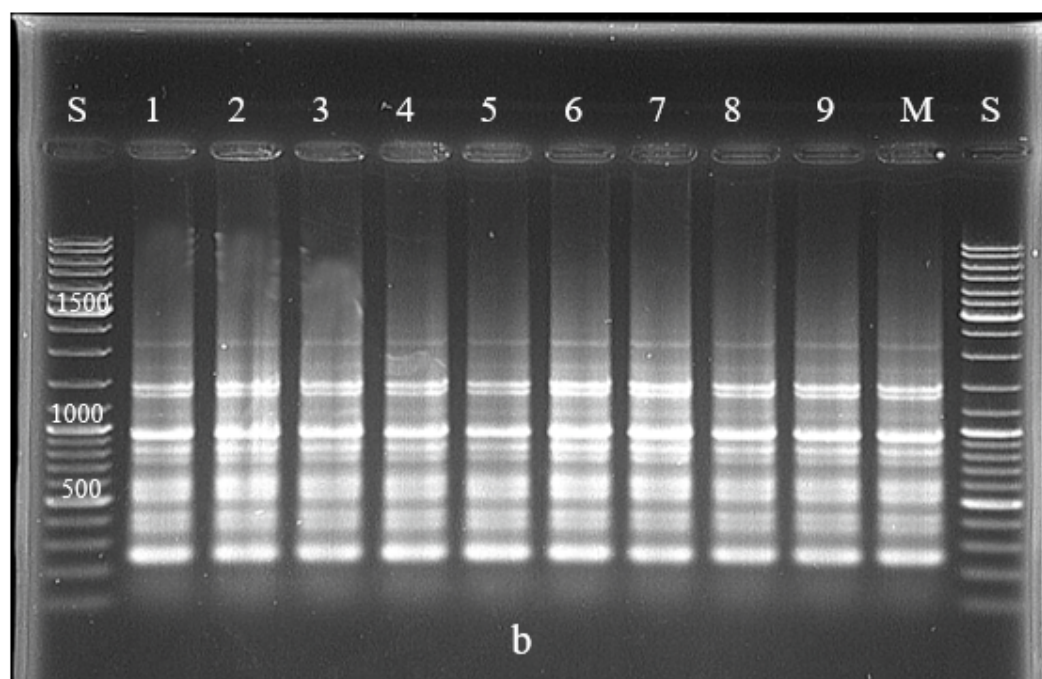
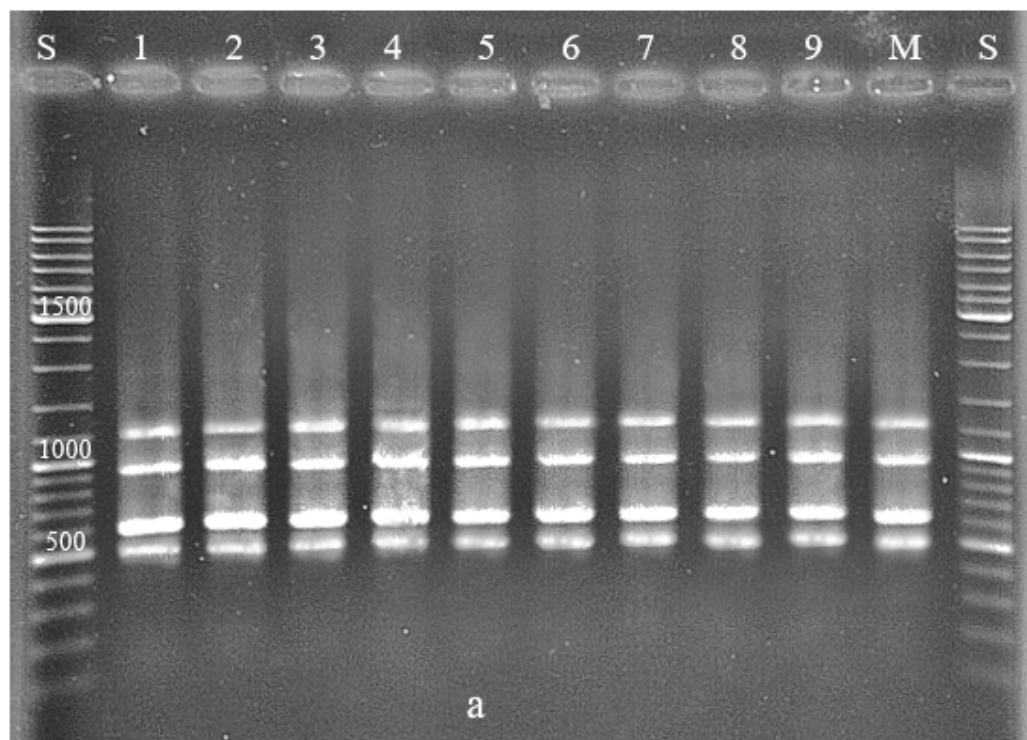


Figure 6

Analysis of RAPD markers produced by the primers OPJ18 (a) and OPC13 (b). The 100–2000 bp size marker is located in lane S. DNA from a selection of regenerated plantlets at random is represented in lanes 1 through 9. Mother plantlet is corresponding to Lane M.

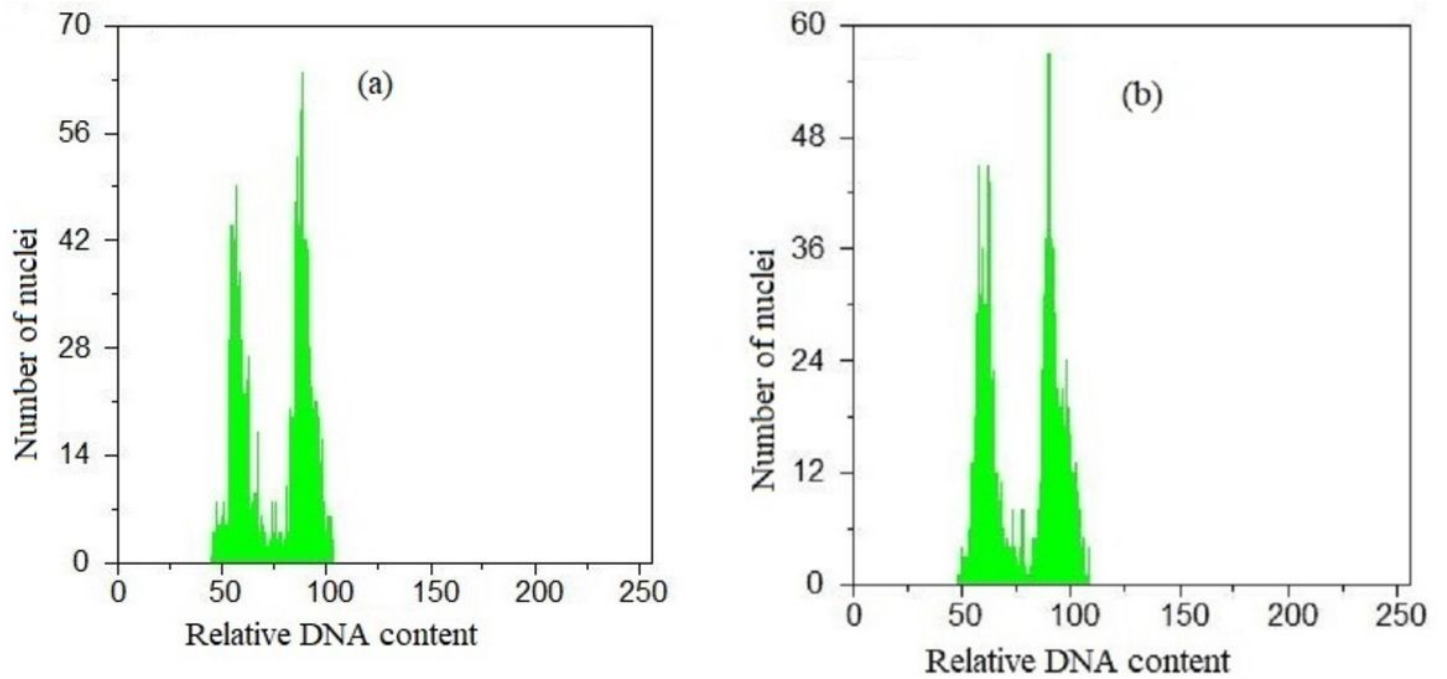


Figure 7

Histogram of the 2C DNA concentration in the donor mother in camelina (a) and the randomly chosen in vitro regenerated plants (b), as determined by flow cytometry. The left peaks in each histogram represented the camelina, and the right peaks represented the internal standard *Solanum lycopersicum* cv. Stupicke (2C DNA = 1.96 pg). The peaks' mean CV% was < 5%.