



An Optimized Protocol for In Vitro Indirect Shoot Organogenesis of Impala Bronzovaya and Zanzibar Green *Ricinus communis* L. Varieties

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Abstract: The castor bean is an important industrial and ornamental crop. In the industry, it is used as a source of castor oil. Moreover, it has a large potential as a feed crop, because the seeds contain a high amount of protein. A main problem with castor bean use is the presence of toxins in the plants. Today, detoxification is carried out using various approaches, including biotechnological methods such as CRISPR/Cas9 technology. A successful application of these methods requires the availability of an efficient in vitro protocol for callus induction and shoot organogenesis. We present the results of in vitro condition optimization for two castor bean varieties (Impala Bronzovaya and Zanzibar Green). Eight different Murashige–Skoog (MS) culture media characterized by different plant growth regulator (PGR) combinations, as well as explant types (hypocotyls, cotyledonous leaves, and cotyledon petioles), were tested. The highest frequency of shoot organogenesis and average number per explant were observed during the cultivation of cotyledon petioles in both varieties on the Murashige and Skoog culture medium (MS) containing 1 or 2 mg/L of zeatin in combination with 0.1 mg/L of 3-indoleacetic acid (IAA). An optimized protocol for in vitro callus induction and shoot organogenesis may be used for biotechnological applications to obtain toxin-free castor bean, as well as *Ricinus communis* L. plants, with new ornamental traits and their combinations.

Keywords: castor bean; callus induction; shoot organogenesis; explants; plant growth regulators (PGR); detoxification



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1. Introduction

From a botanical point of view, the castor bean, *Ricinus communis* L. ($2n = 20$), is a species of the monotypic genus in the Euphorbiaceae family [1,2]. It is widely cultivated as an industrial and ornamental crop in countries with tropical and subtropical climates [3,4]. The potential for the castor bean to be used as an ornamental plant has been studied in various countries. Investigations into the genetic diversity and ornamental and agronomic traits, as well as the breeding of new horticultural varieties, have been carried out [5–7]. The main purpose of castor bean cultivation is seed production. Castor bean seeds contain approximately 60% oil, which is highly valued for the production of lubricants, plastics, cosmetics, paints, varnishes, ethanol, etc. [8–10]. Castor oil has unique properties (which other vegetable oils do not have) due to its ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid). Additionally, the use of castor oil as a biodiesel has good prospects [11,12]. India, China, and Brazil are major castor oil producers in the international market [13].

Castor bean cakes and meals remaining after oil extraction contain high-quality proteins [14]. They may be used as a valuable feed supplement, but their application is limited due to the toxins in castor seeds [15]. Chief among these is ricin. Ricin is known as a toxic protein that consists of two chains (A and B) that are linked by a disulfide bond [16,17].

The chains play different roles when ricin acts on mammalian cells. Chain B allows for the penetration of chain A into the animal cell due to its ability to bind with the components of the membrane surface. In turn, chain A inactivates ribosomes, causing abnormalities in the protein synthesis and, finally, cell death [18,19]. Ricin is extremely dangerous for humans and animals. Ricin poisoning causes cramps, abdominal pain, nausea, and ulceration of the gastrointestinal tract and blood vessels. The ingestion of high doses leads to death [15].

The detoxification of castor oil production waste is an acute problem. There are several detoxification approaches. One is a harsh chemical (primarily leaching with calcium compounds) and temperature treatment [20]. Another is fermentation with microorganisms, such as *Paecilomyces variotii* or *Aspergillus niger* [21,22]. However, both approaches require the construction of added technological lines, as well as the costs of the chemicals and energy. Furthermore, the first approach often results in a decrease in the protein quality and digestibility. Finally, the best approach may be the cultivation of ricin-free varieties. These varieties may be obtained by genetic engineering, resulting in the posttranscriptional degradation of ricin precursors [23], and by the CRISPR/Cas9 genome-editing technique [24].

The application of these biotechnological methods requires the development of an effective protocol for in vitro callus induction, shoot regeneration, and genetic transformation. The genotype, physiological age and origin of the explant, quantitative and qualitative composition of the plant growth regulators (PGR), culture conditions, and many other factors affect the realization of the morphogenetic potential from the somatic cells. Shoot regeneration from the somatic cells is often achieved by organogenesis, with a previous stage of callus tissue formation [25–27]. Currently, there are many in vitro organogenesis protocols for different crops. In these protocols, cotyledons [28,29], segments of epicotyl [30], hypocotyl [31,32], fragments of the stem [33] and peduncles [34], fragments of the leaves [35,36], meristems [37], protoplasts [38], and immature embryos [39] are used as explant sources. The explants are often cultivated on culture media with a wide range of concentrations and types of cytokinins (6-benzylaminopurine (6-BAP), zeatin, kinetin, 2-isopentenyladenine, or thidiazuron (TDZ)), in combination with low concentrations of auxins (3-indoleacetic (IAA), 1-naphthylacetic (NAA), or 2,4-dichlorophenoxyacetic acids (2,4D)) or without them [40–42], to induce the processes of callusogenesis and shoot regeneration.

Many in vitro processes have been previously studied in castor bean varieties by different authors. For example, in a work with the “Shabje” cultivar, Rahman and Bari (2012) used the combination of 2.0 mg/L of 6-BAP + 0.5 mg/L of NAA for the best callus induction from the hypocotyl segments [43]. These authors also suggested decreasing the concentration of NAA in the medium to 0.5 mg/L in order to achieve the best cell culture results. A large number of works were devoted to the in vitro regeneration of castor bean shoots. Protocols with different combinations and concentrations of the PGR (such as BAP, IAA, kinetin, TDZ, and zeatin) have been presented [44–48]. The best results of these experiments are summed up in Table S1. Considering these results, we tried some published PGR combinations in the preliminary experiments with the Zanzibar Green and Impala Bronzovaya castor bean varieties, but our results were worse than in the original publications. We concluded that each new variety requires an optimization of the protocols and experimental testing. Through this connection, we tested various PRG combinations, which showed good results in the preliminary experiments with the studied varieties.

As a result, the optimal culture conditions for the in vitro morphogenesis of two Russian castor bean varieties are presented in this article and will be further used for CRISPR/Cas9 editing in order to obtain ricin-free plants.

2. Materials and Methods

2.1. Plant Material and Obtaining of Aseptic Donor Plants

Castor bean plants of two varieties (Impala Bronzovaya “Gavrish” and Zanzibar Green “Gavrish”) were used for the in vitro experiments. The donor plants were grown from seeds, which were sterilized according to the following protocols. The seeds were treated

in 96% ethanol for 30 min and then immersed in a 5% solution of sodium hypochlorite for 30 min. The treated seeds were washed in distilled water three times for 5 min, peeled from the seed skin, and transferred in vitro on MS culture medium [49] without PGR. Cultivation of the donor plants and explants was carried out in the following conditions: temperature 23–25 °C, illumination 2.5–3.0 klx, and photoperiod 16/8 h (day/night). The obtained seedlings were used for the explant preparation.

2.2. The Preparation of Explants and their Cultivation

The explant segments were aseptically prepared from the middle parts of hypocotyls, with sizes of 10–15 mm, as well as cotyledonous leaves and cotyledon petioles 3–5 mm in length. The explants were cultured in eight different media (MS1–MS8) prepared using different concentrations and combinations of PGRs, as shown in Table 1, where the MS1 was kept as a control with no PGRs, as it was used for the donor plants. The experiment was performed in three replicates, with 10 explants in each. Hypocotyls and cotyledon petioles were placed horizontally on the surface of the medium. The cotyledonous leaves were placed with the abaxial side to the medium. The environmental conditions of the cultivation were the same as for the donor plants. Subcultures were carried out every 15 days.

Table 1. Types and concentrations of PGR used in the MS culture media for the in vitro callus induction and shoot organogenesis of *Ricinus communis* L.

Plant Growth Regulators	Concentration of Plant Growth Regulators in MS Culture Media, mg/L							
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8
Zeatin	–	–	–	–	1	2	–	–
6-BAP	–	2.5	5.0	5.0	–	–	–	–
TDZ	–	–	–	–	–	–	0.25	0.5
GA	–	–	–	1.0	–	–	–	–
IAA	–	0.1	0.1	0.1	0.1	0.1	0.1	0.1

The efficiency of the morphogenesis processes was assessed after three subcultures. Such indicators as the frequency of callus formation, the frequency of shoot organogenesis, and the average number of shoots per explant were considered. The frequency of callus formation was calculated as the ratio of the number of explants that formed callus tissue to their total number, expressed as a percentage. The frequency of shoot organogenesis was calculated as the ratio of the number of explants in which shoot organogenesis occurred to the total number of explants that formed the callus and was expressed as a percentage. The average number of shoots per explant was calculated as the ratio of the number of regenerants to the total number of explants in which shoot organogenesis was observed.

2.3. Statistical Analysis

Statistical processing of the experimental results was carried out using the parametric criteria of Student, Fisher, and Duncan using the AGROS program (version 2.11). The values, expressed as percentages, were converted using the angle–arcsine $\sqrt{X}$ function, where X is the frequency of the callus formation or somatic organogenesis of the shoots. The values of the shoot average number per explant before the analysis of variance were transformed using the expression $\sqrt{(X + 1)}$, where X is the average number of shoots.

3. Results

3.1. Callus Formation and Characteristics

A callus formation was observed in all explant types of the two studied castor bean varieties. The start of the callus formation was at days 5–7 of the first passage. In hypocotyls and cotyledon petioles, a callus was formed laterally at the cut sites (Figure 1a). The callus structures were different in the experiments with high and low concentrations of PGR in the culture media. In the first case, the callus was white, loose, and non-morphogenic. In

the second case, it was green and dense. Further cultivation showed that the callus was morphogenic. At days 11–21 (Table S1), meristematic foci were formed in the petioles and hypocotyls, developing further along the path of hemogenesis (Figure 1c,d). Experiments with cotyledonous leaves as explants showed that they formed a callus on an abaxial surface, where the leaves were in contact with the culture medium (Figure 1b). The callus was light green, dense, and morphogenic. The callus structures without meristematic foci became necrotic by increasing the number of subcultures. All explants died in the MS culture medium without PGR after the second passage.

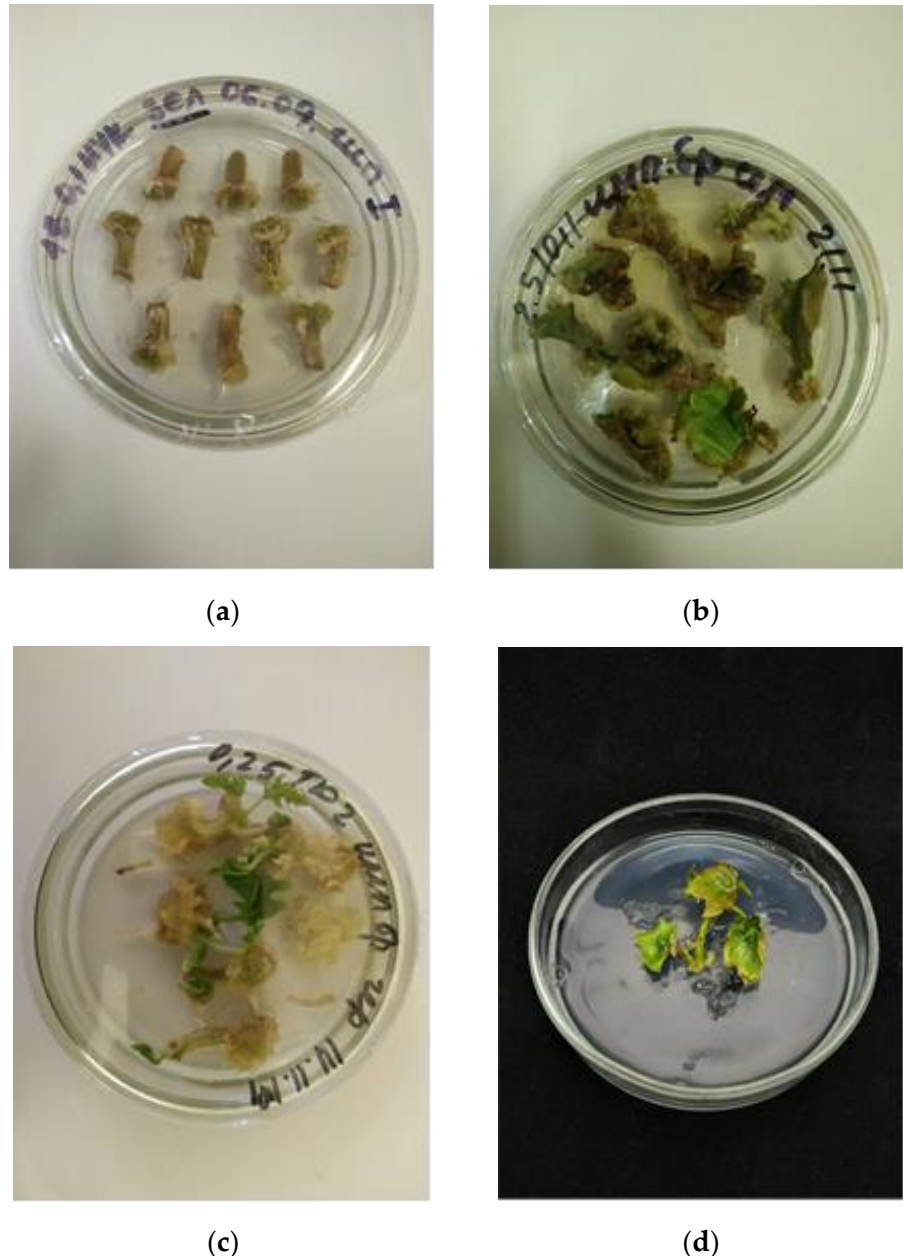


Figure 1. Callus formation and shoot regeneration in castor beans: (a) callus formed laterally at the cut sites of Zanzibar Green hypocotyls, (b) callus formed on an abaxial surface of Impala Bronzovaya cotyledonous leaves, (c) shoot regeneration from the Impala Bronzovaya explants, and (d) shoots from two meristematic foci of the Zanzibar Green explant.

3.2. A Comparative Analysis of Callus Formation Frequencies

A three-factor analysis of variance established the presence of significant differences at the 5% level of significance in the frequency of callus formation between different genotypes, types of explants, and culture media. Moreover, significant differences in the “culture medium $\times$ genotype”, “culture medium $\times$ explant”, and “genotype $\times$ explant” interactions, as well as in the interactions of all three factors, were observed (Table 2).

Table 2. Three-way ANOVA test to evaluate the significance of the culture medium components, castor bean genotype, and explant type on the frequency of callus induction.

Source of Variation	ss	df	ms	F ₀₅	F
Total	106,381.40	143	—	—	—
Variants	101,877.10	47	2167.60	1.63	46.20 *
A (genotype)	14,693.75	1	14,693.76	3.94	313.16 *
B (explant)	2029.57	2	1014.78	3.09	21.63 *
C (cultural medium)	6237.06	7	891.01	2.10	18.99 *
AB	3748.05	2	1874.03	3.09	39.94 *
AC	5439.07	7	777.01	2.10	16.56 *
BC	56,044.40	14	4003.10	1.79	85.32 *
ABC	13,686.16	14	977.58	1.79	20.84 *
Error	4504.34	96	46.92	—	—

Abbreviations: ss—sum of squares, df—degrees of freedom, ms—mean square, F₀₅—critical F value at the 5% significance level ($\alpha = 0.05$), and F—F value, *—F test significant at $\alpha = 0.05$.

Callus formation was observed in the hypocotyls and cotyledon petioles of both castor bean varieties when cultured on a MS medium lacking PGR (MS1). The frequency of callus formation was high in these cases, at around 60% (Figure 2). These observations may be linked to the high concentration of endogenous phytohormones in these explants. Khaliluyev et al. (2014) made the same conclusion, when they described the callus formation from the tomato hypocotyl segments of the “Recordsman” and “Trans” varieties [40]. On the other hand, cotyledonous leaves did not form calluses without exogenous PGR. Similar results were previously obtained by Maramokhin et al. (2018) for *Schisandra chinensis* (Turcz.) Baill. They observed the callus formations from microshoots and the absence of calluses from leaf or internode explants on PGR-free medium [50]. Except for cotyledon petioles, experiments with the MS2–MS8 culture media showed, in most cases, that exogenous PGR significantly increased the frequency of callus formation. In the MS4 medium, the frequency of callus formation with this explant type was as at the MS1 level and significantly lower in other variants.

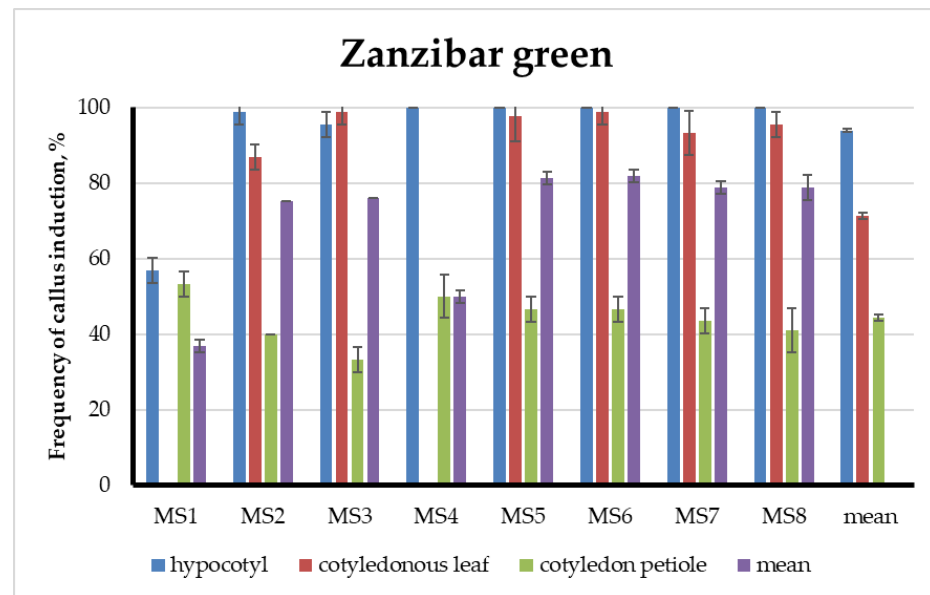
A comparison of the callus formation frequencies revealed that hypocotyls are the best type of explants for the studied castor bean varieties. They showed values of 100% for the callus formation frequency in most of the culture media tested. Cotyledonous leaves showed a lower level of callus induction than hypocotyls (except the MS3 variants in both varieties), but the differences were often not statistically significant. In the case with the MS4 culture medium, cotyledonous leaves of Zanzibar Green and hypocotyls of Impala Bronzovaya did not form a callus. This effect was obviously related to the different actions of GA in the different genotypes, such as, for example, in rye mutants [51] or different *Rht* genotypes of wheat [52].

Overall, MS5 and MS6 (culture media with zeatin as a cytokinin component) were the best for callus formation. Additionally, high values of the callus formation frequency were observed in MS7 and MS8 culture media (with TDZ as a cytokinin component). The observed differences between zeatin and the TDZ variants were not statistically significant.

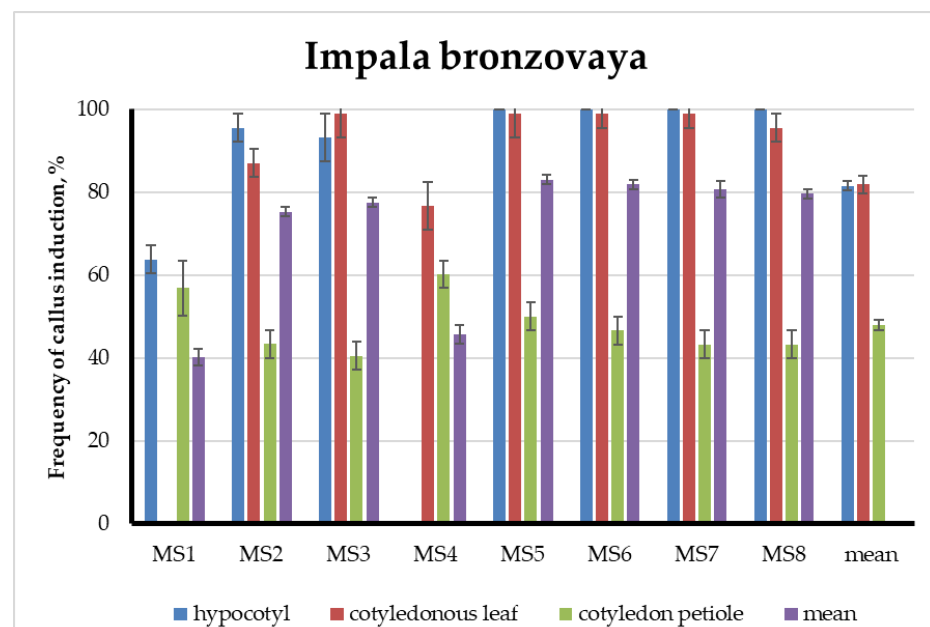
3.3. Somatic Organogenesis of Shoots and Comparative Analysis of Frequencies

In the experiments, callus formation and somatic organogenesis were observed with the same medium in each variant. The start of shoot regeneration was detected on different days of cultivation (from day 11 to day 21; see Table S2). However, such events did not

occur in each variant. The MS1 culture medium without PGR showed a complete lack of somatic organogenesis, regardless of the explant type and genotype (Table 3). The same results were also obtained in MS3, MS4, and MS8 (except for the MS8/cotyledon leaves/Impala Bronzovaya combination). In the MS2 and MS7 culture media, somatic organogenesis was only observed in cotyledon petioles derived from both castor bean varieties. The best results were obtained on MS5 and MS6, where the callus of hypocotyls and cotyledon petioles formed shoots. However, the calluses from cotyledonous leaves did not form shoots in all the tested culture medium–genotype combinations.



(a)



(b)

Figure 2. Influence of the MS culture media supplemented with different types and concentrations of PGR on the callus formation frequency from the explants of Zanzibar green (a) and Impala bronzovaya (b) castor bean varieties.

Table 3. Values of the shoot organogenesis frequency in castor beans, depending on genotype, explant type, and culture medium composition.

Culture Medium	Frequency of Shoot Organogenesis, %							
	Zanzibar Green				Impala Bronzovaya			
	Hypocotyl	Cotyledonous Leaf	Cotyledon Petiole	Mean (Culture Medium) ²	Hypocotyl	Cotyledonous Leaf	Cotyledon Petiole	Mean (Culture Medium) ²
MS 1	0a ¹	0a	0a	0a	0a	0a	0a	0a
MS 2	0a	0a	6.7efghij	2.2bcde	0a	0a	9.2fghijk	3.6defgh
MS 3	0a	0a	0a	0a	0a	0a	0a	0a
MS 4	0a	0a	0a	0a	0a	0a	0a	0a
MS 5	10hijk	0a	11.6ijk	7.2fghi	10.1ghijk	0a	43.3n	17.8j
MS 6	4.6bcdefghi	0a	19.3kl	7.96h	3.5cdefghi	0a	43.3mn	15.6ij
MS 7	0a	0a	16.5jkl	5.5efgh	0a	0a	25.4l	8.46gh
MS 8	0a	0a	0a	0a	0a	0a	6.7defghij	2.2cde
mean (explant) ³	1.8c	0a	6.76d	—	1.7bc	0a	16e	—
mean (genotype) ⁴	2.85a				5.9a			

Means followed by the same letter are not significantly different at $\alpha = 0.05$, according to Duncan's multiple range test. ¹ Influence of the genotype, explant type, and culture medium on the frequency of shoot organogenesis. ² Influence of the genotype and culture medium on the frequency of shoot organogenesis. ³ Influence of the genotype and explant type on the frequency of shoot organogenesis. ⁴ Influence of the genotype on the frequency of shoot organogenesis.

The frequencies of shoot organogenesis varied depending on the medium, explant type, and genotype, from 3.5% (MS6/hypocotyls/Impala Bronzovaya) to 43.3% (MS5 or MS6/cotyledon petioles/Impala Bronzovaya). MS5 and MS6 were the best culture media, with the highest frequency of shoot regeneration. When culturing hypocotyls on these media, an interesting effect was observed. In both castor bean varieties, the shoot regeneration frequency was almost three times lower in MS6 than in MS5. In fact, an increase in the zeatin content from 1 to 2 mg/L led to an inhibition of the shoot organogenesis in hypocotyls. Furthermore, the best type of explant for somatic organogenesis was cotyledon petioles. The mean values also indicated that the calli of the Impala Bronzovaya variety formed shoots more often than those of the Zanzibar Green variety, but the differences were not statistically significant.

The highest values for the average number of shoots per explant were observed with MS5 (2.52) and MS6 (2.58) culture media when cotyledon petioles of Impala Bronzovaya were used as the explant source (Table 4). Independently of the culture medium and explant type, no statistically significant differences were observed between the two tested genotypes (1.2 in Zanzibar Green and 1.3 in Impala Bronzovaya). Independently of the culture medium, significant differences between the explant types (hypocotyls vs. cotyledon petioles) of Impala Bronzovaya were also observed (Table 4).

Table 4. The converted values of the average number of shoots per explant in castor beans, depending on the genotype, explant type, and culture medium composition.

Culture Medium	Values of $\sqrt{(X + 1)}$ Expression, where X Is the Average Number of Shoots per Explant							
	Zanzibar Green				Impala Bronzovaya			
	Hypocotyl	Cotyledonous Leaf	Cotyledon Petiole	Mean (Culture Medium) ²	Hypocotyl	Cotyledonous Leaf	Cotyledon Petiole	Mean (Culture Medium) ²
MS 1	1.00a ¹	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
MS 2	1.00a	1.00a	1.61defghij	1.20ab	1.00a	1.00a	1.61fghij	1.20ab
MS 3	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
MS 4	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
MS 5	2.00hijklm	1.00a	1.77ghijkl	1.60ab	2.00ijklm	1.00a	2.52mn	1.84d
MS 6	1.61bcdefghij	1.00a	2.20lm	1.60bcd	1.61cdefghij	1.00a	2.58n	1.73cd
MS 7	1.00a	1.00a	2.00jkl	1.33ab	1.00a	1.00a	2.16klm	1.40ab
MS 8	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a	1.61efghij	1.20a
mean (explant) ³	1.20a	1.00a	1.40ab	—	1.20a	1.00a	1.70b	—
mean (genotype) ⁴	1.20a				1.30a			

Means followed by the same letter are not significantly different at $\alpha = 0.05$, according to Duncan's multiple range test. ¹ Influence of the genotype, explant type, and culture medium on the number of shoots per explant. ² Influence of the genotype and culture medium on the number of shoots per explant. ³ Influence of the genotype and explant type on the number of shoots per explant. ⁴ Influence of the genotype on the number of shoots per explant.

4. Discussion

In this article, successful callus formation and somatic shoot organogenesis are described for the Zanzibar Green and Impala Bronzovaya castor bean varieties. These varieties are popular as ornamental plants in Russia and may be used for castor bean improvement with the application of in vitro techniques. The experiments were conducted according to a classical scheme that includes culture media without PGR, as well as ones with different concentrations of cytokinins (zeatin, 6-BAP, and TDZ) and auxins (IAA). The start of callus formation was observed at days 5–7, which corroborates the results of some other authors. Gill-Correal et al. observed the emergence of a callus in the epicotyls of the “VERC-02” castor bean genotype on day 8 [48]. Rahman and Bari described a callus starting in the hypocotyls of cv. “Shabje” on days 4–15 [43].

The best results for callus formation in the Zanzibar Green and Impala Bronzovaya castor bean varieties were obtained on MS containing 1 or 2 mg/L of zeatin + 0.1 mg/L of IAA. Combinations of cytokinins and auxins were previously used for the callus production in castor bean by other researchers [43,45,53]. However, they observed both higher and lower callus yields, depending on the genotype tested. Rahman and Bari achieved a 100% frequency of callus formation in the hypocotyls of cv. “Shabje” using a medium with 2.0 mg/L of 6-BAP + 0.5 mg/L of NAA. A high frequency of callus induction was also observed after replacing NAA with 2.4D (91.5%). At the same time, replacing NAA with IAA resulted in a large decrease in the callus formation frequency (54.5%) [43]. Low effects of the cytokinin + auxin application were detected in the studies by Alam et al. and Khafagi [45,53]. As an alternative, one type of PGR is applied for in vitro experiments with castor beans. For example, Alam et al. and Liu et al. used 6-BAP in different concentrations. Khafagi experimented with media supplemented 2.4D only. Gill-Correal et al. combined two different cytokinins in various concentrations (TDZ + zeatin, TDZ + 2ip, TDZ + 6-BAP, and TDZ + kinetin) [45,48,53,54]. Using only 2.4D, Khafagi noted a more intensive growth of the callus compared to the culture media with cytokinins + auxins [53].

The culture medium variants with 1 or 2 mg/L of zeatin + 0.1 mg/L of IAA also showed the best results of somatic organogenesis in the Zanzibar Green and Impala Bronzovaya castor bean varieties. The highest shoot regeneration frequency (43.3%) was observed in cotyledon petioles of the Impala Bronzovaya variety. Furthermore, the highest number of shoots per explant was obtained by culturing cotyledon petioles on the same culture media (2.52 and 2.58 on MS5 and MS6, respectively). Other authors described both higher and lower results for other castor bean genotypes. Gil-Correal et al. achieved a 75.56% frequency of regeneration and 4.3 shoots per explant by culturing epicotyls of the “VERC-02” genotype on a medium containing 1 mg/L of TDZ + 0.5 mg/L of zeatin. On the other hand, the 1 mg/L of TDZ + 0.5 mg/L of 6-BAP combination resulted in a large decrease in the regeneration frequency (37.78%) and number of shoots per explant (0.8) [48]. Studying a castor bean genotype from the Rajshahi University Botanical Garden, Alam et al. found an 85% regeneration frequency for a culture medium with 3 mg/L of 6-BAP and 36% by using 4 mg/L of 6-BAP + 0.5 mg/L of NAA [45]. Moreover, the number of shoots per explant varied from 12.56 to 4.9. It is possible that such values were achieved due to the use of large explants, including cotyledonous leaves, cotyledon petioles, and hypocotyls.

In conclusion, a very high callus induction frequency for both the Zanzibar Green and Impala Bronzovaya castor bean varieties was obtained, while a good shoot regeneration efficiency was only found for Impala Bronzovaya. However, the best types of explants for the formation of calluses (hypocotyls and cotyledonous leaves) were inferior to the cotyledon petioles in terms of regeneration, even on the best variants of culture media. Thus, the application of plant genetic engineering by *Agrobacterium*-mediated or bioballistic transformation requires the use of all the studied explant types or large explants that consist of cotyledonous leaves, cotyledon petioles, and hypocotyls for the maximal yield of the transgenic shoots.

5. Conclusions

In the experiments with the Impala Bronzovaya and Zanzibar Green castor bean varieties, the following conclusions were made: Hypocotyls were the best type of explants for callus formation and showed values of 100% in most of the culture media tested. Cotyledonous leaves showed a lower level of callus induction than hypocotyls. Cotyledon petioles were the best type of explant for somatic organogenesis and showed higher values of shoots per explant than the others. Thus, it is advisable to use hypocotyls and cotyledonous petioles of the studied varieties in further experiments related to the optimization of the in vitro protocols. The best culture media for callus induction and somatic organogenesis contained 1 or 2 mg/L of zeatin in combination with 0.1 mg/L of IAA.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7050105/s1>: Table S1: The best results of in vitro experiments with *Ricinus communis* L.; Table S2: Days of the regeneration start in different explants of Zanzibar Green, and Impala Bronzovaya castor bean varieties, depending on the variant of the cultural environment.

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