Germination and morpho-physiological analysis of seedlings pre-treated with different concentrations of colchicine in soybean (*Glycine max* (L.) Merr.)

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Wider genetic diversity has the potential to improve crop productivity of soybean, especially under environmental stress conditions. The pre-treatment of soybean seeds with antimitotic agents to establish improved genetic pool may also contribute in the enhancement of germination, seedling development, morpho-physiological growth and yield. In this study, two soybean genotypes viz. TGx1835-10E and Dundee were imbibed in solutions containing different concentrations of colchicine (0.0, 0.1, 0.5 and 1%) to evaluate the variations in germination, morphometric and physiological parameters. The seeds were imbibed for 6, 12 and 24 hours before sowing for germination in plastic pots containing moistened sterile vermiculite. The variance components expressed as means and mean percentage of total variations showed that colchicine concentration and imbibitional duration were the most important sources of variation for all traits, followed by the genotypes. Significant responses were detected for various germination parameters, seedling morphology and physiological contents such as; chlorophyll content, total phenolics, flavonoids as well as total protein and DNA content in the two genotypes used.

Keywords: colchicine; germination; growth; morpho-physiological parameters; soybean.

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Introduction

Glycine max (L.) Merr. (Fabaceae) is a valuable leguminous pulse crop, widely known as soybean. This crop contains larger amounts of carbohydrates, proteins, unsaturated fats, essential oils, vitamins, and other compounds that have health promoting effects [1]. However, soybean has a narrow genetic diversity, making it highly sensitive and vulnerable to the various biotic and abiotic stress factors. Thus, soybean breeders continue to focus on improving growth, quality and quantity of yields, phytochemicals and macromolecules contained in the seeds. Some of the methods used for this purpose include hybridization, genetic engineering, and mutagenesis. Over the years, research also focused on the use of colchicine, as a potential antimitotic mutagenic agent that prevent microtubules' function in chromosome separation [2]. Colchicine as one of mutagenic agent specifically interferes with the structure and orientation of mitotic and spindle fibers. Such effects cause subsequent increases in the growth characteristics such as size of leaves, fruits, and seeds in plants successfully pre-treated with this chemical. This kind of genetic modifications have long gained popularity in many breeding systems. The approach still presents a viable alternative to genetic

engineering, especially because it has received considerable attention to consumers and researchers, since concerns of genetic instability and possible carcinogenic activities are highly minimized.

In recent years, colchicine was widely used to derive many varieties of food and ornamental crops including grain legumes such as soybean and cowpea [3]. Both *in vitro* and *in vivo* protocols were tested, usefully obtaining new traits, creating genetic variability, and supplementing conventional breeding in combating stress. As reported by Essel *et al*. [3], colchicine is the most preferred mutagenic agent because it is less fatal in plant cells compared to animal tissues, and it efficiently results in larger number of fertile seeds, fruits, and a more desired plant growth. The need to exploit this technique for combating climate change and food insecurity in developing countries should not be over emphasized. Seed germination, morphological and physiological analyses are pivotal in understanding the role and effects of colchicine in plant growth and development. Furthermore, investigations are necessary to elucidate the responses of different genotypes, especially to develop stress resistant varieties. The continued use of mutagenic agents to develop stress resistant crops is perhaps the most fundamental service to mankind and the increasing populations. For the above mentioned reasons, studies investigating the potential influence of colchicine on growth factors such as germination, seedling development, secondary metabolites, chlorophylls, DNA, and proteins remain a prerequisite. Therefore, this study made the attempt to evaluate the differences in the quantity of above mentioned parameters between two soybean cultivars (Dundee and TGx1835-10E) pre-treated with varying concentrations of colchicine for 6, 12, and 24-hrs of seed imbibition.

Materials and Methods

Chemicals and materials

Soybean (*Glycine max* (L.) Merr.) seeds cultivar Dundee and TGx1835-10E were obtained from the Department of Biodiversity (Botany) and multiplied by planting at Amaloba nursery at the University of Limpopo, from September 2018 to March 2019. Colchicine (AR) powder, Folin-Ciocalteau, gallic acid, quercetin, and glucose standard were purchased from Prestige Laboratory Supplies, Republic of South Africa (RSA). Eppendorf centrifuge tubes, aluminum nitrate ($Al(NO_3)_3$), sodium nitrate ($NaNO_3$), sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), acetone, methanol, and phenol solution were purchased from Rochelle Chemicals and Lab Equipment (RSA). Gallenkamp Orbital Shaker and JENWAY's UV/VIS Spectrophotometer were purchased from Lasec SA and International Laboratory Supplies Ltd.

Pre-treatment and germination of seeds

The seeds of soybean were evaluated for germination by initially surface disinfecting them using chlorine gas for 16 hours as described by Mangena *et al*. [4]. After sterilization, the seeds were then pre-treated with different concentrations of colchicine by pre-soaking for 6, 12, and 24 hours as shown in Table 1. Colchicine pre-treated seeds were then sown in 30 cm plastic pots containing heat sterilized vermiculite, which is a hydrous phyllosilicate mineral, used as a growth medium due to its non-toxic effect, neutral pH, air-moisture retention capacity, and nutrient free. Seed cultures were then incubated for 10 days in a growth room under controlled environmental conditions. The growth room was equipped with white florescence light LED lamps at 120–200 µmol/m^{2}s light intensity, 24±2°C, and 16 hours photoperiod. Germination was considered as the emergence of the epicotyl and the parameters were assessed as indicated on Table 2.

Table 1. Determination of the effect of colchicine and duration of exposure on seed germination and morpho-physiological parameters in soybean.

Soybean Cultivar Name	Seed Treatment ID	Colchicine Level (%)	Period of Imbibition (hours)
Dundee	A1	0.1	6
TGx1835-10E	A2	0.1	6
Dundee	A3	0.5	6
TGx1835-10E	A4	0.5	6
Dundee	A5	1.0	6
TGx1835-10E	A6	1.0	6
Dundee	B1	0.1	12
TGx1835-10E	B2	0.1	12
Dundee	B3	0.5	12
TGx1835-10E	B4	0.5	12
Dundee	B5	1.0	12
TGx1835-10E	B6	1.0	12
Dundee	C1	0.1	24
TGx1835-10E	C2	0.1	24
Dundee	C3	0.5	24
TGx1835-10E	C4	0.5	24
Dundee	C5	1.0	24
TGx1835-10E	C6	1.0	24
Dundee	CD	– –	– –
TGx1835-10E	C10E	– –	– –

Table 2. Description of formulae used to study germination and seedling parameters following *in vivo* pretreatment of seeds in various concentrations of colchicine.

Parameter	Symbol and Unit	Equation	Description	Reference
Final Germination Percentage	FGP (%)	$\mathrm{FGP} = \frac{NG}{n} \times 100$	*NG*- total number of germinated seeds; *n*- is the total number of tested seeds.	ISTA [13]
Mean Daily Germination	MDG	$MDG = \frac{NG}{t}$	*NG*- total number of germinated seeds; *T*- total number of days.	Czabator [14]
Mean Germination Time	MGT (days)	$MGT = \sum F * \frac{x}{\sum F}$	*F*- seed germinated on day x.	Kader [15]
Germination Speed	GS (%/day)	$GS = N1/t1 + N2/t1 \ldots\ldots\ldots..$	*N*- the number of germinated seeds; *t*- is the number of days.	Czabator [14]
Peak Value	PV	$PV = \frac{HSG}{t}$	*HSG*- highest number of seeds germination; *t*- number of days.	Czabator [14], Kader [15]
Germination Value	GV	$GV = PV \times MDG$	*PV* - peak value; *MDG*- mean daily germination, calculated as indicated above.	Gairola *et al*. [16]
Germination Index	GI	$GI = \sum(GT/Tt)$	*GT*- total number of germinated seeds; *Tt* period for the final and last germination count.	Islam *et al*. [17]
Coefficient of Velocity of Germination	CVG	$CVG = N1 + N2 + \cdots \frac{Nx}{100} \times N1T1 + \cdots + NxTx =$	*N*- is the number of seeds germinated each day; *T*- number of days from corresponding to the germinated seeds.	Kader [15]
Emergence Rate	ER (%)	$ER = \left(\frac{GT_3}{GT_7}\right) \times 100$	GT_3- number of seeds germinated 3 days after sowing; GT_7- number of seeds germinated after 7 days of sowing.	Islam *et al*. [17]
Seedling Vigor Index	SVI	$SVI = \left(\frac{SL \times FGP}{100}\right)$	*SL*- seedling length (mm); *FGP* – final percentage germination.	Kader [15], Islam *et al*. [17]

Table 3. Description of formulae used to determine total chlorophyll, phenolics, flavonoids, carbohydrates, DNA, and protein content following *in vivo* pretreatment of seeds in various concentrations of colchicine.

Parameter	Symbol and Unit	Equation	Description	Reference
Total Chlorophyll	Chla+b (mg/mL)	$Chla+b = 7.05\Delta A_{661.6} + 18.09\Delta A_{644.8}$	*Chla+b* -concentration of total chlorophyll in *mg/mL*	Boyer [5]
Total Phenolics	PT (mg GAE/g extract	$PT = c \times V/m$	*c*- is the concentration of gallic acid in *µg/mL*, *V*- volume of extract in *mL*, *m*- is the weight (*g*) of extracts.	Nikolova *et al*. [18]
Total Flavonoids	TF (mg Quer/g extract	$TF = As \times mc \times 10/Ac \times ms$	A_s- absorbance of extract, A_c- standard quercetin absorbance, m_s- is the weight of extract (*g*), *mc*- is the weight of quercetin (*g*)	Nikolova *et al*. [18]
DNA and Protein content	NA (µg/mL)	$NA = (A_{260} - A_{280}) \times D$	*NA*- nucleic acids, A_{260}- is the *NA* concentration absorbance at 260 nm, A_{280} *D*- is the dilution factor (50 µg/mL). Proteins were estimated from reading at A_{280}.	Barbas *et al*. [11], Bhusnure *et al*. [12]
Total Carbohydrates	CHO (mg/g)	$CHO = \frac{C}{Ac} \times As \times D$	*C*- concentration of glucose, *Ac*- glucose absorbance, *As*- absorbance of the sample, *D*- dilution factor.	Kafeel *et al*. [19]

Determination of phytochemicals and nucleic acids

Plant chlorophyll pigments were isolated and determined using a procedure as described by Boyer [5] with modifications. Seedling samples were ground into fine powder in liquid nitrogen using a mortar and pestle. The ground tissues were immediately transferred into 50 mL centrifuge containers and stored at -80°C until use. Total chlorophyll content was then extracted from plant tissue homogenates of 0.1 g using 100% acetone, and then the acetone extract filtrates were quantified using a spectrophotometer at 661.6, 644.8, and 470 nm. Average absorbance for each plant extract and levels of total chlorophyll content were calculated according to the equation on Table 3.

The concentration of phenolic contents was quantified based on a colorimetric assay method [6, 7]. A 0.1 g of sample was extracted using 80% methanol and mixture was centrifuged at 3,500 rpm for 10 min. Plant extracts were then mixed with Folin-Ciocalteau reagent and 1.5 mL of 20% sodium carbonate (Na_2CO_3). The mixture was vortexed, incubated for 1 hour and the absorbance measured at 765 nm. Total phenolic content was then determined as gallic acid equivalents (mg GAE per gram of extract) using the formula indicated on Table 3.

Total flavonoid content was determined according to the procedure by Marinova *et al*. [8] and Zhishen *et al*. [9]. The 0.1 g samples were weighted, and 15 mL of 80% methanol was added for the extraction on an orbital shaker. The samples were centrifuged at 3,500 rpm for 10 minutes. About 200–500 µL of the supernatants were mixed with 2 mL distilled water and then further mixed with 1 mL of 5% sodium nitrate, vortexed, and incubated for 5 minutes at room temperature. A 500 µL of 10% aluminum nitrate solution was added to the mixture, allowed to stand for 5 minutes before adding 1 mL of 1M

NaOH, and the absorbance read at 510 nm. The absorbance of quercetin solutions used as standards was also measured at 510 nm, and the flavonoid content was calculated as quercetin equivalents (mg Quer/g of extract) as shown on Table 3.

Total carbohydrate content was determined using phenol-sulphuric acid method as described by Masuko *et al*. [10] with slight modifications. Sample extracts were diluted to 2,000 µL in 10 mL tubes and mixed with 1 mL of 5% phenol solution. A 2 mL of sulphuric acid was added to the mixture, vortexed, and allowed to stand for 10 minutes at room temperature. The samples were then incubated in a water bath set at 30°C for 30 minutes. After the incubation, absorbances were measured at 490 nm using a spectrophotometer, and concentrations of total carbohydrates were calculated using the equation shown in Table 3.

Nucleic acid content was determined as described by Barbas *et al*. [11] and Bhusnure *et al*. [12]. Ground samples (0.2 g) were mixed with 8 mL CTAB (cetyl trimethylammonium bromide) extraction solution, mixed, and incubated for 1 hour with occasional agitation. An equal volume of acetone (80%) was added, vortexed, and centrifuged for 5 minutes at 10,000 rpm at 4°C. The supernatant was collected and then washed several times with CTAB solution and acetone, followed by 30 minutes incubation in a water bath at 65°C. The mixture was then centrifuged for 5 minutes at 3,000 rpm, the supernatant was decanted, and the pellet was resuspended in TE buffer (prepared by mixing 10 mM Tris-Cl pH 8.0, 0.1 mM EDTA, and 1 M NaCl) to a 1 mL per gram of sample plant material. Sample extracts were then spectrophotometrically quantitated at OD_{260}/OD_{280} ratio to determine the nucleic acid contents. The amounts of DNA and proteins were calculated using formula found in Table 3 with equivalent DNA ratio of 1.8 and protein ration of 2.0.

Growth conditions and statistical analysis

Soybean seeds were surface sterilized using chlorine gas for 16 hours and imbibed for respective periods (Table 1) at room temperature. All seed cultures were incubated in a growth room under 120-200 µmol/m^2s light intensity, 24±2°C, 50–60% humidity, and 16 hours photoperiod. Analysis of variance (ANOVA) was performed using version 25 to calculate individual means. The samples' means were further compared using t-test at 5% confidence level.

Results and Discussion

Effect of colchicine on germination

The percentage germination of soybean seeds cv. TGx1835-10E and Dundee, pre-treated with varied concentrations of colchicine under two different durations were presented in Figure 1 and Table 4. Germination rates on pre-treated seeds ranged between 0 to 100%, with cultivar Dundee achieving the highest percentage on seeds imbibed for 12 hours with 0.1% colchicine (Figure 1). Germination further declined to 80, 70, 60, 50, and 48% for treatment A5, A1, A3, B5, and C1 for 6, 12, and 24 hours of incubated soybean seeds at 0.1–1.0% colchicine. There was almost a complete polynomial decrease in percentage germination with both the increase in colchicine concentration and period of incubation. As reported by Gairola *et al*. [16] germination remain quite complex to evaluate because several independent factors like temperature, light, oxygen, and water affects each stage of the germination process in different ways. According to Dhakhanamoorthy *et al*. [21], if these factors are adequately available, each determines a fraction of the germinated seeds and the rate of the

Table 4. Mean comparison of germination parameters of soybeans cultivar Dundee and TGx1835-10E seeds pre-treated with varying concentration of colchicine.

Treatments	MGT	MDG	GS	GI	GV	PV	CVG
A1	2.19±0.13	59.5±1.28*	5.95±1.28	79.0±0.53	5.95±1.29	1.00±2.20*	175±0.81
A2	1.40±0.18	35.6±0.70	3.56±0.70	46.0±0.31	2.55±0.02	0.70±1.30****	111±0.67
A3	1.93±1.09	60.3±1.73*	6.31±1.73*	81.0±0.55	5.41±1.09	0.90±2.10	150±0.58
A4	0.00	0.00	0.00	0.0	0.00	0.00	0.00
A5	2.46±1.41	63.8±1.28	6.38±1.28*	84.0±0.56	7.29±1.92	1.102.40±	196±0.36
A6	1.20±0.70*	27.9±0.46	2.79±0.46	36.0±0.24	1.59±0.39	0.60±1.10**	95±0.65
B1	3.40±1.84	100±2.19	11.10±2.19	146.0±0.98	15.85±0.55	1.40±4.60	276±0.51
B2	1.20±0.67*	32.5±0.38**	3.25±0.38**	43.0±0.29	1.86±0.64	0.60±1.50**	97±0.67
B3	2.00±1.09	55.6±0.60	5.56±0.60	75.0±0.49	4.76±0.35	0.90±2.70	162±0.21*
B4	0.70±0.36	18.5±0.20	1.85±0.20	25.0±0.17	0.53±0.74	0.30±0.90	54±0.70
B5	1.60±0.88**	40.4±0.45	4.21±0.45***	56.0±0.37	3.01±0.39*	0.70±2.00****	120±0.98
B6	1.07±0.58	32.8±0.50**	3.28±0.50**	45.0±0.30	1.41±0.83	0.40±1.50	87±0.70
C1	2.10±1.21	53.2±1.03	5.32±1.03	69.0±0.46	5.32±1.03	1.00±2.00*	167±0.36*
C2	1.60±0.92**	42.1±0.86	4.21±0.86***	59.0±0.37	3.01±0.07*	0.70±1.60	128±0.39
C3	0.00	0.00	0.00	0.0	0.00	0.00	0.00
C4	0.00	0.00	0.00	0.0	0.00	0.00	0.00
C5	0.00	0.00	0.00	0.0	0.00	0.00	0.00
C6	0.00	0.00	0.00	0.0	0.00	0.00	0.00
CD	2.79±1.60	76.0±1.64	7.60±1.64	101.0±0.68	9.78±1.92	1.30±2.80***	223±0.95
C10E	2.60±1.50	69.0±1.45	6.90±1.45	90.0±0.60	8.88±0.39	1.30±2.50***	207±0.94

Values with asterisks (*) are not significantly different ($P>0.05$) using t-test. MGT: mean germination time; MDG: mean daily germination; GS: germination speed; GI: germination index; GV: germination value; PV: peak value; CVG: coefficient of velocity of germination.

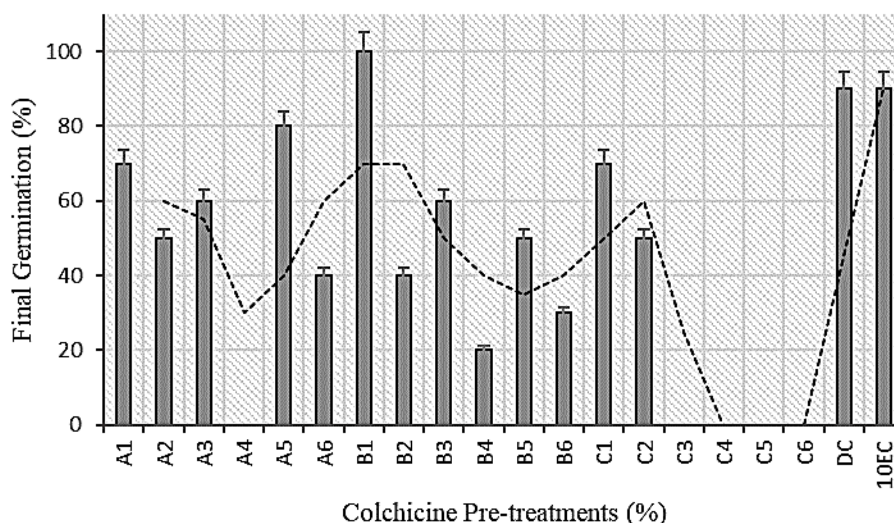

Figure 1. Mean final germination percentage of soybean cv. Dundee and TGx1835-10E seeds pre-treated with different concentrations of colchicine under different periods of incubation in soybean.

germination. Similarly, this study had clearly demonstrated the negative effects of colchicine on germination and seedling development. Percentage germination was consistently high in the control (92% in Dundee and 90% in TGx1835-10E) compared to the colchicine pre-treated seeds, with A4, C3, C4, C5, and C6 recording 0% germination (Figure 1). The results also indicated

that colchicine can be highly fatal to plant cells, as an effect already indicated in animal cells [3].

Reduction in other germination parameters were further observed with increasing seed imbibition period and colchicine concentration. Lower mean germination time (MGT) and mean daily germination (MDG) were recorded, which subsequently affected other germination parameters as indicated on Table 4. For example, treatment B4 had low MGT, which was further accompanied by decrease in GI and CVG when compared to the controls and seeds treated with minimum amounts of colchicine at minimum imbibition period. Although colchicine was reported to be well tolerated by plants and mostly resulting in improved morphological and reproductive characteristics [20], observations made in this study showed that pre-treatment of seeds with colchicine induced significant variations amongst treatment and the control. Similar findings were made by Essel *et al*. [3] in cowpea [*Vigna unguiculata* (L.) Walp] after pre-soaking dry and quiescent seeds in varying concentrations of colchicine (0.05 g/dL, 0.10 g/dL, 0.15 g/dL, and 0.20 g/dL) for 3 hours. The findings made in this study also illustrated significant differences in means of recorded germination based on the genotype. Soybean cultivar Dundee (A1, A3, A5, B1, B3, B5, and C1) mostly recorded a high mean number of germination parameters as showed on Figure 1 and Table 4 compared to TGx1835-10E.

Effect of colchicine on seedling phytochemicals and nucleic acid

Colchicine has been reported to have caused inhibitory effects on seedling development leading to poor plant growth [21]. In this study, the reduction in germination percentage as a result of colchicine exposure and poor survival of germinated seeds led to a dramatical decrease in seedling growths. It was observed that high amount of colchicine inhibited radicle and epicotyl emergence in seeds pre-treated with an increased amount of colchicine (0.5–1%). Soybean seeds imbibed for germination on 1% colchicine showed poor growth and mass deaths of seedlings. Furthermore, there was significant variation among all treatments regarding the period of imbibition. It was observed that seedling establishments was more successful in A1 and A2 incubated for 6 hours compared to seeds pre-treated for more than this period. Pande and Khetmalas [22] also reported such growth inhibitory effects on *Stevia rebaudiana* using mutagenic sodium azide and colchicine at 0 to 0.25% for 12 and 24 hours at room temperature. The reduction in germination rates with subsequent negative effects on seedling growth were also reported by Widoretno [23] in *Pogostemon cablin* and Benth and Khalili *et al*. [24] in *Gerbera jamesonii* Bolus cv. Mini Red. The pre-treatment of seeds with colchicine also increased hypocotyl girth as reported by Essel *et al*. [3]. However, studies that used lesser amounts of colchicine for prolonged duration reported significantly varied results on the contrary. Such reports include that of Udensi and Ontuni [25], which showed improved growths, especially under prolonged incubation periods for germination and seedling establishment.

The significant differences among the treatments were not only limited to germination and seedling morphology but also the variations observed in the total content of chlorophyll, phenolics, flavonoids, carbohydrates, and nucleic acids. The varied compositions of these phytochemicals were determined by decrease/increase in absorbance using spectrophotometric quantifications. Therefore, getting systematic results of these phytochemicals in Dundee and TGx1835-10E

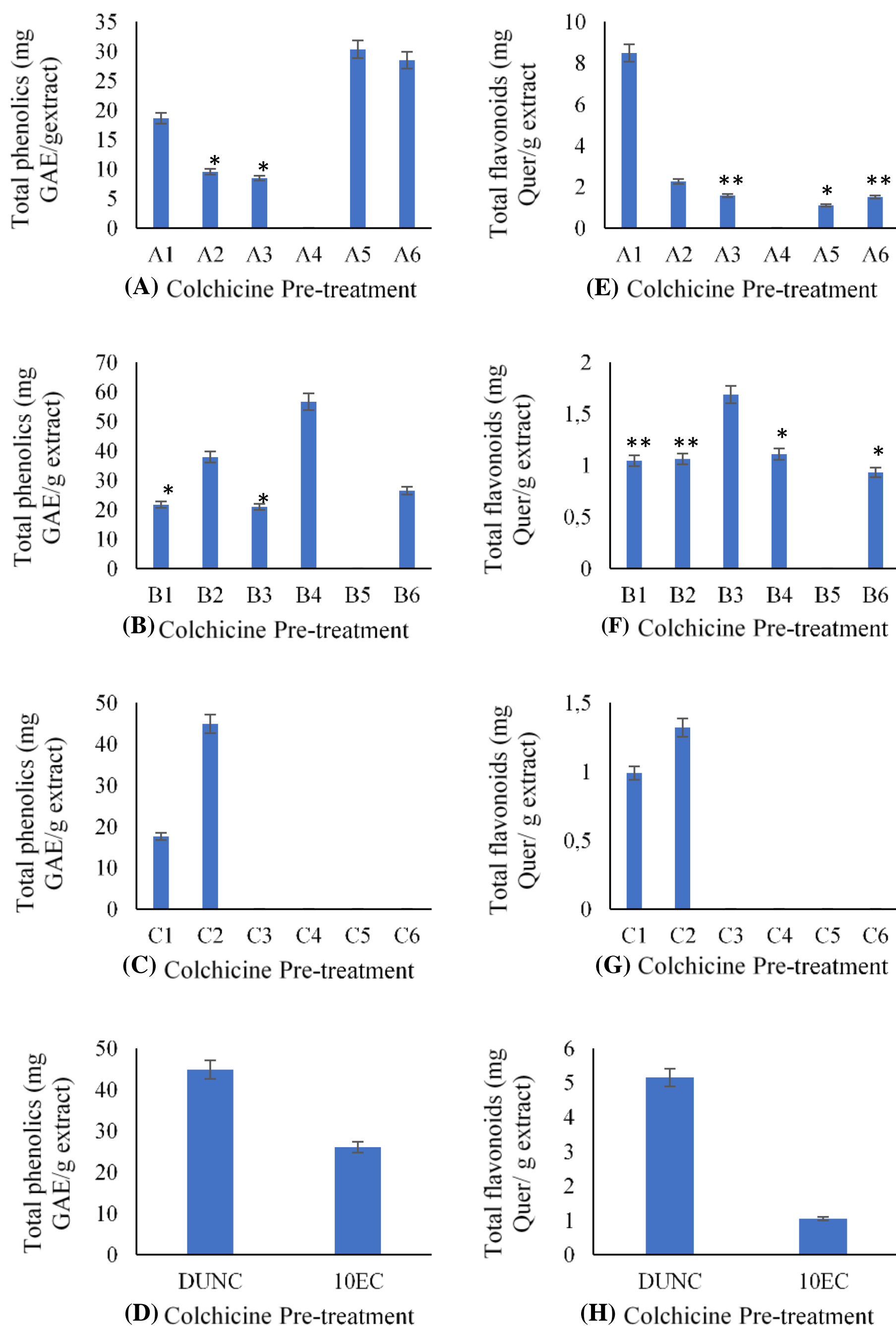

Figure 2. Total phenolic content expressed as gallic acid (GAE) equivalents [A-C) and flavonoid content expressed as quercetin (Quer) equivalents (E-G) of the methanol extracts in soybean pre-treated with 0.1 (A/E), 0.5 (B/F) and 1% (C/G) colchicine, respectively. Values with asterisks (*) are not significantly different (*P*>0.05) using t-test.

soybean cultivars used remain highly important for research and development of legumes against biotic and abiotic stress. The results of analyzed samples and their activities were illustrated in Figure 2 and Table 5.

Table 5. Mean comparison of total phytochemical and nucleic acid contents found in 7-day seedlings germinated from seeds pre-treated with varying concentration of colchicine in soybean cv. Dundee and TGx1835-10E.

Colchicine Pre-treatments	Total Chlorophyll (mg/mL)	Total Carbohydrates (mg/g DW)	DNA (µg/mL)	Proteins (µg/mL)	DNA-Protein Precipitation (µ/mL)
A1	5.74±0.38	1.07±0.18*	0.920	1.377*	2.297
A2	5.45±0.36	0.93±0.15**	2.511	1.196	3.707
A3	3.41±0.23	1.07±0.18*	2.914	1.383*	4.297
A4	0.00	0.00	0.00	0.00	0.00
A5	6.41±0.44	0.70±0.12	1.900**	1.902	3.802*
A6	2.04±0.13*	0.99±0.163**	2.686	1.275	3.961
B1	7.50±0.51	0.80±0.13	4.107	0.858	4.965
B2	6.48±0.42	1.18±0.19	0.605	1.265	1.870
B3	2.03±0.13*	1.55±0.26***	1.793*	1.656	3.449
B4	2.70±0.17**	1.51±0.25***	1.773*	1.616	3.389
B5	0.00	0.00	0.00	000	0.00
B6	2.71±0.18**	1.52±0.19***	1.609	1.273	2.882
C1	6.99±0.45	1.19±0.17	1.922**	1.311	3.233
C2	3.78±0.25	0.98±0.16**	1.854	0.295	2.149
C3	0.00	0.00	0.00	0.00	0.00
C4	0.00	0.00	0.00	0.00	0.00
C5	0.00	0.00	0.00	0.00	0.00
C6	0.00	0.00	0.00	0.00	0.00
CD	8.05±0.43	0.84±0.14****	2.746	1.082**	3.828*
C10E	9.68±0.35	0.80±0.13****	2.664	1.085**	3.749

Values with asterisks (*) within columns are not significantly different ($P>0.05$) using t-test. DW: dry weight of crushed sample extract.

In terms of total chlorophyll content, almost all samples were varied and significant, except A6 (2.04±0.13) and B3 (2.03±0.13) as well as B4 (2.70±0.17) and B6 (2.71±0.18) giving similar mean readings as shown in Table 5. This was the case despite the fact that these emanated from the different colchicine pre-treatments and imbibition periods. It can also be seen that the highest total chlorophyll content was observed in B1 (7.50±0.51) amongst the treatments, and the maximum in the controls CD (8.05±0.43) and C10E (9.68±0.35). Results indicated that colchicine impacted negatively on chlorophyll content since the minimum was observed on pre-treated seeds and the maximum on the controls. These findings were in conflict with Amiri *et al*. [26] who reported that chlorophyll content in treatment 22.5 µM/48 h was more than in the treatment 22.5 µM/24 h of colchicine. This further indicated that imbibition period can also have an impact on total chlorophyll content as well.

Based on Figure 2 results, the highest content of phenolics were obtained in B4 and C2, both treated with increasing amounts of colchicine for 12 hours. These were even higher than those obtained in the controls. Among all the treatments, the highest phenolic content was found in soybean seeds pre-treated with 0.5% colchicine B4 (56.67 mg GAE/g extract), followed by C2 (44,85 mg GAE/g extract), A5 (30.32 mg GAE/g extract), and A6 (28.47 mg GAE/g extract). Overall, phenolic contents were significantly higher in cultivar TGx1835-10E treatments than in Dundee, which demonstrated individual treatment achievement. The differences in phenolic content, however, were not found to be

statistically significant for all the pre-treated seeds, especially between treatment A2 and A3 as well as B1 and B3. Such variations could be observed in a wide variety of plant species and organs, often influenced by growing conditions [27].

The total flavonoids content in different colchicine treatments are also shown in Figure 2 (E–H). Among colchicine pre-treated germinated seedlings, the highest amount of flavonoid content was observed in A1 (8.48 mg Quer/g extract), B3 (1.69 mg Quer/g extract), and C2 (1.319 mg Quer/g extract). Whereas in the controls, Dundee recorded the highest flavonoid content (5.15 mg Quer/g extract) than TGx1835-10E (1.05 mg Quer/g extract). As plants are potential sources of these phytochemicals [28], these results have also shown variations according to colchicine concentration and at a lesser extend according to cultivar differences.

The results regarding total sugar content of all the colchicine pre-treated seeds and the control are also presented in Table 5. Mean carbohydrates values ranged from 0.70 to 1.55 mg/mL in pre-treated seeds and 0.80 to 0.84 mg/mL in the control. Overall, total carbohydrate content lies between 0.7 to 1.5 mg/mL with a clear reduction based on colchicine level and imbibition period. The concentration of sugars obtained were negatively influenced by the poor growth responses of colchicine pre-treated seedlings. This has further affected the ability of the plant to produce starches and soluble sugars as a product of photosynthesis [19]. The concentration of plant nutrients/carbohydrates plays a key role in various metabolic activities of the plant that are essential to promoting seedling growths. Furthermore, the present research findings also indicated that sufficient nucleic acids were also expressed. Colchicine appeared slightly non-toxic to DNA and protein formation, except for A1 and C2 which recorded significantly lower amounts of 0.92 µg/mL and 0.29 µ/mL for DNA and Protein, respectively. The values also showed that the solutions of nucleic acids had less contaminations. Some of the reported contaminants may include phenolics and lipids [29]. Of all the treatments, B1 yielded a high amount of DNA than proteins, followed by A3 with high amounts of both DNA and proteins, followed by A5 which had a good quantity of both as indicated in Table 5.

Conclusion

The results demonstrated significant variations in terms of germination, seedling growth, phytochemicals, and nucleic acids extracted from the samples. There were notable polynomial increases with the increase in the amount of colchicine and imbibitional duration used. Increase in the concentration of colchicine proved detrimental to seed germination and seedling development. However, findings suggest that seeds could be exposed to minimum levels of colchicine with less imbibition period in order to increase the chance of producing better germination and seedling establishment for subsequent polyploidization in soybean.

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