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Evaluation of Genistein Content in Chickpea (*Cicer arietinum* L.) and Mungbean (*Vigna radiata* L.) Sprouts Germinated Under Different Conditions

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Abstract

Cicer arietinum L. (chickpea) and *Vigna radiata* L. (mungbean) are two important pulses, being frequently used by majority of the population all over the world. Sprouts of both pulses are also gaining importance due to the presence of high level of dietary fibers, vitamin B-Complex, vitamin A, vitamin C, omega-3 fatty acids and proteins. Earlier, only the influence of sprouting with water and its effect on certain bioactive constituents were studied but no report on the effect of different growth conditions was presented. Therefore, the present study has been carried out to evaluate the growth and genistein content of chickpea and mungbean sprouts under different growth conditions (BOD, *in vitro*, *in vivo)* along with prohexadione treatments. Among the three studied genotypes of chickpea, CSCD-884 genotype was the best genotype for the genistein content. In case of mungbean, V. RMW 344 genotype was the best genotype for genistein content. With the change in growth condition, *in vitro* group gives best results in mungbean sprouts (28.32 fold higher) while in chickpea sprouts BOD group gives best result (12.8 fold higher). Also, a lower concentration of prohexadione-pure treatment enhances the genistein content in both the chickpea and mungbean sprouts at BOD condition. A significant increase was also observed in chickpea sprouts while treatment with prohexadion-calcium at *in vitro* condition. This study also reveals that mungbeans can supplement higher levels of genistein in comprision to chickpeas if sprouted at controlled culture conditions. This finding could expand the potential for the development of both pulses sprouts as a functional food by giving stress treatment and enhancing the total isoflavonoid contents.

Key words: *Cicer arietinum* sprout, *Vigna radiata* sprout, Elicitation, Prohexadione, Genistein, Isoflavonoid

1. Introduction

Pulses have high nutritional value and play a significant role in traditional human diets throughout the world. Both the seeds as well as sprouts of pulses are tremendous examples of functional food, lowering the risk of numerous diseases and exerting health-promoting effects (Arora and Ramawat, 2018). In addition, sprouting has been reported to improve the nutritive value of seeds through enhancing the bio-availability and digestibility of nutrient (Erba et al., 2018). Numerous investigations have shown that germination is an inexpensive and effective way to improve the nutritional quality of legumes as it increases amino acids, total dietary fibers, and total soluble sugars, antioxidant content (Gómez-Favela et al., 2017; Awika and Duodu, 2017). *Cicer arietinum* L. (Chickpea) and *Vigna radiata* L. (mungbean) are the important pulse crops grown and consumed as a common food in all over the world, especially in the Afro-Asian countries (Sen et al., 2018). Over the past decades, chickpea and mungbean (both seed as well as sprout) have received considerable attention for their different mineral ingredients as well as isoflavone content (Siddiqui et al., 2017; Pajak et al., 2014). Remarkably, isoflavonoids have been reported as the main bioactive components in both chickpeas as well as mung bean. The major bioactive constituents in chickpea and mungbean seed are formononetin (4′-O-methyl ether of daidzein), biochanin A (4′-O-methyl ether of genistein), ononin (formononetin glucoside), sissotrin (biochanin A glucoside), genistein, trifolirhizin, vigradiatain, and 2′-Hydroxydaidzin (Bai et al., 2016; Megías et al., 2016; Tang et al., 2015). We have an interest in elevating the isoflavone contents of both chickpea and mungbean sprouts, in view of a study by Gao et al. (2015) who reported that diversity in isoflavone content could show change upon the experimental conditions as well as the different chemical treatments. Similarly, elicitors and phytohormones enhance the synthesis of phytochemical compounds in a number of fruits, vegetable and herb species (Arora, 2016; Baenas et al., 2014; Arora et al., 2010). Prohexadione calcium (Phd~Ca) (BAS 125W or 9054 W) belongs to a new class of compounds acylcyclohexdiones and works as a plant growth regulator (Reasor et al. 2018). Expenditure of Phd-Ca significantly affected pulp weight, core weight, pulp thickness in *Psidium guajava* L. (Chang, 2016) and also increase the size of the fruit in Red apple trees (Szot et al.,2016). However, earlier only the influence of sprouting with water, light and their effect on certain bioactive constituents were studied (Kanthaliya et al. 2016; Shi et al., 2010) but no report on the effect of different growth condition on isoflavone contents was presented. Therefore, in this study, we carried out a quantitative analysis of isoflavone (genistein) contents in chickpea and mung bean sprouts by growing them in different conditions (BOD, *in vivo*, and *in vitro*) as well as under prohexadione treatment. Employing this analysis, we determined the optimal germination conditions to maximize the genistein contents for the purpose of nutraceutical benefits of both chickpea and mungbean sprouts.

2. Material and Methods

Collection of seeds: Chickpea genotypes C.RSG-888 (Anubhav-Resistance for rainfed, Moderately resistant to wilt & root rot), C.RSG-963 (Aadhar- resistant to wilt, Dry root rot, B.G.M. & Collar rot, Pod borer, & Nematodes), C.CSCD-884 (Moderate resistant to wilt, dry root rot) and mungbean genotypes (V.RMG-62, V.RMW-344,V.RMN-492) were kindly provided by Agricultural Research Station (ARS), Durgapura, Jaipur, Rajasthan in India.

Reagent: Authentic standards of genistein and analytical $AlCl_3$ were used of Sigma-Aldrich. Methanol and other chemical were used of the molecular or analytical grade of Himedia, India.

Study of genistein content in three genotypes of both pulses in BOD growing state: Seeds of both pulses (all three genotypes) were placed on a 5cm thick cotton bed in the magenta box (PLANTON, Tarson) with 20 ml double distilled water in BOD incubator on

24±0.1°C Temperature. The seeds of all three genotypes were sprouted up to 6th day from incubation day as the 1st day (Control).

Study of genistein content in three different growth conditions of both pulses: Chickpeas and mungbeans seed were germinated under different experimental conditions, including germination in the BOD at 24°C, in the *in vivo* conditions during their growing season that is from October to March, and in culture room in MS ¼ (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose, pH 5.8. These cultures were incubated at 25 ± 0.2°C under a 16 h d-1 photoperiod with 50 μmol. m-2 s-1 irradiance provided by cool white fluorescent tubes (Philips, India) with 60-65% relative humidity.

Effect of Prohexadione Pure on genistein content in both Pulses in BOD growing state: Chickpea and mungbean sprouts were investigated for growth and genistein content with and without treatment of Phd~Ca (1mg/l & 5 mg/l). Seeds were placed on a 5cm thick cotton bed in the magenta box (PLANTON, Tarson) with 20 ml double distilled water in BOD incubator on 24±0.1°C Temperature.

Effect of Prohexadione-Ca on genistein content in both Pulses in in vitro growing state: Both the chickpea and mungbean sprouts were investigated for growth and genistein content with and without treatment of Phd~Ca (500mg/l). Both the pulses were germinated up to 6th day in culture room on MS ¼ (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose and pH 5.8. These cultures were incubated at 25 ± 0.2°C under a 16 h d-1 photoperiod with 50 μmol. m-2 s-1 irradiance provided by cool white fluorescent tubes (Philips, India) with 60-65% relative humidity.

Preparation of extract and solution: Each day (till 6 days) germinated seeds were dried in 60˚C in the oven for 12 hours after that ground in pastel-motor. The extract was prepared by the 100mg dried powder of sprouts in 5 ml methanol and shaking on a test tube rotator for 6 hours on the 70 rpm speed at room temperature (24°C - 26°C). After that sonication for 5 min was done by sonicator (Sonar) and centrifuged for 10 min on 7000g. An aliquot of the solution is used for the determination of genistein content. The genistein content was determined according to the spectrophotometer method described by César et al. (2008), and a standard curve was prepared using genistein authentic compound of Sigma.

Statistical Analysis: All values were expressed as the mean ± standard deviation (X±SD). Data were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc Dunnett's test, using the Prism software. The difference of $p<0.05$ was considered significant.

3. Results and Discussion

Study of genistein content in three genotypes of both pulses in BOD: Sprouting may be considered a normal biotechnological module and could be used to increase the nutritional value of food products. In the present study, up to the 6th day, 100% sprouting was observed in all the three genotypes of both the pulses incubated in BOD. Interestingly, these 6th day sprouts stand much longer and healthy in comparison to other day sprouts. In the studied three genotypes of chickpea the maximum genistein content was observed at sixth day sprouts of C. CSCD-884 (84.67mg/100g) in comparison to C.RSG-888(61.35 mg/100g) C.RSG-963(69.35mg/100g) genotypes. However, in the case of mungbean genotype, the highest genistein content was observed in a sixth-day sprout of V.RMW-344(41.09mg/100g) in comparison to V.RMG-62(38.79mg/100g) and V.RMN-492(34.21mg/100g) genotypes. These results revealed that genistein increased from 7-11 folds in the case of chickpea sprout and 1.6-2.6 folds in mung bean sprout in contrast to control. Similar results were obtained by Quinhone Júnior and Ida (2015), they found that after 168 h of germination, the total content of aglycones in the soybean sprouts represented 2.5% of the total isoflavones and was 2.9 fold higher than ungerminated seeds. In the same way, Pajak et al. (2014) has reported that germination increased the total phenolic (TP) and flavonoid (TF) levels, as well as the amino acid of the seeds and influenced the profile of free and bound flavonoid in mung beans, radish, broccoli, and sunflower. In relation to ungerminated seeds, a previous study showed that the genistein content in soybean sprouts increased up to 1-3 times after the 7th day of germination for soybean cultivars while the daidzein content increased up to 3-9 time (Kim et al., 2006). Also, Urbano et al. (2005) reported that germination of *Pisum sativum* seeds for 3 days significantly improves palatability and nutritive utilization of these seeds in form of protein and other constituents. Table 1 and Table 2 summarises the genistein content among three studied genotypes of both the mung bean and chickpea. According to these results, the best genotype (C.CSCD 884 for chickpea and V.RMW 344 for mung been) were selected for further evaluation of genistein content under different growth conditions.

Table 1. Effect on growth and genistein content in different genotypes of *chickpea* sprouts in BOD. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus Ist day)

Harvest day	Dry weight(g)			Genistein Content (mg/100g)		
	C.CSCD 884	*C.RSG 888*	*C.RSG 963*	*C.CSCD 884*	*C.RSG 888*	*C.RSG 963*
1st day	1.25±0.02	1.33±0.01	2.11±0.10	7.237±0.26	7.916±0.69	9.767±0.36
2nd day	1.38*±0.02	1.49*±0.03	1.89±0.09	13.160*±0.96	9.548**±0.98	10.228**±0.85
3rd day	1.47*±0.03	1.33±0.01	3.42**±0.11	35.128**±1.23	24.241**±1.51	27.553**±1.05
4th day	1.57*±0.05	1.54*±0.06	2.11±0.13	53.011**±1.56	36.584**±1.20	43.254**±1.19
5th day	1.38*±0.01	1.34*±0.01	2.02±0.17	70.168**±1.78	51.653**±1.68	58.781**±1.97
6th day	1.44*±0.03	1.56*±0.03	1.75±0.08	84.673**±1.95	61.356**±1.10	69.345**±1.85
F value	27.81^{b}	32.02^{b}	76.67^{b}	1519^{b}	1205^{b}	1165^{b}

Number of seeds = 10, ^{a} Non-significant (P> 0.05), ^{b} Significant (P<0.01), * or **Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Table 2. Effect on growth and genistein content in the different genotype of *mungbean* sprouts in BOD conditions. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus Ist day)

Harvest day	Dry weight (g)			Genistein Content (mg/100g)		
	V.RMG 62	*V.RMW 344*	*V.RMN 492*	*V.RMG 62*	*V.RMW 344*	*V.RMN 492*
1st day	0.310±.01	0.190±.03	0.230±.01	15.698± 1.12	16.223±.956	20.570±1.18
2nd day	0.360*±0.02	0.390**±.02	0.300*±.0.02	26.341**±1.08	20.205*±1.02	21.658*±1.36
3rd day	0.360*±0.01	0.380**±.02	0.310*±.0.02	30.211**±0.26	25.369*±1.30	23.754*±1.85
4th day	0.350*±0.03	0.320*±0.01	0.250*±.01	31.135**±1.12	37.175**±1.90	25.566*±1.87
5th day	0.309±0.01	0.318*±0.01	0.302*±0.02	38.532**±1.68	39.158**±2.10	32.351*±1.98
6th day	0.313±0.02	0.342*±0.04	0.307±0.02	38.796**±1.52	41.099**±2.10	34.216*±1.98
F value	11.94^{b}	53.75^{b}	15.68^{b}	128.24^{b}	146.19^{b}	23.02^{b}

Number of seeds = 20, ^{a} Non-significant (P> 0.05), ^{b} Significant (P<0.01), * or **Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Study of genistein content in three different growth conditions of both pulses: A number of studies have been scrutinized for the influence of germination techniques on the nutritional composition of several legume species. In the present study, to conclude the effect of different growth condition on genistein content in both pulses, seeds were incubated in BOD, *in vivo*, and *in vitro* situations. The maximum genistein content in chickpea sprouts was observed in BOD condition (91.28mg/100g) in comparison to *in vivo* (58.17mg/100g) as well as *in vitro* conditions (42.36mg/100g) at 7th day (Table 3). Remarkably, genistein content in this group was 2.15 fold higher than *in vitro* group and 1.56 fold higher than *in vivo* group. In case of mungbean sprout, the highest genistein content was observed in *in vitro* group (181.25mg/100g) in comparison to *in vivo* (120.81mg/100g) and BOD (52.56mg/100g) groups at 7th day (Table 4).

Table 3. Effect on growth and genistein content in different growth conditions in *chickpea* sprouts (*C.CSCD 884*)**.** The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 1st day)

Harvest day	Dry weight(g)			Genistein content(mg/100g)		
	BOD	***In vivo***	***In vitro***	**BOD**	***In vivo***	***In vitro***
1st day	1.33±0.02	1.68±0.03	1.090±0.01	6.687±0.26	4.256±0.78	3.658±0.46
2nd day	1.49*±0.01	1.88*±0.04	1.42*±0.02	11.160*±0.96	9.823**±0.92	7.469**±0.69
3rd day	1.33±0.01	1.99*±0.05	1.52*±0.03	33.076**±1.23	15.667**±1.01	11.749**±0.88
4th day	1.54*±0.03	1.80*±0.04	1.32*±0.02	50.712**±1.56	27.926**±1.13	13.459**±0.63
5th day	1.34*±0.02	2.23*±0.06	1.33*±0.03	72.272**±1.78	52.813**±1.75	24.761**±1.22
6th day	1.56*±0.03	2.07*±0.06	1.53*±0.03	85.590**±1.95	50.934**±1.17	35.470**±1.43
7th day	1.73*±0.03	1.78*±0.03	1.69*±0.04	91.281**±2.38	58.174**±1.65	42.369**±1.46
F value	83.29^{b}	44.08^{b}	105.4^{b}	1560^{b}	968.9^{b}	603.4^{b}

Total number of seed = 10, a Non-significant (P> 0.05), b Significant (P<0.01), ∗ or **Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Table 4. Effect on growth and genistein content in different growth condition in *mungbean* sprouts (*V. RMW 344*). The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 1st day)

Harvest day	Dry weight(g)			Genistein content(mg/100g)		
	BOD	***In vivo***	***In vitro***	**BOD**	***In vivo***	***In vitro***
1st day	0.700±0.01	0.710±0.03	0.690±0.03	18.799±0.95	2.945±0.009	6.486±0.08
2nd day	0.710*±0.02	0.720*±0.03	0.720*±0.03	19.421*±1.09	4.915*±0.03	17.787**±1.26
3rd day	0.720*±0.01	0.670±0.02	0.730*±0.03	22.820*±1.15	17.092**±1.11	31.369**±1.54
4th day	0.690±0.01	0.540±0.01	0.670±0.02	24.282*±1.31	33.445**±1.78	45.853**±1.98
5th day	0.640±0.01	0.560±0.02	0.700*±0.02	38.646**±1.56	59.041**±1.34	65.927**±2.20
6th day	0.630±0.02	0.530±0.02	0.620±0.02	45.698**±2.10	78.727**±1.60	159.731**±3.50
7th day	0.540±0.02	0.450±0.02	0.560±0.02	52.562**±2.98	120.814**±3.90	181.256**±4.20
F value	28.99^{b}	47.10^{b}	12.31^{b}	189.7^{b}	1660^{b}	2401^{b}

Total number of seed = 20, ^{a} Non-significant (P> 0.05), ^{b} Significant (P<0.01), * or **Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

It is also observed that genistein content in mung bean sprout exist 4 to 5 fold higher in comparison to chickpea sprouts at *in vitro* group. Similarly, Vidal-Valverde et al. (2002) found that germination modified the nutritional composition of legumes and variability in these compositions depends on the variety of legume and germination conditions. Also, Kim et al. (2006) reported an increase in the malonylglucoside content in seven soybean cultivars germinated with light (110 times higher) and without light (33 times higher) for 120 h at 25°C. However, the malonyldaidzin, malonylgenistin and malonylglycitin content declined 7.7, 2.0 and 1.4 times after 168 h of germination at 25°C with light (Shi et al., 2010). In the same way, Khattak et al. (2007) also mentioned that germination time up to 48 h significantly reduced the phytic acid content from 1.01% to 0.6% while beyond that time it increased significantly and reaching the maximum value in *Cicer arietinum* L. sprouts. Furthermore, Agostina et al. (2008) suggested that variability in isoflavone content in *Lupinus albus* cultivars depend on altered growth conditions as well as growth stage.

Effect of prohexdione-pure on genistein content in both pulses in BOD growing state: The lower concentration of prohexdione pure increase the dry weight of chickpea sprouts. Up to 1.25 fold increments observed in dry weight at lower concentration (1mg/l) while 1.18 fold increments observed at higher concentration (5mg/l) in contrast to control at 6th day. Interestingly, a significant increase in genistein content was observed at both concentrations of treatment. The maximum genistein content (100.15mg/100gm) was recorded at higher concentration of prohexdione pure treatment in comparison to control (85.59mg/100gm) and 1mg/l concentration (86.53mg/100gm) (Table 5).

Table 5. Effect on growth and genistein content of *chickpea* sprouts *(C.CSCD 884)* treated by Prohexadione Pure in BOD. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 1st day)

Harvest day	Dry weight(g)			Genistein content(mg/100g)		
	Control / non treated	Treated		Control	Treated	
		1mg	5mg		1 mg	5 mg
1st day	1.43±0.02	1.42±0.03	1.53±0.05	6.687±0.26	3.719±0.15	2.155±0.12
2nd day	1.41±0.02	1.40±0.04	1.46±0.01	11.160*±0.96	24.384**±1.36	29.899**±1.74
3rd day	1.44*±0.02	1.36±0.01	1.41±0.01	33.076**±1.23	36.573**±1.64	54.560**±2.10
4th day	1.95*±0.09	1.49*±0.06	1.55*±0.37	50.712**±1.56	63.905**±2.34	51.847**±1.95
5th day	1.38±0.11	1.34±0.02	1.43±0.02	72.272**±1.78	77.367**±2.64	76.683**±2.89
6th day	1.36±0.02	1.70*±0.05	1.61*±0.03	85.590**±1.95	86.530**±2.36	100.155**±2.51
F value	39.85[b]	26.95[b]	3.492[a]	1560[b]	836.5[b]	820.7[b]

Total number of seed = 10, [a] Non-significant (P> 0.05), [b] Significant (P<0.01), * or ** Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

On the other hand, a decrease was observed in dry weight of mung bean sprouts. The highest dry weight was observed at 1st day sprouts at both concentrations of treatment whereas in control state the highest dry weight was observed at 3rd day sprouts. Moreover, 1.10 fold increases in genistein content were observed in the lower concentration of prohexdione pure (1mg/l) at 6th day sprouts in comparison to control while 0.88 fold decreases were observed at higher concentration (5mg/l) (Table 6).

Table 6. Effect on growth and genistein content of *mungbean* sprouts (*V.RMW 344)* treated by perhexiodione pure in BOD. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 1st day)

Harvest day	Dry weight(g)			Genistein content(mg/100g)		
	Control/ non treated	Treated		Control/ non treated	Treated	
		1 mg	5mg		1mg	5mg
1st day	0.700±0.01	0.770±0.03	0.830±0.04	18.799±0.95	19.022±0.89	19.311±1.02
2nd day	0.710*±0.02	0.710±0.03	0.720±0.02	19.421*±1.09	23.149*±1.23	18.690±0.08
3rd day	0.720*±0.01	0.740±0.03	0.690±0.02	22.820*±1.15	21.468*±1.15	17.155±0.09
4th day	0.690±0.01	0.740±0.38	0.670±0.02	24.282*±1.31	24.428*±1.24	29.435*±1.22
5th day	0.640±0.01	0.620±0.31	0.630±0.02	38.646**±1.56	42.776**±2.14	34.150*±1.35
F value	8.211[a]	7.957[a]	19.28[a]	129.300[b]	139.000[b]	196.800[b]

Total number of seed = 20, [a] Non-significant (P> 0.05), [b] Significant (P<0.01), * or ** Means are significantly different when compared with control (Day 1st) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Similar results were also obtained by Swieca et al. (2014), they found that phenylalanine treatment raises flavonoid contents up to 1.6 fold in lentil sprouts. Moreover, Treutter et al. (2010) have reported increased phenolic content and unaffected biomass in *Actinidia arguta* leaves after application of Prohexadione-Ca. This compound is known to suppress gibberellic acid biosynthesis and induces genes for polyphenols biosynthesis (Rademacher 2000). Similarly, Mendoza-Sanchez et al. (2016) reported that salicylic acid treatment rise epigallocatechin (63fold), rutin (41fold) and quercetin (16.6 fold) flavonoids in *Phaseolus vularis* seed during sprouting. Jadhav et al. (2016) reported that 0.5-1.25 % caffeine treatment gives the best results which can be reconfirmed by plant height, dry weight, as well as additional nutritional constituents in *Vigna radiata* L. Previously, an increase in isoflavonoid content has been observed when developing embryos and other plant organs of soybean seed were fed with phenylalanine (Dhaubhadel et al., 2003). Thus the addition of growth regulators have a profound effect on the biosynthetic pathway and increased content can be obtained by additions of these compounds.

Effect of prohexdione-Ca on genistein content in both pulses in vitro growing state: In recent years, several studies have shown that the sprouts of legumes after germination have more apparent biological activities as well as secondary metabolites despite having relevant biosynthetic enzymes are activated during the initial stages of germination. A significant increase was observed in both dry weights as well as genistein content in chickpea sprouts. The highest dry weight in control state was observed at 6th day while in treatment it will be highest at 4th day. Interestingly, maximum genistein content was observed in sprouts treated with prohexdione Ca at 500mg/l treatment (46.22 mg/100g) in comparison to control (35.47mg/100g) at 6th day. In the case of mung bean sprouts, a significant decrease was observed in both the genistein content and dry weight. The maximum genistein content was observed at control state (159.73mg/100g) while 1.12 fold decreases were observed in treatment with prohexadion Ca at 6th day (Table 7 and Table 8).

Table 7. Effect on growth and genistein content of *chickpea* sprouts (*C.CSCD 884*) treated by prohexadione-Ca in *in vitro* condition. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 2nd day)

Harvest day	Dry weight(g)		Genistein content(mg/100g)	
	Control	500mg	Control	500mg
2nd day	1.42±0.02	1.49±0.01	7.469±0.69	4.775±0.12
3rd day	1.52*±03	1.36±0.02	11.749*±0.88	14.775**±1.00
4th day	1.32±0.02	1.67*±0.03	13.459*±0.63	20.620**±1.69
5th day	1.33±0.03	1.54*±0.03	24.761**±1.22	30.908**±2.24
6th day	1.53*±0.03	1.47±0.02	35.470**±1.43	46.226**±2.29
F value	27.88^{b}	48.23^{b}	376.30^{b}	267.00^{b}

Total number of seed = 10, ^{a} Non-significant (P> 0.05), ^{b} Significant (P<0.01), * or **Means are significantly different when compared with control (Day 2nd) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Table 8. Effect on growth and genistein content in *mungbean* sprouts (*V.RMW 344*) treated by prohexadione-Ca in *in vitro* condition. The data were analyzed by one-way ANOVA followed by Dunnett multiple comparison test (comparing all versus 2nd day)

Harvest day	Dry weight(g)		Genistein content (mg/100g)	
	Control	500 mg	Control	500mg
2nd day	0.720±0.03	0.720±0.04	17.787±1.26	16.991±1.04
3rd day	0.730*±0.03	0.675±0.03	31.369*±1.54	28.551*±2.40
4th day	0.670±0.02	0.675±0.03	45.8539**±1.98	30.477*±2.85
5th day	0.700±0.02	0.780*±0.03	65.927**±2.20	49.965**±3.15
6th day	0.620±0.02	0.650±0.02	159.731**±3.50	141.724**±4.11
F value	6.384^{a}	6.654^{a}	1907^{b}	922.8^{b}

Total number of seed = 20, ^{a} Non-significant (P> 0.05), ^{b} Significant (P<0.01), * or **Means are significantly different when compared with control (Day 2nd) at (P<0.05) or (P<0.01), according to Dunnett multiple comparison test. Mean data without * are non-significantly different from the control value

Similarly, Swieca and Baraniak (2014) reported that H_2O_2 and mannitol treatment give rise to both phenolic and antioxidant constraints (8-12 fold) in lentil sprouts in contrast to control. Also, Shirvani et al. (2016) recommended that sprouting caused a significant increase in organic acids and minerals (except potassium). However, according to Phommalth et al. (2008), the dry weight of sprouts of the *AGA3* and *Pungsannamulkong* cultivars reduced with germination time and showed the slightest values after the 168 h of germination. Furthermore, the view about a significant decrease in genistein content in mung bean sprouts was supported by the concentration-dependent oxidation of the plant extracts (Tarzi et al., 2012).

4. Conclusion

Among the three studied genotypes of chickpea, CSCD-884 genotype was the best genotype for genistein content. However, in case of mung bean, V. RMW 344 genotype contains high genistein content. Therefore, these genotypes were chosen for further enhancement of genistein content under different growth condition. With the change in growth condition, *in vitro* group gives best results in genistein content in mung bean sprouts (28.32 fold higher) while in case of chickpea sprouts BOD group give the best result (12.8 fold higher). In addition, a lower concentration of prohexadione-pure treatment enhances the genistein content in both the chickpea and mung bean sprouts at BOD condition. Remarkably, a significant increase was also observed in genistein content in chickpea sprouts while treated with prohexadion-calcium at in vitro condition. The present study reveals that chickpea and mungbean are potential sources of genistein and legume sprouts are a better source of isoflavones than the seeds. Prohexadione-calcium can be used to control vegetative growth with no change in the nutritional qualities of both pulses. It discloses that mungbeans can supplement higher levels of genistein contents than chickpeas if sprouted at controlled culture conditions. Conclusively, we can say that enhancement of sprout quality by elicitation with abiotic stresses is low-cost and easy biotechnology and it seems to be an alternative to conventional techniques applied to improve the health-promoting bioactive constituents as well as bioactivity of low-processed food. This finding could expand the potential for the development of both pulses sprouts as a functional food by giving stress treatment and enhancing the total isoflavonoid contents.

Conflict of Interest Statement: We declare that we have no conflict of interest.

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