

Original Research Article

Chemical-induced dormancy breaking of freshly harvested potato minitubers and its effect on plant growth.

Comment [B1]: its effect on subsequent growth and yield.

Abstract

Tuber dormancy is an important phenomenon in potato seed production cycle and its duration can positively affect the number of crops and crop performance during the seed production. The present study was conducted to investigate the effect of different factors which include potato genotype (Roko and SM kaghan varieties), chemical treatments i.e GA₃, thiourea, Thiourea+GA₃ and sugar in combination with light and dark storage conditions. It was found that between varieties, Roko responded better than SM kaghan to various chemical treatments regarding number of days to dormancy breaking. It was found that the growth hormone GA₃ (gibberellic acid) in combination with thiourea can significantly increased sprouting percentage to 100% under dark conditions after 31 days of application. However, it was observed that after sowing of the treated tubers the emergence percentage, growth performance and minituber yield did not show any significant effect of the three experimental treatments in comparison with control. It is therefore concluded that tuber treatment with thiourea+GA₃ and store in dark can decrease the dormancy duration without having any negative effect on plant physiological growth and tuber yield.

Comment [B2]: evaluate

Comment [B3]: genotypes

Comment [B4]: under

Comment [B5]: planting

Key words: Potato, minitubers, Dormancy, thiourea, GA₃, sprouting %

Comment [B6]: key words should be words which are not appearing in the title.

Introduction

Potato (*Solanum tuberosum* L.) is considered as an important food crop worldwide and a good source of energy, fats, proteins, minerals and vitamins (Ekin 2011; King and Slavin 2013). Among the root and tuber crops, it ranks first followed by cassava, sweet potatoes and yams (Hawkes, 1990; FAO, 2008). There are a number of constraints that negatively affect potato production in the world and among them lack of quality seed potato has vital significance (Gul et al., 2020). In Pakistan, it is also an important vegetable and is grown on 0.19 million hectares, with an estimated annual production of 4.6 million tons, at an average yield of 24.2 tons per hectare (Bashir et al., 2021). Potato is grown round the year in Pakistan as autumn, spring and summer crop. Seed potatoes varies in their economic dormancy period regarding country and cropping patterns. The year round potato cropping in Pakistan shortens the interval between harvesting and planting consecutive crop and the growers usually adapted the autumn to autumn cycle by storing the seed of autumn crop of medium to long dormancy period genotypes for the coming autumn crop which declines the seed quality (Khan, 2004; Haider et al., 2019).

Comment [B7]: First mention the major constraints which limit potato production.

Comment [B8]: How much is the world average?

Comment [B9]: It needs rewriting.

Dormancy is the final stage of tuber life serving to preserve tubers as organs of vegetative reproduction under unfavorable growth conditions. Endogenous hormones have been proposed to play a significant role in tuber dormancy regulation. Generally dormancy is divided into three categories. During deep dormancy (endodormancy) growth is stopped under the influence of internal physiological factors even under ideal conditions. The second category of dormancy

i.e. par dormancy is forced or induced dormancy in which growth is arrested by unfavorable external factors. The third stage of dormancy is ecodormancy during which meristematic activity is halted by external environmental factors. Immediately after harvest tubers are in the state of deep dormancy and cannot sprout even under favorable environmental conditions (Suttle, 2011).

After the termination of the deep dormancy tubers can sprout but they remain at rest under unfavorable external conditions (at temperature 0 to 4°C). In the beginning of sprouting, lateral buds are in the state of forced dormancy (depending on internal physiological factors) because they experience a correlative inhibition from the apical bud sprouting earlier (Suttle, 2007; Teper-Bamnlker, et al., 2012).

The tuber dormancy duration and sprouting time have significant economic importance in seed production cycle. The tuber dormancy period depends on potato cultivar characteristics (genotype) and also on growing season or pre-harvest and post-harvest conditions such as temperature and light. Depending on the genotype tuber storage at high temperature (up to 30°C) and humidity (up to 90%) favors dormancy breaking and early bud outgrowth and temperature above 35°C can immediately break dormancy inducing a condition called psychopathy or heat sprouting (Suttle, 2007).

Considering the storability of potato the term dormancy is significantly important. The tuber dormancy duration gives information on how long the potato can be stored. It helps in selecting varieties for short to long term storage. Regarding process and fresh market potatoes, detrimental quality concerns raised if sprouting begins which causes changes in carbohydrate status, weight loss, increase in respiration rate and storage management issues such as impeded airflow. Therefore controlling the length of the dormancy period is of considerable economic importance especially in case of seed potato. New strategies are needed because current cold storage techniques are often problematic. Seed growers may need to accelerate or retard sprout development depending upon the time of year and intended seed market. When working with seed production programs it is essential to standardized different chemicals and their concentrations needed for dormancy breaking for routine applications because different types of genetic materials (varieties) react differently to various chemicals that promote sprouting. Usually late maturing clones have a long dormancy that is difficult to break than that of early maturing clones.

Comment [B10]: reference

Early potato planting requires dormancy breaking. Various methods for chemical regulation of dormancy period has been developed which includes tuber treatment with Rindite (Kim et al., 1999), bromoethane (Alexopoulos et al., 2009), carbon disulfide (Salimi et al., 2010), thiourea (Mani et al., 2013), ethanol (Claassens, et al., 2005), or bromoethane favors dormancy breaking (Alexopoulos, et al., 2009). Moreover, cytokinins and GA₃ (gibberellins) reactivate meristematic and sprouting activity shortly before dormancy termination (Hartmann et al., 2011; Suttle, 2004). The response of potato genotype to different chemical treatments and storage methods may also be differed due to genetic factors, type of chemicals and storage conditions.

Comment [B11]: reference

Comment [B12]: what about GA₃ and sugar?

In seed production programs it is indispensable to standardize different protocols and chemicals used for dormancy breaking. The common way of producing pre-basic seed of potato is the production of mini-tubers in the greenhouse from in vitro plantlets produced through tissue culture. Generally for certified seed production cycle the mini-tubers harvested during the month of May have to be planted usually in the first week of June in Hilly areas suitable for seed

production. So there is not enough time between harvest and planting to break dormancy naturally. Therefore, it is anticipated to develop an effective technique easy to apply in order to induce seed-tuber dormancy breakage in different potato varieties. The aim of this study is to develop an effective protocol in order to analyze the performance of various chemical treatments in interaction to certain environmental factor (light;dark) and plant genotypes to find a potential relation between treatments applied in the storage phase and to record the dormancy breakage duration of each variety and to evaluate potato crop performance after plantation.

Materials and Methods:

This study was conducted in the greenhouse at Hazara Agriculture Research Station, Abbottabad. The research experiment was carried out in two phases: In the first phase (at storage level) minitubers were treated with different chemicals while in the second phase the same treated minitubers were sown in the green house and evaluated for plant growth and yield performance.

This research is based on 3 factorial randomized complete design with two replications. Experimental factors were chemical treatments as factor one with five levels (control, 50 ppm gibberellic acid, 1% thiourea, thiourea +GA₃ and Sugar treatment (100g sugar in 200ml water), potato cultivars as the second factor with two levels (SM Kaghan and Roko) and light as the third factor with two levels (light, dark conditions). Freshly harvested 100 minitubers of uniform shape and size of each variety was selected. The weight of the each selected tuber was between 30-40 gram.

The tubers were soaked in the 1% Thiourea solution for 1 hour and air-dried. For GA₃ treatment tubers were soaked in 50ppm solution for 30 minutes and air dried. For sugar treatment 1:2 ratio sugar solution (dissolving 100g of table sugar in 200ml of water) was prepared according to the procedure described by Zaghum et al., 2021 and tubers were sprayed thoroughly with this solution and air-dried. For thiourea+GA₃ treatment first tubers were soaked in thiourea (1%) solution for 1 hour then transferred to GA₃ solution and soaked for 30 minutes, removed and air dried while for control the tuber were treated with distilled water. After each treatment application the treated minitubers were placed in plastic trays and kept in the dark and light conditions. Data were recorded on sprouting (%), number of sprouts per tuber and any tuber rotting (%).

In the second phase of the experiment the same treated tubers when showed sprouting were sown in earthen pots (18x20cm) filled with 4 kg of sterilized peat moss soil and data on plant growth in terms of plant height, number of branches and tuber yield per plant were recorded. The recorded data was analyzed with the help of computer software Statistix8 Version 8.1.

Results

Potato minitubers of both varieties i.e SM Kaghan and Roko for each respective treatment were observed regularly to ensure the emergence of any sprout. Only the sprout that reached a length of about 2mm was counted as the first sprout from that particular tuber. Data pertaining to sprouting percentage of each respective treatment interaction showed that after 22 days of treatments application only 10% sprouting was first seen in potato variety Rokominitubers placed in Dark and treated with Thiourea + GA₃ (Figure 1). Any other treatment interaction has not shown sprouting in both varieties.

Comment [B13]: planted

Comment [B14]: why it is two? In green house trials, mostly more than three replications are recommended.

Comment [B15]: Mention the concentration

Comment [B16]: Genotypes. Please be consistent

Comment [B17]: storage condition

Comment [B18]: delete it.

Comment [B19]: What was your base to set such treatments? you did not even mention the concentration of these chemicals important for dormancy breaking of potato tubers in the introduction section.

Comment [B20]: reference

Comment [B21]: what about ethanol? How did you dissolve GA₃ solution?

Comment [B22]: What about sprout length, dormancy period, fresh and dry mass of sprouts which were important parameters at this stage?

Comment [B23]: During the storage period, the internal temperature and relative humidity of the storage room should be recorded every day using thermo hygrometers. What about in your case?

Comment [B24]: that

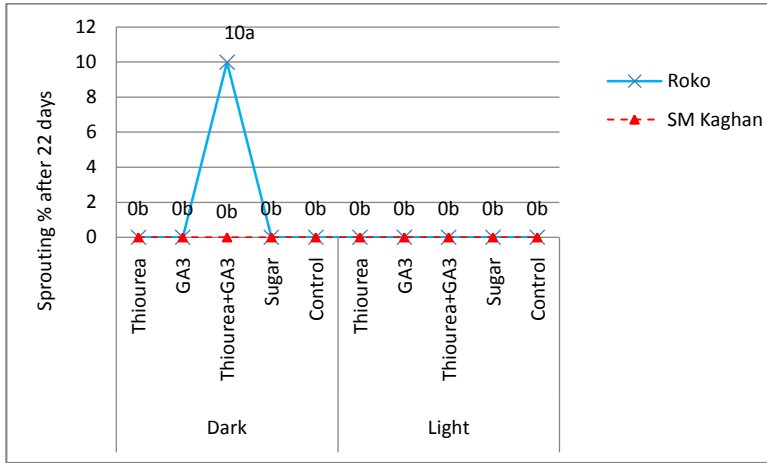
Comment [B25]: the following parameters were also important at this phase:

1. Days to emergence
2. Days to physiological maturity
3. Average tuber weight
4. Average tuber number
5. Total dry biomass
6. Stem number
7. Specific gravity and dry matter content

Comment [B26]: what type of experimental design was used?

Comment [B27]: Cite in the reference section

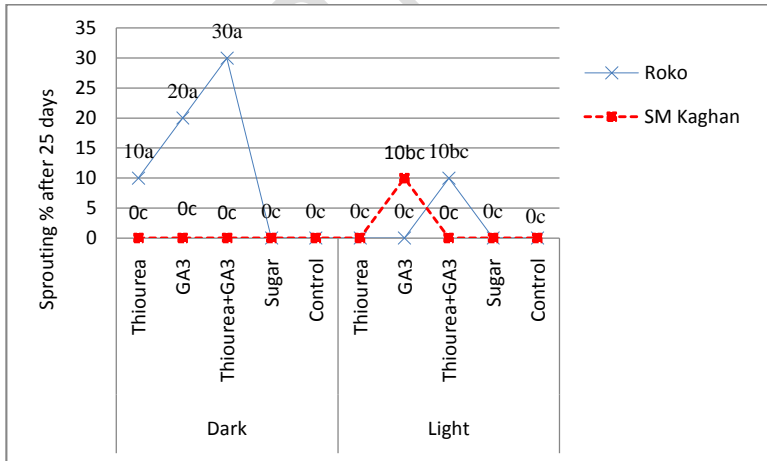
Comment [B28]: Observed



Mean values accompanied by different letters are significantly different at $P \leq 0.05$

Figure 1: Mean comparison of sprouting % in potato minitubers under interactive effect of genotype (variety) x chemical treatments x storage condition after 22 days of chemical application.

After 25 days figure 2 showed 10% sprouting in Roko variety treated with thiourea under dark conditions followed by 10% and 20% sprouting in GA₃ treatment under light and dark conditions, respectively. 30% sprouting was recorded in Thiourea + GA₃ treatment in dark environment while only 10% tubers showed sprouting in the same treatment but under light conditions in Rokominitubers. Whereas variety SM Kaghan showed 10% sprouting in GA₃ treatment under light conditions (Figure 2). In the sugar treatment and control no sprouting was observed.



Mean values followed by different letters are significantly different at $P \leq 0.05$

Figure 2: Mean sprouting % in potato minitubers under interactive effect of genotype (variety) x chemical treatments x storage condition after 25 days of treatment application.

Comment [B29]: Why you need to consider recording of 10%? Why you need to start after 22 days and end at 34 days of treatment application?

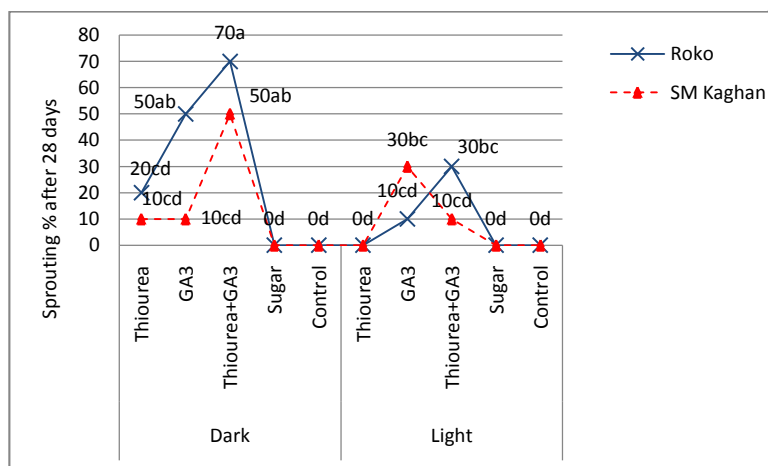
Comment [B30]: treatment

Comment [B31]: please check this data again. Otherwise, it is confusing.

Comment [B32]: Some of them are similar in value but they have different mean separation letters.

Comment [B33]: What is your base for scheduling this period for recording the sprouting percentage?

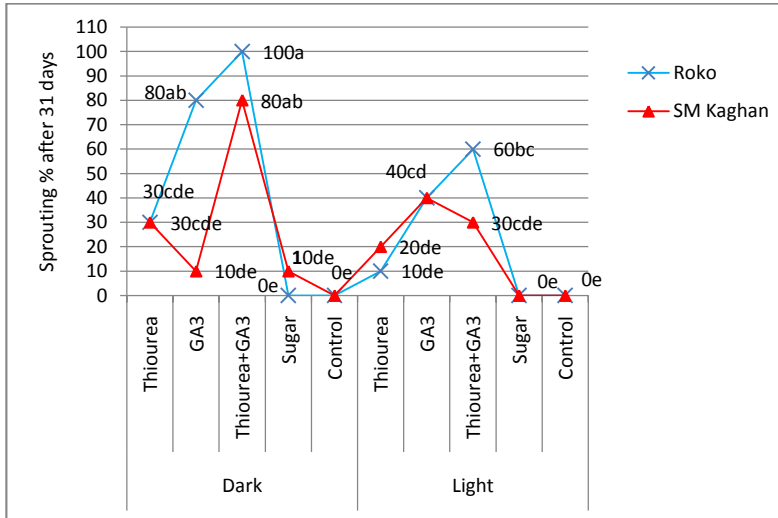
The analyzed data for 28 days after treatments application showed a significant interaction effect of experimental factors on sprouting percentage and considerable increase in sprouting percentage was observed in both varieties for Thiourea + GA₃ treatment under dark conditions in which 70% sprouting occurred in Roko followed by 50% in SM Kaghan (Figure 3). In GA₃ treatment under light conditions both varieties showed sprouting with 30% and 10% in SM Kaghan and Roko, respectively. However, no sprouting was recorded in sugar treatment and in control.



Means followed by different letters are significantly different at $P \leq 0.05$

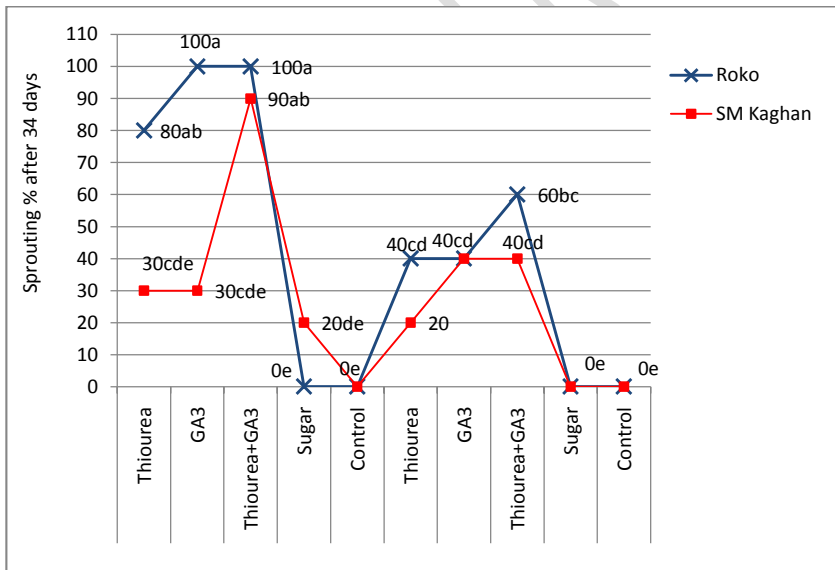
Figure 3: Mean sprouting % in potato minitubers under interactive effect of genotype (variety) x chemical treatments x storage condition after 28 days of treatment application.

It was observed that Thiourea along with GA₃ significantly affected the tubers dormancy as compared to other treatment combinations after 31 days and 100 % tubers were sprouted in Roko and 80% in SM Kaghan under dark conditions (Figure 4). Second highest sprouting percentage (80%) was recorded in GA₃ treatment in dark for potato variety Roko followed by 60% sprouting in thiourea+GA₃ treatment under light conditions (Figure 4). Moreover, breaking of dormancy in SM Kaghan variety was also observed in sugar treatment and 10% tubers showed sprouting under dark conditions in this interaction. No sprouting was observed in both varieties for control (Figure 4).



Means followed by different letters are significantly different at $P \leq 0.05$

Figure 4: Mean sprouting % in potato minitubers under interactive effect of genotype (variety) x chemical treatments x storage condition after 31 days of treatment application.



Means followed by different letters are significantly different at $P \leq 0.05$

Figure 5: Mean sprouting % in potato minitubers under interactive effect of genotype (variety) x chemical treatments x storage condition after 34 days of treatment application.

Analyzed data presented in Figure 5 revealed a significant increase in sprouting (100%) in GA₃ treatment alone and in thiourea+GA₃ treatment in dark environment in Roko variety after 34 days. However SM kaghan minitubers showed 90% sprouting in thiourea+GA₃. The lowest sprouting i.e 20% was recorded in sugar treatment under dark conditions and in thiourea treatment in light conditions in SM kaghan (Figure 5). It was observed that tubers treated with sugar treatment did not showed good response in both varieties under dark and light conditions even after 34 days of treatment application. No sprouting was observed in control for both varieties during this period (Figure 5).

By comparing the varietal response to the chemical treatments applied it was found that minitubers of potato variety Roko showed comparatively better sprouting % whereas the dormancy breakage and the respective sprouting percentage in potato variety SM Kaghan was comparatively low in all the interactions except in sugar treatment in which only SM Kaghan showed 20% tuber sprouting only under dark conditions (Figure 5).

Table 1: Mean number of sprouts per minituber under interactive effect of genotype (variety) x chemical treatments x storage condition after 34 days of treatment application.

Variety	Treatments									
	Thiourea		GA ₃		Thiourea + GA ₃		Sugar		Control	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
Roko	1.83a bcd	1.17de f	2.30ab	1.17def	2.50a	2.00ab c	0g	0g	0g	0g
SM Kaghan	1.00ef	1.50cd e	1.50cd e	1.67bcd e	1.50cd e	1.00ef	1.00e f	0g	0g	0g

Means followed by different letters are significantly different at $P \leq 0.05$

The results in Table 1 showed that the application of growth regulators on minitubers of different varieties have a significant effect on the total number of sprouts. It is worth noting that the difference created by the gibberellic acid alone and in combination with thiourea in turn was different from the other treatments regarding to the average number of sprouts in dark as well as light conditions with highest mean number of sprouts i.e 2.50 were found in Roko minitubers in thiourea+GA₃ treatment followed by 2.30 mean sprouts number in GA₃ treatment alone under dark conditions (Table 1). The least mean number of sprouts (1.00) was recorded in SM kaghan variety minitubers in thiourea and sugar treatment under dark and in thiourea+GA₃ treatment under light conditions (Table 1).

Comment [B34]: Not necessarily

Table 2: Mean emergence % of the minitubers of potato variety Roko and SM Kaghan under interactive effect of genotype (variety) x chemical treatments x storage condition after 10 and 15 days of sowing.

No. of days	Variety	Treatments									
		Thiourea		GA ₃		Thiourea + GA ₃		Sugar		Control	
		Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
10 days	Roko	40b	10cd	30bc	10cd	30bc	10cd	0d	0d	50ab	25bcd
	SM	0d	0d	0d	0d	10cd	0d	0d	0d	50ab	75a

Comment [B35]: (Roko and SM Kaghan)

Comment [B36]: planting

Comment [B37]: how do we know whether the main effects or interaction effects are significant or not without putting the anova table or presenting the anova report?

	Kaghan										
15 days	Roko	100a	100a	100a	100a	100a	100a	0c	0c	100a	100a
	SM Kaghan	100a	100a	100a	100a	100a	100a	100	0c	75b	100a

Means followed by different letters are significantly different at $P \leq 0.05$

In the current research project after dormancy breaking of minitubers of both potato varieties, the sprouted tubers were sown in pots in the green house to study the interactive effect of various experimental treatments (factors) including an untreated sprouted control on the plant growth and yield. The tubers of Roko and SM kaghan in sugar treatment under light conditions were not sprouted (Fig 5) and were not sown except the tubers of dark conditions of SM kaghan of the same treatment. It was observed that after 10 days of sowing in both varieties the control showed higher emergence percentage (Table 2). In potato variety roko 40% emergence occurred in Thiourea treatment under dark conditions whereas 10% emergence in SM kaghan minitubers occurred in thiourea+GA₃ treatment under dark conditions (Table 2).

Data recorded for percent emergence after 15 days of sowing showed a considerable increase in emergence % i.e 100% in both varieties including control (Table 2) except 75% emergence observed in minitubers of SM kaghan control under dark conditions.

Comment [B38]: planted

Comment [B39]: it needs rewriting

Comment [B40]: What about sugar treatment?
(there was 0% emergence)

UNDER PEER REVIEW

Table 3: Mean plant growth parameters under interactive effect of genotype (variety) x chemical treatments x storage condition after 30 and 60 days of emergence.

No. of days	Varieties	Mean Growth parameter	Treatments									
			Thiourea		GA ₃		Thiourea + GA ₃		Sugar		Control	
			Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
30 days	Roko	Shoot length (cm)	15.51cde	15.13de	18.00abcd	19.16abc	19.67ab	18.03abcd	0g	0g	17.22abcd	17.48abcd
		No. of branches	5.80abcd	6.65abcd	7.60abc	7.30abcd	8.60a	8.80a	0e	0e	5.60abcd	4.75cd
	SM Kaghan	Shoot length (cm)	12.57e	18.00abcd	19.40abc	17.13abcd	20.47a	19.90ab	8.40f	0g	16.05bcde	16.16bcde
		No. of branches	5.00cd	6.50abcd	8.75a	5.15bcd	8.42ab	7.30abcd	4.00d	0e	5.50abcd	5.00cd
60 days	Roko	Shoot length (cm)	42.23abc	39.25abcdef	43.42ab	39.70abcde	46.91a	41.26abcde	0l	0l	41.77abcd	39.56abcde
		No. of branches	8.95cde	8.65de	10.50abcd	10.50abc	11.50ab	12.50a	0g	0g	11.37abc	9.95bcd
	SM Kaghan	Shoot length (cm)	33.97cdefg	35.30bcdefg	36.65bcdefg	32.065fg	33.38efg	38.58bcdef	29.65g	0h	36.56bcdefg	33.85defg
		No. of branches	7.00ef	8.00bcd	10.20abcd	9.00cde	10.65abcd	9.3500bcde	7.60ef	0g	9.81bcd	8.40de

Comment [B41]: I think it is 'h'

Comment [B42]: 8.40 is higher than 8.00. so, check the mean separation letters.

Means followed by different letters are significantly different at $P \leq 0.05$

Statistical analysis of data regarding plant height/shoot length showed that the two potato varieties responded differently to different treatments after sowing the sprouted minitubers and

Statistical analysis of the data after 30 days of emergence showed no significant difference among different treatment interactions including control in potato variety Roko except Thiourea+GA₃ dark conditions treatment in which significantly highest mean shoot length (19.67 cm) was observed. The lowest mean value (15.13 cm) was observed in Thiourea treatment with light conditions (Table 3). In SM Kaghan variety the highest plant height (20.47cm) was recorded in Thiourea+GA₃ treatment interacted with dark conditions followed by 19.40 cm in GA₃ dark conditions treatment (Table 3). In the sugar treatment significantly lowest plant height (8.40cm) was observed in SM kaghan variety plants developed from minitubers placed in dark conditions whereas no data was recorded in the sugar treatment of roko variety as no tubers were found sprouted and were not sown (Fig. 5; Table 2). In the control no significant difference was found in plant height between the two varieties, however plants developed from tubers of dark condition have comparatively higher shoot length (Table 3).

Comment [B43]: Delete it.

Comment [B44]: But statistically similar with GA3 alone and control treatments (shares similar mean separation letter)

Comment [B45]: What about 19.90?

Comment [B46]: Your result did not show this.

Comment [B47]: storage

The interaction effect among varieties x chemical treatment x light and dark condition on plant height after 60 days of emergence showed that between varieties the potato variety Roko showed higher plant height in all treatment interactions than SM kaghan except in sugar treatment (Table 3). Among treatments with in Roko variety the plants of dark conditions of thiourea+GA₃ and GA₃ treatment showed higher plant height (46.91cm) and (43.42cm), respectively. whereas in SM kaghan the thiourea+GA₃ treatment of light conditions has highest shoot length i.e 38.58 cm than any other interaction followed by 36.65 cm in GA₃ with dark conditions. The lowest mean plant height (29.65cm) was recorded in sugar treatment dark conditions of SM kaghan variety (Table 3).

Comment [B48]: The result should be presented as three factor interaction. Otherwise, the way you presented the data was totally wrong. You have to report the result by considering genotype as one of the factor and it should not be reported separately. Please do not report the results of the two genotypes as a separate result.

The data collected on mean number of branches per plant after 30 days (Table 3) showed that no significant difference found in mean number of branches of variety Roko in all the interactions including control, however the thiourea+GA₃ treatment in both light and dark conditions showed highest mean number of branches i.e 8.80 and 8.60 followed by 7.60 and 7.30 in GA₃ treatment under dark and light conditions respectively (Table 3). In the potato variety SM kaghan highest mean branch number (8.75; 8.42) was recorded in GA₃ and thiourea+GA₃ treatments with dark conditions, respectively. The least number of branches (4.00) were recorded in sugar treatment (Table 3). A similar trend in increase in mean number of branches of both varieties was observed after 60 days of emergence in all the treatment interactions in both potato varieties (Table 3).

Comment [B49]: What do you mean?

Comment [B50]: from

Statistical analysis showed that highest mean tuber number i.e 5.16 was recorded in thiourea+GA₃ treatment of SM kaghan variety under light conditions followed by 5.00 and 4.83 mean tuber number in control and in GA₃ interacted with light conditions, respectively of the same variety SM kaghan (Table 4). Whereas, in variety Roko highest mean tuber number (4.25) was obtained in control under light conditions followed by GA₃ under dark condition. The lower mean tuber number i.e 3.00 was recorded in individual Thiourea and GA₃ treatments under light condition (Table 4). Regarding tuber size the data in Table 4 showed that the number of small size tubers is greater in both varieties than medium and large size tubers with highest mean small tubers (4.00) produced in thiourea treatment under light conditions of potato variety SM kaghan.

Comment [B51]: the sharing of similar mean separation letters indicate most of the results are statistically non-significant which contradicts with your discussion. So, please check your result again.

Statistically no significant difference was found among various experimental factors regarding mean tuber yield per plant in both potato varieties (Table 5). However, among the variety, treatment and light/dark interaction, the maximum average tuber weight (42.37g) was recorded in potato variety SM kaghan for thiourea treatment under dark conditions followed by thiourea+GA₃ treatment light conditions.

Comment [B52]: But they are statistically similar.

Discussion

Major problem in potato seed production cycle is the in time availability of sprouted seed. Seed dormancy lead to poor sprouting which results in delayed planting and poor emergence. In the present study a significant percentage of minitubers of potato varieties i.eRoko and SM kaghan showed sprouting earlier when treated with GA₃ either individually or in combination with thiourea when placed in dark. By comparing dormancy period of tubers treated with Thiourea and GA₃ individually it was noticed that GA₃ was found superior and just after 28 days of treatments application higher percentage of minitubers started sprouting (Figure 3). This may be due to the fact that the exogenous application of GA₃ enhances the endogenous level of gibberellins which affects the synthesis of amylase enzyme thereby enhances starch break down causing quick sprout growth as demonstrated by Krochko et al., 1998; Zhang et al., 2014). Carrera et al., 2000 studied the effect of gibberellins on potato tuber dormancy and reported that internal growth regulators such as gibberellic acid, abscissicacid andcytokinins are responsible for dormancy termination and commencement of sprouting under invitro conditions and Demo, 2002 found that the maintenance of seed quality in terms of seed health and vigor are related to gibberellins application. The previous scientific research showed that tuber treatment with GA₃ caused breakdown of starch and accumulation of renewable sugars in potato tubers that can stimulate germination and consequently dormancy breaking (Alexopoulos et al. 2007).

Table 4: Mean number of tubers per plant at the time of harvesting under interactive effect of genotype (variety) x chemical treatments x storage condition.

Variety	Treatments										
	Tuber category	Thiourea		GA ₃		Thiourea + GA ₃		Sugar		Control	
		Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
Roko	Large	0.60ab	0.17b	0.20b	0.17b	0.40ab	0b	0b	0b	1.00a	0.25b
	Medium	0.87abc	1.50ab	1.10abc	1.83a	1.10abc	1.33ab	0c	0c	0.75abc	1.25ab
	Small	1.87abcd	1.33bcd	2.90abc	1.00cd	2.60abc	2.67abc	0d	0d	1.75abcd	2.75abc
	Mean Total	3.33ab	3.00b	4.20ab	3.00b	4.10ab	4.00ab	0c	0c	3.50ab	4.25ab
SM Kaghan	Large	0.50ab	0.50ab	0.50ab	0.17b	0.32ab	0b	0b	0b	0b	0b
	Medium	1.25ab	0c	1.50ab	0.83abc	1.37ab	1.33ab	0.50bc	0c	1.00abc	1.50ab
	Small	1.25bcd	4.00a	1.00cd	3.83a	1.93abcd	3.83a	3.00abc	0d	2.50abc	3.50ab
	Mean Total	3.00b	4.50ab	3.00b	4.83ab	3.62ab	5.16a	3.50ab	0c	4.75ab	5.00a

Means followed by different letters are significantly different at $P \leq 0.05$

Comment [B53]: Please reconsider the table results again because:
 1. Similar values have different mean separation letters.
 2. Some of the mean separation letters did not consider the value. For instance, 1.25ab and 1.00a

Table 5: Average tuber yield per plant(grams) under interactive effect of genotype (variety) x chemical treatments x storage condition.

Variety	Treatments									
	Thiourea		GA ₃		Thiourea + GA ₃		Sugar		Control	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light
Roko	39.98a	38.10a	34.60a	34.88a	41.29a	39.35a	0b	0b	40.73a	38.75a
SM Kaghan	42.37a	30.10a	41.62a	37.91a	40.84a	42.35a	24.85a	0b	37.95a	36.52a

Means followed by different letters are significantly different at $P \leq 0.05$

Our results are consistent with the findings of Rehman et al., (2001) who showed that number of days required for 50% sprouting of potato minitubers can be reduced by the application of GA₃ under laboratory conditions. By comparing the two varieties it is clear from the data presented in Figure 5 that Roko variety showed greater sprouting % than SM kaghan variety in treatments including GA₃. These results are in line with the findings of Shibairo et al., 2006 who reported differences among genotype response to GA₃ and found that potato seed tubers of genotype Kenya Sifa showed delayed in dormancy breaking and had least sprouting %. Carrera et al., 2000 elaborated the reasons for such differences as that ectopic expression of gene coding for GA biosynthetic enzyme GA sub 20-oxidase resulted in high GA content of potato tuber and premature sprouting. Genotypic differences could therefore be attributed to inherent factors such as amounts of growth regulators present in each genotype. It is possible that the quantities of GA₃ present in some genotypes may be too low and therefore external supplementation is needed to prop up successive sprout growth.

The tubers of both varieties expressed very poor response to sugar treatment under both dark and light conditions and only 20% tubers showed sprouting in SM Kaghan variety under dark conditions. These findings are in contradiction to the results reported by Zaghum et al., 2021 who demonstrated that treating potato tubers with 300grams sugar in 600ml water solution breaks the dormancy of potato tubers within 12-16 days at open light conditions. Contrary to this it was noticed in the present study that the tubers treated with sugar solution caused decay tubers mostly by fungal deterioration of tuber eyes.

The variations observed in dormancy period of different genotypes i.e. Roko and SM kaghan in each treatment under the effect of dark and light conditions showed that after 31 days dormancy was broken in maximum tubers under dark. Demo et al., 2004 demonstrated that seed storage under diffused light conditions can delay dormancy breaking by delaying tuber ageing process and reduce apical dominance. It was evident from the results of our study that tuber storage under dark and light conditions in interaction with the use of chemical treatments affected the dormancy period of potato varieties Roko and SM Kaghan which normally take 80-90 and 100-120 days for complete tuber dormancy elimination respectively. Greater number of minitubers sprouted in Roko than SM Kaghan cultivar in just 28 days which could be stated due to different physiological characteristics of different varieties; may be one is long duration (120 days crop) (SM Kaghan) whereas Roko has normal 90 days crop maturity. Differences in this characteristic have been observed in different varieties in the other experiments in literature. Salimi et al., (2010) observed that regarding the genotypes, potato cultivars Burren and Agria achieved longest and shortest dormancy period after various chemical treatments.

Throughout the experiment, we can observe the significant effect of Thiourea + GA₃ treatment in Dark on dormancy breaking of tubers. The dormancy period was shorter in the tubers of both varieties treated with Thiourea + GA₃ and placed in Dark conditions as compared to other treatment interactions particularly tubers placed under light, while in control no sprouts were emerged in all treatment combinations of the two varieties. During the experiment it was also observed that sprouts developed from the tubers of the two varieties treated with gibberellic acid and placed in dark conditions were found slender and elongated as compared to untreated control. This might be due to the fact that breaking dormancy naturally requires greater physiological age, which affects membrane integrity (Knowles & Knowles, 1990) and metabolic activity of cell division and elongation (Taiz & Zeiger, 2002). Alexopoulos et al., 2008 reported

Comment [B54]: Sprouting percentage was better by thiourea + GA3 treatment but your discussion is mainly focused on GA3. So, would you reconsider it?

Comment [B55]: caused

that potato tubers treated with gibberellic acid produced seedlings that were thinner and longer than those that sprouted naturally.

It was also observed in our study that the plant's growth and tuber yield in different experimental treatment interactions did not differ significantly and that the sprouted tubers after sowing showed 100% germination, good growth and production performance. Barrani et al. (2013) showed that when seed potatoes were treated either dormant or sprouted with various concentrations of gibberellic acid, emergence of plants from treated seed was more rapid than from untreated seed tubers.

Comment [B56]: this

Comment [B57]: not necessarily

Comment [B58]: planting

Statistically plant height was non-significantly ($p \geq 0.05$) affected by cultivar, GA_3 and thiourea and storage conditions i.e light and dark (Table 3). However, data recorded on growth parameters of potato minitubers revealed that plants developed from tubers treated with GA_3 individually and along with thiourea showed increase in plant height of the two cultivars i.e. Roko, and SM kaghan and the response did not vary significantly between light and dark conditions storage. The process of plant growth occurred as cell division and cell elongation. The comparatively increase in plant height might be due to GA_3 effect on the elongation of internodes and shoot growth. Reza et al. (2015) demonstrated that the GA_3 causes increase in cell number and cell size resulting on a significant effect on growth. Chindi and Tsegaw (2019) reported that GA_3 applied on the foliage increased plant growth (8%) as compared to the control, and it may be related to the effect of internodes and shoot growth. But in our results no significant difference was found between treatments \times Cultivar \times light/dark storage interaction which can be due to the reason that no foliar application was done in this experiment. Plant height in sugar treatment was found lower than control in SM Kaghan variety, the possible reason for this might be that the sugar is not a hormone or growth promoting agent and therefore showed no effect.

Comment [B59]: in

Comment [B60]: this study

Non-significant differences observed regarding mean number of branches of both varieties in all the interactions including control, however effect of GA_3 was strongly enhanced by thiourea, since the most efficient plant growth and higher number of branches were recorded in thiourea+ $GA_3 \times$ dark/light conditions in both varieties (Table 3).

Non-significant differences observed in the yield parameters between varieties, chemical treatments and storage environment. GA_3 , thiourea and sugar treatment didn't cause any significant change in number of tubers and total yield in two potato cultivars (Tables 4 & 5). However, average tuber yield of both varieties in treatments including GA_3 or Thiourea is comparatively higher. Moreover, potato variety SM-kaghan showed greater tuber yield (42.37g; 42.35g) than Roko when treated with thiourea individually and along with GA_3 in combination, respectively. The possible reason can be the genetics of the two varieties which affected their yield performance. Germchi et al., 2011 studied the application effect of Thiourea in three levels as 0, 0.5, and 1% on potato minitubers dormancy breaking in Marfona cultivar in greenhouse and found that 1% thiourea increased tuber number per plant and increasing tuber yield in Marfona minituber production. However, the utilization of GA_3 individually and with other hormones in solving dormancy problem has not been optimized for all potato genotypes and there are still many constraints on the information of potential yields obtained after breaking dormancy.

Comment [B61]: but statistically non-significant

Comment [B62]: delete it.

Comment [B63]: Please check the result table again.

Conclusions and recommendations

On the basis of results it was concluded that Thiourea along with GA₃ shortens the dormancy period of tubers as compared to other treatment combinations. Gibberellins i.e GA₃ can break the tuber dormancy to about 31 days if stored in dark environment. Tubers placed in light conditions (diffused light) can take much longer time to sprout even treated with GA₃ or Thiourea or both. It was also concluded that the dormancy breaking treatments did not affect the plant growth and yield in comparison with control. Visual observation concluded that storage in light conditions produced shoots with better quality than ones in dark conditions.

It is recommended that the different concentrations of gibberellins can be tested alone and with different concentrations of thiourea in combination with different environmental conditions temperature, humidity and ventilation of storage place for different potato varieties to find a comprehensive protocol to shorten the dormancy duration in certified seed potato production cycle for those areas where there is only one potato crop per year.

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Comment [B64]: Conclusion and recommendation

Comment [B65]: Delete it.

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