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Optimization of Seed Potato (*Solanum tuberosum* L.) Tuber Dormancy and Sprouting Capacity through Integrated Gibberellic Acid and Benzylaminopurine Application

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors conducted and managed the literature searches, and designed the study. All the authors wrote, supervised and reviewed the study, the statistical analysis, and the first draft of the manuscript. All authors read, agreed and approved the final manuscript.

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ABSTRACT

Lack of sprouted seed potato tubers at planting is a major drawbacks for potato production in Kenya as most potato varieties remain dormant longer than three weeks while the window of planting is two weeks. To develop strategies that can enhance earlier sprouting a study was done to determine the effects of application of gibberellic acid (GA) and benzylaminopurine (BA) either

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singly or in combination sequenced in a time dependent manner on potato tubers genotypes Asante, Dutch Robjn and Kenya Sifa dormancy breaking. The tubers were stored in diffuse light conditions at Kenya Agriculture and Livestock Research Organization (formally Kenya Agricultural Research Institute), National Potato Research Centre Tigoni. The treatments included three potato varieties, Gibberellic acid (GA) applied at 0, 50, 100, 300 ppm and cytokinins (Benzylaminopurine) (BA) applied at 0, 50, 75, 100 ppm. The experiment was laid in a factorial arrangement. Tubers treated with GA alone or with a combination of GA and BA (GA+BA) showed faster dormancy termination and sprout growth than the control and those treated with BA alone. Tubers treated with GA or GA+BA had longer sprouts and number of sprouts per tuber than the control and BA treated tubers in all genotypes. Application of BA compared to GA alone decreased the number of sprouts by 1.46 and 0.89, 1.0 and 0.94, and 0.86 and 1.15 in Asante, Dutch robyjn and Kenya Sifa both in Trial I and II respectively. The highest sprout vigour score of 5.0 and 4.0, 4.33 and 3.67, and 3.67 and 3.33 and the greatest number of sprouts of 5.52 and 4.27, 4.07 and 3.74, and 2.13 and 2.43 was observed when BA was applied just before sprouts emergence at 9 weeks where 15 ppm gibberellic acid and then 15ppm BA after 9 days (GA.9BA) with Asante, Dutch robyjn and Kenya Sifa both in Trial I and II respectively. Both GA and BA may have been involved in initiating dormancy termination and sprout growth probably by synergistically enhancing both cell division and enlargement. Therefore GA or a combination of GA and BA can be adopted for potato dormancy termination and sprout growth. However, BA is more effective when applied toward the end of dormancy breakage.

Keywords: Potato; seed; giberrelic acid; benzylaminopurine; dormancy; sprouting.

1. INTRODUCTION

In Kenya the demand for potato seed is high and seed tubers are often needed before sprouting occurs. Due to the high seed demand, it may be necessary to break dormancy, or rest period, soonest after harvesting seed potato. After lifting, potato tubers undergo between two to three months (8-10 onths) dormancy period and will not sprout [1,2]. In tubers destined for processing, maintenance of tuber dormancy is a critical aspect of successful potato storage as it allows for many months of storage with or without sprouting inhibitors application. However, for tubers destined for use as seed, rapid termination of dormancy is desirable. Dormancy in potato is the physiological state of the tuber in which sprout growth will not occur within two weeks, even when the tuber is stored under ideal conditions for sprout growth [3]. Timely availability of well-sprouted seed potato tubers at the onset of rains is critical for attaining high yields. In major potato regions in Kenya with a bimodal rainfall, the window of planting is two weeks. However, most potato varieties are dormant for longer than three weeks [4] due genetic background, stage of tuber development, environmental and management conditions during tuber growth and storage [5]. Therefore, it is important to terminate dormancy in freshly harvested potatoes to enable provide adequate and sprouted seed stocks to enhance early planting and increase potato productivity. Potato tubers sprouting initiation results from a variety of biochemical changes in hormonal concentration, respiration rate, onset of nucleic acid synthesis, cell division and enlargement [6,7].

The dormant seed tubers can be induced to sprout by treating with cytokinins and gibberellins (GA). Gibberellins [4,8] and cytokinins promote growth whereas abscisic acid (ABA) and ethylene are sprout growth inhibitors. Gibberellins and cytokinins regulate the termination of endodormancy, whereas both abscisic acid (ABA) and ethylene are required for dormancy induction but only ABA is needed to maintain bud dormancy [1]. Exogenous GA [3] or a combination of GA and BA [9] can promote potato tuber sprouting. GAs (typically GA3) are often used in seed certification programs where rapid replanting of seed tubers is required for pathogen testing [10]. In field grown tubers, endogenous cytokinins begins to increase near the end of the dormancy period [11], while endogenous GA increase during sprouting [12]. Cytokinins is the primary factor in the switch from innate dormancy to non-dormant state in the potato tuber buds but probably do not control the subsequent sprout growth [1,13] while gibberellins have a stimulating role in sprout development [8,14]. The role of cytokinins might be primarily in regulation of cell division [15,16] which is a necessary step for both tuberization and sprouting.

Dormancy release in harvested seed tubers using external agents can enable provide timely and adequate seed stocks for use by the farmers or in seed multiplication programs and rapid postharvest agronomic or pest testing procedures. This study therefore tested the effects of combined application of gibberellic acid and benzylaminopurine on potato tuber dormancy and subsequent sprouting.

2. MATERIALS AND METHODS

2.1 Potato Treatment and Growth in the Field

The experiment was conducted at National Potato Centre, Tigoni from November 2008 to July 2009. Tubers sprout 8-10 weeks after harvest depending on variety and conditions during growth and at harvest [2]. Tubers of three genotypes with different dormancy periods; 'Asante' (short dormancy), Dutch Robyjin (medium dormancy), and 'Kenya Sifa' (long dormancy) [17] were grown through the conventional method from November 2008 to February 2009 Trial I and April to July 2009 Trial II. After harvest, tubers were washed and dried in air for 24 h. Ten days after harvest, 20 randomly selected tubers (mean weight 5±1 g) of each genotype were either immersed for 30 minutes in distilled water, or in an aqueous solution of 15 ppm gibberellic acid (GA), or 15 ppm benzylaminopurine (BA), or in their combination and was in a sequence of 3 days for 9 days (Table 1). The experiment was laid out in a randomized complete block design (RCBD) with three replications. The potato tubers from each treatment were put in paper trays, labeled and put in diffuse light store (DLS).

2.2 Dormancy and Sprouting Determination

During storage, data on dormancy period, number, length and vigour of sprouts were determined. Tuber sprouting was defined as when a tuber had at least one visible sprout of at least 2 mm long [1]. For dormancy period, the buds of all seed tubers from each treatment were observed after every week for eight weeks. The number of sprouted tubers was counted and recorded after which planting was done. Sprouting was recorded as a percentage of the number of sprouted tubers in a sample. Dormancy was considered to have broken when its sprouting was 80% and dormancy period was given by the duration from when the sample was treated to time when sample tuber dormancy was broken [4]. For the number and length of sprouts, five tubers from each treatment were picked at random after every week and the number of sprouts per tuber and the length of the longest sprout per tuber noted. Sprout vigour was determined as a 5 point rating score based on sprout base thickness and sprout length where; 1= Very low vigour (where half or more of the tubers in a treatment sample had produced sprouts of at least 1mm base diameter and 2 mm long), 2= low vigour (where half or more of the tubers in a treatment sample had produced sprouts of at least 2 mm base diameter and 3 mm long), 3= medium vigour (where half or more of the tubers in a treatment sample had produced sprouts of at least 3 mm base diameter and 4 mm long), 4= high vigour (where half or more of the tubers in a treatment sample had produced sprouts of at least 4 mm base diameter and 4 mm long) and 5= very high vigour (as described in score 4 but had green colouration, firm and had no defects).

Table 1. Sequence of application of gibberellic acid and benzylaminopurine

Treatments	Sequence of PGR's application
D. water	Distilled water (Control)
GA	15 ppm gibberellic acid alone
BA	15 ppm benzylaminopurine (BA) alone
GA.BA	15 ppm gibberellic acid and 15 ppm BA (0 days)
GA.3BA	15 ppm gibberellic acid and then 15 ppm BA after 3 days.
GA.6BA	15 ppm gibberellic acid and then 15 ppm BA after 6 days.
GA.9BA	15 ppm gibberellic acid and then 15 ppm BA after 9 days
BA.3GA	15 ppm BA and then 15 ppm gibberellic acid after 3 days
BA.6GA	15 ppm BA and then 15 ppm gibberellic acid after 6 days
BA.9GA	15 ppm BA and then 15 ppm gibberellic acid after 9 days

2.3 Data Analysis

Data analysis was done by use of Analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat. Treatments means which exhibited significant difference were compared using the Fisher's protected LSD test at 5% probability level. The means of sprouts length and sprouts per tuber between various treatments were subjected to repeated measures analysis and presented by use of line graphs.

3. RESULTS

3.1 Sprouting Percentage

Sprouting in GA alone, GA.BA and GA then BA treated tubers commenced during the 2nd week for Asante and Dutch Robjn in both seasons compared with the control (Figs. 1 and 2). Tubers treated with GA followed by BA application after 3 days took 2 weeks to end dormancy. There was no significant difference in sprouting% between the control and tubers treated with BA alone in all genotypes in both seasons which took 4 weeks for Asante and 5 weeks for Dutch Robjin. Sprouting in Kenya Sifa commenced during the fourth week for tubers treated with GA alone and those treated with GA followed with BA. However, tubers of Kenya Sifa which were treated with BA followed with GA took 5 weeks to give visible sprouts. The control and tubers treated with BA alone took 7 weeks during the first season and 8 weeks for the 2nd season. Tubers treated with GA alone, or GA followed by BA, or BA followed with GA after 3 days completed sprouting after 3 weeks in both seasons for Asante and Dutch Robjin but the same took 6 weeks for Kenya Sifa.

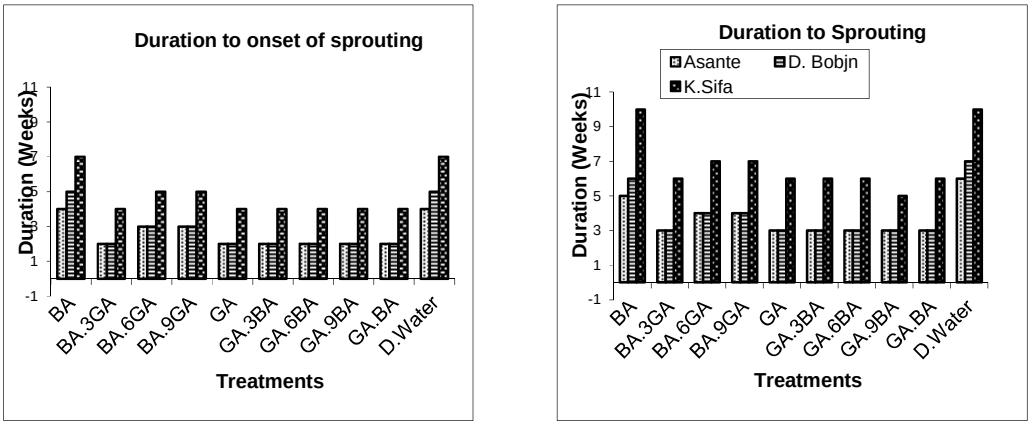

Fig. 1. Time to onset of sprouting and to attain 80% sprouting of tubers under diffuse light conditions after sequent application of gibberellic acid and benzylaminopurine (Trial I)

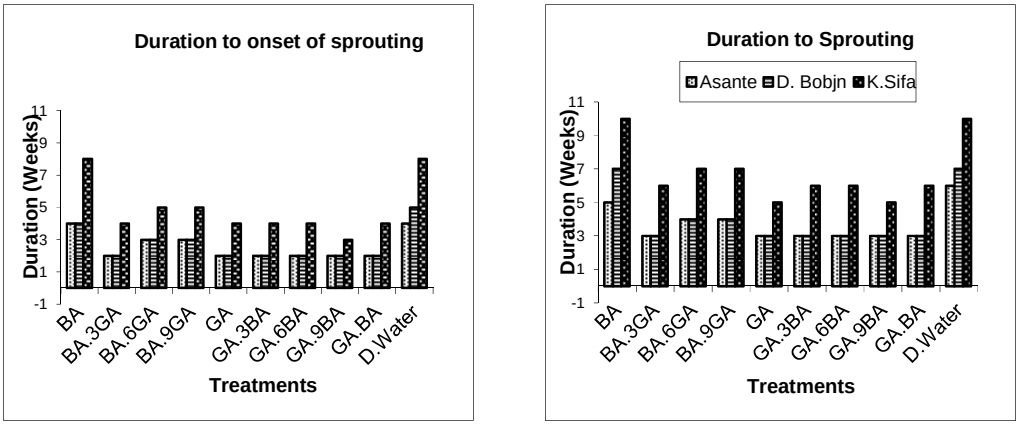

Fig. 2. Time to onset of sprouting and to attain 80% sprouting of tubers under diffuse light conditions after sequent application of gibberellic acid and benzylaminopurine (Trial II)

NB: BA.xGA = Apply cytokinins then gibberellins after xdays, GA.yBA= Days, GA.yBA = Apply gibberellins then cytokinins after y days

However, when BA was applied 9 days following GA, sprouting ended after 5 weeks in Kenya Sifa. Sprouts were first seen after 4 weeks in tubers treated with BA followed with GA application after 6 and 9 days for Asante and Dutch Robjin and 7 weeks for Kenya Sifa. Tubers treated with BA alone and the control took the same duration to end sprouting but varied according to the varieties taking 5 weeks, 7 weeks and 10 weeks for Asante, Dutch Robjin and Kenya Sifa respectively in both seasons.

3.2 Sprout Length

Sprout length differed significantly (*P*=0.05) among treatments and genotypes in both seasons. Mean sprouts length in tubers treated with BA alone was not significantly different from that of the control. Tubers treated with BA first then GA at different time interval exhibited significant time dependent and decreasing difference with GA application. Tubers treated with GA alone or a combination of GA then BA had sprout which were significantly longer than the control in both seasons for all genotypes (Figs. 3 and 4). However, mean sprouts length was higher in the combination than in GA then BA than when BA was applied before GA. Sprout length in tubers showed the longest sprouts where BA was applied after 9 days from GA in all genotypes compared to the control Asante genotype had the longest sprouts while Kenya Sifa had the shortest. Sprouts were longer in tuber where GA was applied first then BA than when application was started with BA in all genotypes in both seasons.

3.3 Sprouts per Tuber

The number of sprouts per tuber was significantly different (*P*=0.05) for both the treatments and genotypes in both seasons compared to the control (Table 2). Sprouts per tuber differed significantly depending on whether GA or BA was applied first in all genotypes. More sprouts per tuber were noted when GA was applied first then BA at different time intervals than when application of BA was done before GA (Figs. 3 and 4).

However, tubers treated with BA followed with GA exhibited no significant difference with time of application of GA. Similarly, the number of sprouts per tuber for GA treated tubers and those treated with GA then with BA did not differ significantly except for Asante and Kenya Sifa when BA was applied after 9 days. There were no significant differences for sprouts per tuber between tubers treated with BA alone and the control in Asante and Kenya Sifa genotypes. The highest number of sprouts per tubers was observed when GA was applied followed by BA after 9 days, while the lowest was noted in control and in tubers treated with BA alone. Asante had the highest number of sprouts while Kenya Sifa had the lowest.

3.4 Sprout Vigour

Sprouts vigour score varied significantly among treatments and genotypes in both seasons compared with the control (Table 3). Vigour score increased with duration of storage in all treatments and genotypes in both seasons. No significant difference was observed between Asante and Dutch Robyjin during the 3rd week in all treatments but sprouts vigour differed significantly from the 6th when a combination of GA and BA was applied. Asante produced the strongest sprouts while Asante gave the weakest. Tubers treated with GA then BA gave significantly stronger sprouts at 9th week than those treated with BA followed by GA except for Asante and Dutch Robyjin in season 1. Sprouts vigour of BA treated tubers exhibited no significant difference than the control in all genotypes. For tuber treated with GA then BA strongest tubers were observed when was applied the 9th day compared to earlier applications in all genotypes.

4. DISCUSSION

The results obtained from this study showed that GA alone or in combination with BA caused more rapid breakage of dormancy and sprouting in tubers than the control treatment. Visible sprouts were observed during the second week after treatment for Asante and Dutch Robyjn and 4th week for Kenya Sifa compared to 4th and 7th week for the control respectively. This was in agreement with [4] who observed that sprouting commenced during the 2nd week after treatment with GA. The visible sign of sprouting in GA and GA+BA treated tubers were reported to occur 10 days after application [9] and application of gibberellic acids reduced the duration of potato tuber dormancy and exhibit premature sprouting [3,14]. However, there are different opinions from different researchers whether GA is the cause agent for dormancy breakage. According to Mikitzel [8] and Suttle [14], GA have only stimulating roles in tuber sprouting and development and initiate sprouting only after dormancy is complete.

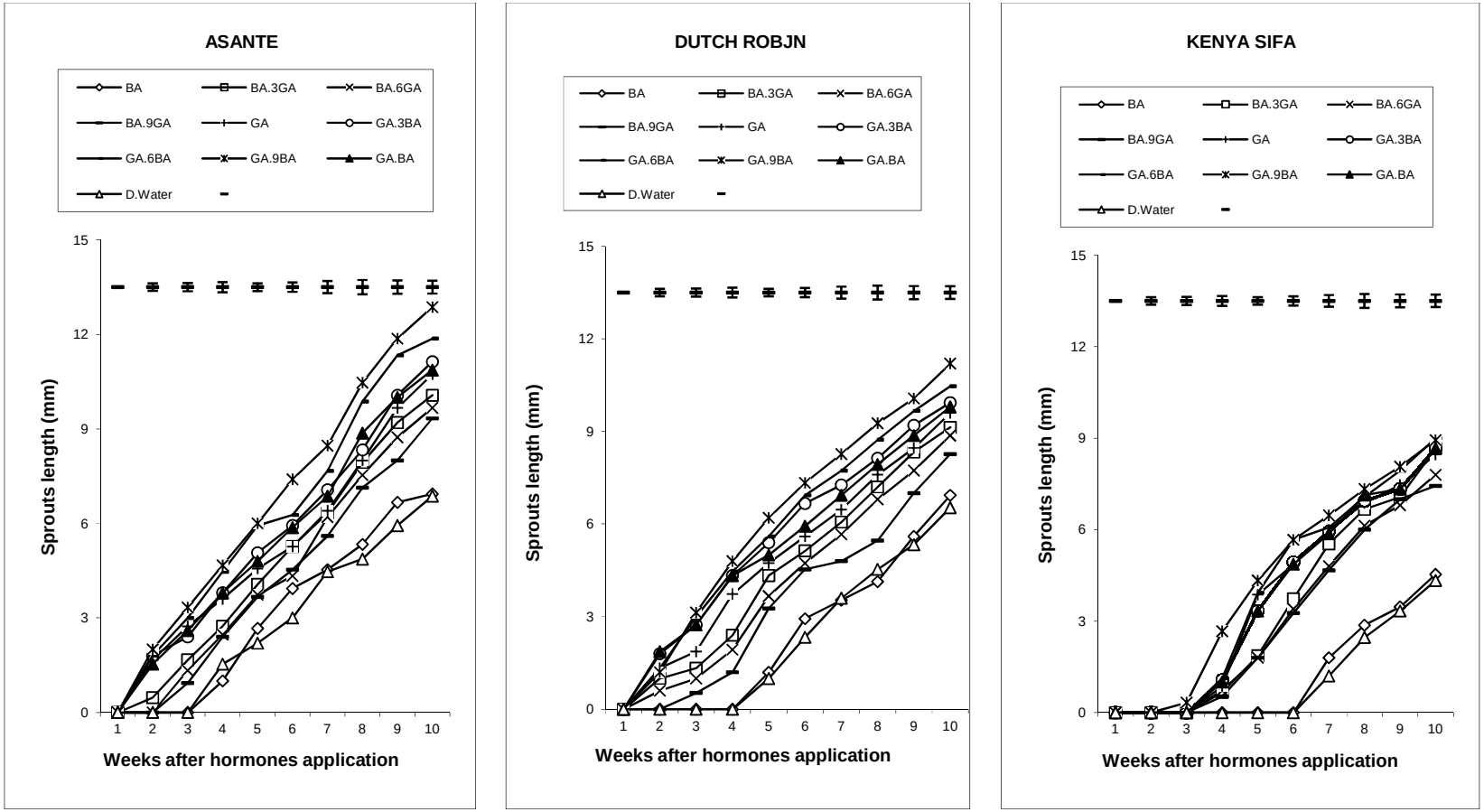

Fig. 3. Effects of sequence of application of gibberellic acid and benzylaminopurine on sprouts length of potato tuber stored under diffuse light conditions (Trial I)

NB: BA.xGA means applying 15 ppm benzylaminopurine then 15 ppm gibberellic acid after x days. GA.yBA means applying 15 ppm gibberellic acid then 15 ppm benzylaminopurine after y days. Vertical bars are standard error (SE) of the means

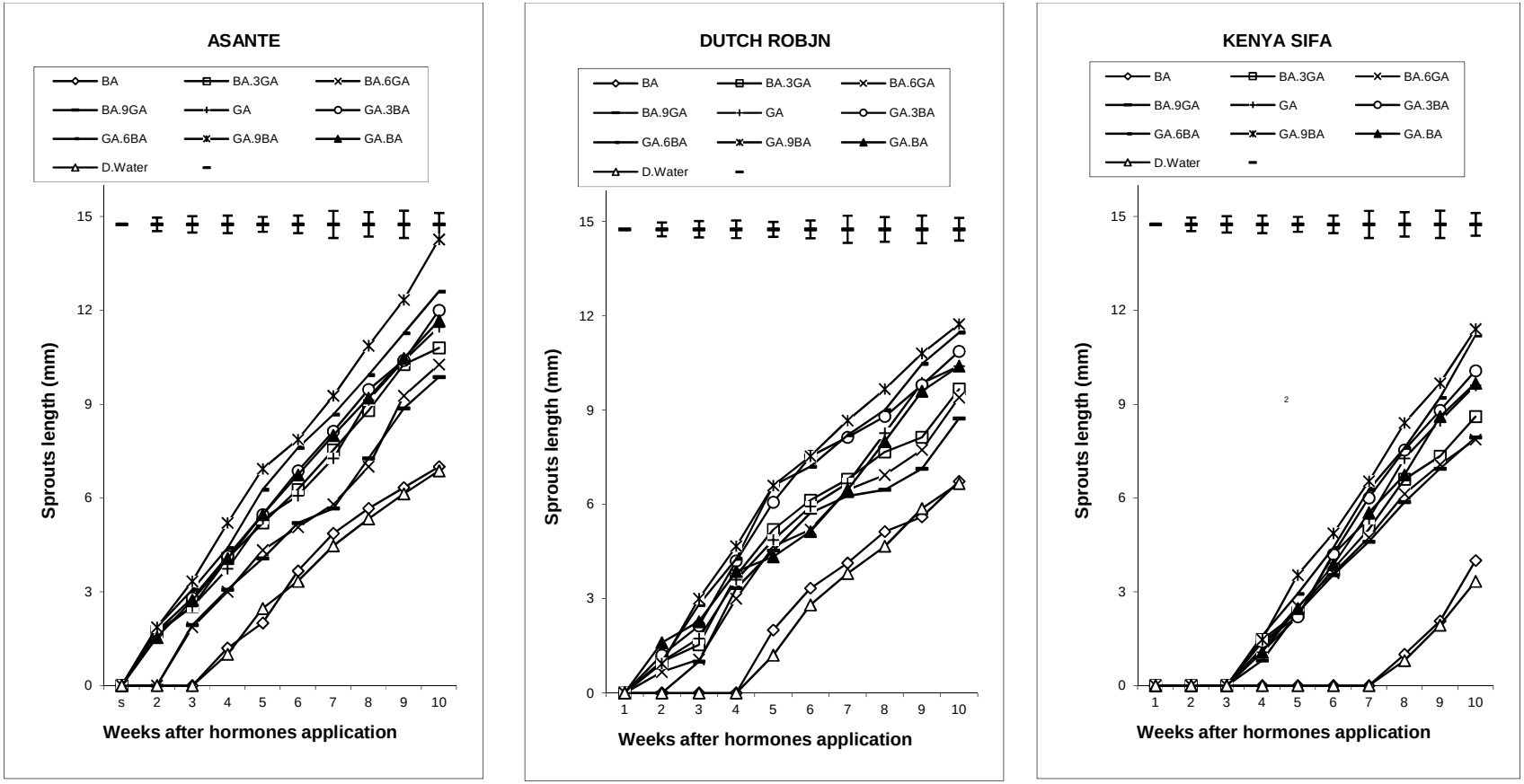

Fig. 4. Effects of sequence of application of gibberellic acid and benzylaminopurine on sprouts length of potato tuber stored under diffuse light conditions (Trial II)

NB: BA.xGA means applying 15 ppm benzylaminopurine then 15 ppm gibberellic acid after x days. GA.yBA means applying 15 ppm gibberellic acid then 15 ppm benzylaminopurine after y days. Vertical bars are standard error (SE) of the means

Table 2. Effects of gibberellic acid and benzylaminopurine on number of sprouts per tubers of potato tubers of potato genotypes Asante, Dutch Robyjn and Kenya Sifa under diffuse light conditions in 2008 and 2009

Trial I	**Treatments**									
Genotypes	**BA**	**BA.3GA**	**BA.6GA**	**BA.9GA**	**GA**	**GA.3BA**	**GA.6BA**	**GA.9BA**	**GA.BA**	**D. Water**
Asante	3.05	3.32	3.18	3.23	4.51	3.9	4.07	5.52	3.7	2.94
Dutch Robyjn	2.27	3.17	3.15	3.15	3.27	3.67	3.92	4.07	3.35	1.9
Kenya Sifa	0.87	1.65	1.64	1.6	1.73	1.93	2.03	2.13	1.87	0.85
$LSD_{0.05}$ Treatment (T)	0.06									
$LSD_{0.05}$ Genotype (G)	0.1									
$LSD_{0.05}$ T X G	0.29									
CV%	4.2									
Trial II										
Asante	3.01	3.43	3.39	3.4	3.9	3.83	3.91	4.27	3.8	3
Dutch Robyjn	2.17	2.8	2.65	2.6	3.11	3.53	3.54	3.74	3.17	2.03
Kenya Sifa	0.97	1.63	1.63	1.71	2.12	2.02	2.01	2.43	2.08	0.088
$LSD_{0.05}$ Treatment (T)	0.122									
$LSD_{0.05}$ Genotype (G)	0.067									
$LSD_{0.05}$ TXG	0.207									
CV%	6.5									

NB: BA.xGA means tubers were treated with benzylaminopurine then with gibberellic acid after x days; GA.yBA means tubers were treated with gibberellic acid then with benzylaminopurine after y days; G= Genopype; T = Treatment; $LSD_{0.05}$ =least significant difference at 5% probability level; and CV% = percent coefficient of variation

Table 3. Effects of sequence of application of gibberellic acid and benzylaminopurine on sprouts vigour of potato tubers stored under diffuse light conditions in 2008 and 2009

Trial I	Genotypes								
	Asante			Dutch Robyjn			Kenya Sifa		
	Sprouts vigour score			Sprouts vigour score			Sprouts vigour score		
Treatment	**3 Wks**	**6 Wks**	**9 Wks**	**3 Wks**	**6 Wks**	**9 Wks**	**3 Wks**	**6 Wks**	**9 Wks**
BA	0.00	2.67	3.67	0.00	2.67	3.67	0.00	1.00	2.67
BA.3GA	1.00	3.00	4.67	1.00	2.67	4.00	0.00	1.67	3.67
BA.6GA	1.00	3.00	4.67	1.00	2.67	4.00	0.00	1.33	3.33
BA.9GA	1.00	3.00	4.67	0.67	2.67	4.00	0.00	2.00	3.33
GA	1.33	3.33	4.67	1.67	3.00	4.00	0.00	2.33	3.67
GA.3BA	2.00	3.00	4.67	2.33	3.00	4.00	0.00	2.33	3.67
GA.6BA	2.00	3.00	4.47	2.00	3.33	4.00	0.00	2.67	3.67
GA.9BA	1.67	3.00	5.00	2.33	3.00	4.33	0.00	2.67	3.67
GA.BA	1.33	3.33	4.67	2.00	3.00	4.00	0.00	2.00	3.67
D.Water	0.00	2.67	3.53	0.00	2.67	3.53	0.00	0.00	2.67
$Lsd_{0.05}$ Treatment.	0.28	0.39	0.31	0.28	0.39	0.31	0.28	0.39	0.31
$Lsd_{0.05}$ Genotype	0.15	0.27	0.15	0.15	0.27	0.15	0.15	0.27	0.15
Trial II									
BA	0.00	2.33	3.33	0.00	1.00	2.67	0.00	0.00	2.00
BA.3GA	1.00	2.67	3.33	1.00	2.00	2.67	0.00	1.00	2.67
BA.6GA	1.00	2.33	3.33	1.00	2.33	3.00	0.00	1.00	2.67
BA.9GA	1.00	2.33	3.33	1.00	1.67	3.00	0.00	1.00	2.67
GA	1.00	2.33	3.67	1.00	2.33	2.67	0.00	1.33	2.47
GA.3BA	1.00	2.33	3.67	1.00	2.33	3.33	0.00	1.67	3.00
GA.6BA	1.00	2.67	4.00	1.00	2.67	3.33	0.00	1.67	3.00
GA.9BA	1.00	3.00	4.00	1.00	2.33	3.67	0.00	2.00	3.33
GA.BA	1.00	2.67	3.33	1.00	2.00	2.67	0.00	1.33	2.67
D. Water	0.00	1.67	3.00	0.00	1.00	2.33	0.00	0.00	1.67
$Lsd_{0.05}$ Treatment.	0.00	0.41	0.36	0.00	0.41	0.36	0.00	0.41	0.36
$Lsd_{0.05}$ Genotype	0.00	0.24	0.25	0.00	0.24	0.25	0.00	0.24	0.25
CV%	0.00	13.1	7.3	0.00	13.1	7.3	0.00	13.1	7.3

NB: BA.xGA means applying 15 ppm benzylaminopurine then 15 ppm gibberellic acid after x days; GA.yBA means applying 15 ppm gibberellic acid then 15 ppm benzylaminopurine after y days; $LSD_{0.05}$ =least significant difference at 5% probability level; and CV% = percent coefficient of variation

The application of BA in the absence of GA had no visible effect on the duration of dormancy and sprouting, number of sprouts and sprouts length compared with the control. This is in agreement with the view of [1] who observed that application of cytokinins in the early stages of tuber storage had no effect on potato dormancy and sprouting. These findings are in tandem with [9] observations that BA did not affect both duration of dormancy and sprout growth.

The sprout length and number of sprouts per tuber from tubers treated with GA alone or in combination with BA was higher than the control. However, sprouts from GA+BA treated tubers were more and longer than those of tubers treated with GA alone. This is in agreement with results found by [8] while working on tubers derived from true potato seeds found similar results. Physiologically, cytokinins are primarily involved in cell division [15,16] while gibberellins facilitate cell elongation. Growth is a combination of cell division and cell elongation. During dormancy, meristems are arrested in the G1 phase of cell division [1]. BA facilitate dormancy break by acting on G1 by introducing G0 cells to enter the cell cycle and by inducing D-cyclins to bind to Cdk proteins making it possible for the transition from G1 phase to S-phase to take place [18]. BA is also involved in transition from G2 to M-phase through the introduction of cdc-2-kinase [19]. However, translocation of BA in plants is weak and the presence of other hormones will increase the effectiveness of its effect. GA is known to facilitate the movement of cytokinins to the buds enhancing cell division [9]. The increase in the number of cells resulting from cell division create a sink for the carbohydrates hence provoking further starch breakdown so that the resulting sugars may be transferred to the sprouts, thus accounting for increased growth. Gibberellins and BA therefore work synergistically by enhancing both cell division and elongation since more cells resulting from cell division can increase the rate of growth. Tubers which were treated with BA first then with GA at different time intervals exhibited decreasing sprouts length and number of sprouts per tuber with duration of time of application of GA. The highest results were recorded when both GA and BA were applied at the same time while tubers treated with GA 9 days after BA application gave the shortest sprouts and least sprouts per tuber. All tubers treated with GA prior to sequential application of BA sprouted during the second week after treatment but both sprout length and number of sprouts per tuber increased with duration to BA treatment with highest results observed when BA was applied 9 days after GA application. These results agree with the [1] findings that exogenous application of cytokinins does not break potato dormancy when applied during the early stages of storage but is more affective when applied towards the end of dormancy. Sprouts from GA treated tubers were long and thin than BA treated tubers and the control. This was similar to [4] who also observed that GA treated tubers gave long but slender sprouts. However, results from this study revealed that a combination of GA and BA results to higher sprout growth and vigour compared to when each hormone was applied alone at the same rate. This was more pronounced in Kenya Sifa with long dormancy period.

5. CONCLUSIONS AND RECOMMENDATIONS

From the above results, it can be concluded that GA or a combination of GA and BA may be involved in dormancy termination and sprout growth. However, their combination has a greater effect on tuber sprouts length, sprouts number and vigour than GA alone. GA and BA works synergistically by enhancing both cell division and enlargement resulting to faster physical growth. BA alone has no effect on dormancy termination and sprouts growth. However, a higher impact on both tubers sprouts number and length from a combination of BA and GA than in GA alone indicates that BA influences the rate of growth. Use of BA is more effective when applied toward the end of dormancy breakage. GA or its combination with BA gives more long but slender sprouts than the control. GA or a combination of GA and BA can be used commercially for the dormancy breakage of potato seed tubers. However, tubers should be planted quickly after sprouts emergence so as to avoid the danger of sprout breakage and desiccation during planting. To maximize the results, BA should be applied towards the end of dormancy period (9 days after GA application).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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