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PLANT GROWTH REGULATORS AND ELECTRIC CURRENT BREAK TUBER DORMANCY BY MODULATING ANTIOXIDANT ACTIVITIES OF POTATO

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Potato (*Solanum tuberosum* L.) is an important food crop in Pakistan and produced twice or more in a year. Its tubers undergo a certain period of dormancy after the harvest and are incapable to sprout which can limit their usage as seed for the next season. Therefore, this study was planned to break the tuber dormancy using plant growth regulators and electric current. For this purpose, three experiments were conducted from April 2015 to July 2016 with the objectives: i) to screen out genotypes based on their dormancy duration and ii) to assess the effects of two dormancy breaking methods on seed tuber dormancy breakage, hydrogen peroxide contents and antioxidative activities at one and three week storage. The first experiment comprised of 22 equal number of white and red skin potato genotypes. Mean dormancy ranged from 36 to 85 days in these genotypes, including FD51-5 and PRI Red in short-term dormancy group; Sante and FD73-49 in medium-term dormancy group and FD69-1 and FD8-1 in long-term dormancy group. The second experiment consisted of above screened genotypes and three levels of benzyl aminopurine (30, 60 and 90 mgL^{-1}) and gibberellic acid (10, 20 and 30 mgL^{-1}). The solution of 60 mgL^{-1} benzyl aminopurine significantly reduced the dormancy duration in all genotypes but did not have a significant effect on the sprout outgrowth. While, 20 mgL^{-1} GA_3 produced maximum sprout length with non-significant effect on dormancy duration. The genotype × PGR interaction was more pronounced in short and medium-term dormancy genotypes than in long-term dormancy genotypes. The third experiment also consisted of above six genotypes with five levels of electric current (0, 20, 40, 60 and 80 volt). The use of electric current was most effective at 80 volt for shortening tuber dormancy and inducing sprout length. Hydrogen peroxide contents, superoxide dismutase and ascorbate peroxidase activities after treatment with plant growth regulators and electric current increased with dormancy progression. In contrast, catalase and peroxidase activities decreased. Our results indicate that enhanced antioxidative activity is closely associated with the effect of applied treatments, storage period, genotypes and genotype × storage period. Nevertheless, further research should be continued to evaluate the combined effect of BAP and GA_3 on dormancy termination.
Keywords: *Solanum tuberosum* L., BAP, GA_3, electric current, multiple cropping, antioxidants

INTRODUCTION

Potato is an important vegetable crop in the world due to its nutritional contents and occupies 4th position in production after cereals like rice, wheat and maize (Hancock *et al.*, 2014). In Pakistan, potato is cultivated on 0.18 million hectares, providing an estimated annual production of 4.00 million tons, averaging 22.4 ton/ha. Pakistan ranks 20th in terms of production and 16th for export quantity (FAOSTAT, 2016). It is cultivated in Pakistan throughout the year in a wide range of agro-ecological zones classified by physiography, climate and water availability (Rauf *et al.*, 2007). The autumn crop is the main crop grown from mid-September to January, summer crop from April to September and spring during the months of January to May (Saljoqi, 2009). The harvest from the autumn crop usually uses as seed source for summer and subsequent autumn crops. It is not used for spring crop because tubers are naturally dormant (Brown and Scheidegger, 1995). Similarly, tubers harvested from summer crop are used as seed only for spring crop but not for autumn crop, due to dormancy. Tuber dormancy is the physiological state in which autonomous sprouting does not occur even under favourable environment (Reust, 1986). As a result of short time between harvesting and planting of consecutive crops, Pakistani farmers have adapted autumn to autumn seed potato cycle. It would be of great value to develop effective dormancy breaking procedures to ensure seed potato availability for planting of subsequent potato crop.

Little research is available on the involvement of antioxidant enzymes in tuber dormancy break of potato (Mani *et al.*, 2014). Among these enzymes, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) play a key role in maintaining cell metabolism *via* the ascorbate-glutathione cycle (Iba, 2002). SOD in the

antioxidant defence system, is assumed as the first line of defense as it controls the cellular concentration of superoxide (O_2^-) and H_2O_2 (Agarwal and Shaheen, 2007). The excessive quantity of H_2O_2 is removed by CAT while APX does the fine modulation. Both enzymes are thought to belong to two distinct groups of scavenging enzymes based on their affinities for H_2O_2 (Mittler, 2002). Relating to the changes in antioxidative activities observed during three week storage, an increase in hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and ascorbate peroxidase (APX) was measured (Zabrouskov *et al.*, 2002).

Different methods are used for breaking potato tuber dormancy depending on the germplasm and resources available (Muthoni *et al.*, 2014). Chemicals used for breaking dormancy include respiratory inhibitors, sulfhydryls, anesthetics or end products of glycolysis. Rindite, bromoethane, thiourea and carbon disulphide are some of examples of widely used chemicals to terminate tuber dormancy but they are either not completely effective or toxic to humans and environment. Some hormones, such as BAP and GA_3 are also used for bud break and sprouting of seed potatoes and are safe for human use (Allen *et al.*, 1992).

BAP, a cytokinin can increase the activity of SOD, which in turn causes an increase in H_2O_2 production and ultimately results in oxidative stress (Durmus and Kadioglu, 2005). A positive correlation exists between H_2O_2 and activation of genes (1 GA ox, ox2 GA and AGA 3ox), whose transcription products are involved in biosynthesis of active gibberellins (Kloosterman *et al.*, 2005). Gibberellins assist inducing of bud activation and elongation after dormancy break, but their endogenous concentrations are perhaps not associated with maintenance of dormancy (Suttle, 2004a; Hartmann *et al.*, 2011). The effect of GA_3 on sprout length has been reported by Fernie and Willmitzer (2001), Lim *et al.* (2004) and Salimi *et al.* (2010). GA_3 is found more efficient in increasing sprout length than BAP (Alexopoulos *et al.*, 2007).

Electric current can alter the antioxidative activity such as CAT, SOD, POD and APX (Haghighat *et al.*, 2014). It causes uncoupling of free radicals in membranes and enhances ROS generation and can be exploited to break dormancy (Sahebjamei *et al.*, 2007).

The potato varieties mostly grown in Pakistan have moderate to deep dormancy and need to be stored at cold temperatures to enable fall harvest and summer planting. But the country does not have enough cold storage facilities (Ahmad *et al.*, 2005). Methods to shorten tuber dormancy are needed to enable multiple cropping in Pakistan. The objective of the current project was to screen selected potato genotypes for short-term tuber dormancy and to evaluate plant growth regulators and electric current to break dormancy for the screened genotypes.

MATERIALS AND METHODS

***Plant material*:** Twenty-two potato genotypes including an equal number of white and red skin genotypes were obtained from Potato Research Institute (PRI), Sahiwal, Pakistan. Healthy tubers without any damage, disease, disorder or deformation were selected for this study.

***Screening trial*:** In screening trial, tubers of all genotypes were kept in cardboard boxes at ambient temperature for 85 days. Each genotype had four replications and every replicate consisted of six tubers. Temperature and humidity during the experiment were recorded. Total six screened genotypes were selected from both white and red skin potatoes on the basis of their sprouting behaviour and used to study the effect of various concentrations of PGRs and electric current in breaking their tuber dormancy.

***Application of PGRs and electric current*:** Ten days after harvesting, tubers were dipped in 30, 60 or 90 mgL^{-1} solution of BAP and 10, 20 or 30 mgL^{-1} solution of GA_3 for 30 hours. For control, tubers were dipped in the distilled water. For each treatment, 12 tubers were used and replicated four times. As the skin of potato tubers is almost impermeable to the chemicals, parenchyma tissues were exposed to different solutions of PGRs by a limited cut (15 mm diameter × 10 mm depth). After treatment the treated tubers were stored under ambient conditions.

Electric current was applied to tubers at 20, 40, 60 or 80 volts. The needles at both apical and stem ends of tubers were penetrated about 15 mm inside the flesh and electric current was applied for 24 hrs. For control, tubers were injected by needles and electric current was applied without any voltage. Once treated, the tubers were stored under ambient conditions.

Both trials were laid out according to completely randomized design (CRD) under factorial settings and data was recorded as under:

***Dormancy duration and sprout length*:** Data regarding sprout length and dormancy duration were taken from six tubers in each replication, on a daily basis. A measuring tape was used to measure sprout length in mm.

***Activities of antioxidative enzymes and H_2O_2 contents*:** Tuber antioxidative enzymes and H_2O_2 contents were determined from six tubers after one and three weeks of storage after treatment to determine if changes happen during dormancy transition. Frozen tuber samples (1 g) of potato were grinded with the help of mortar and pestle and homogenized in 2 ml phosphate buffer of pH 7.2 at room temperature and then centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant was collected and used for the analysis of antioxidative enzymes. Activities of antioxidative enzymes [POD (EC 1.11.1.7), CAT (EC 1.11.1.6) and SOD (EC 1.15.1.1)] were determined by using the method described by Ali *et al.* (2016) while APX (EC 1.11.1.11) was analysed by method used by Egley *et al.* (1983) and were expressed as U

mg^{-1} protein. For H_2O_2, 1 g of each sample was thoroughly homogenized with 1 ml TCA, centrifuged for 10 minutes and supernatant was collected. Then, 0.5 ml of supernatant, 0.5 ml phosphate buffer (pH 7.0) and 1ml potassium iodide were dissolved in plastic tubes. Aliquots from each sample were distributed in 96 well plates and their absorbance was recorded at 390 nm. H_2O_2 contents were expressed as µmol g^{-1} fresh weight.

***Protein extraction*:** The method outlined by Bradford (1976) was followed for protein extraction, and extracted protein was expressed as mg/g fresh weight of tuber. Bovine serum albumin (Sigma Aldrich Pty. Ltd., Castle Hill, Australia) was used as a standard protein.

***Statistical analysis*:** The experimental data were subjected to analysis of variance (ANOVA) using Statistix 9® for Windows (Analytical Software, Tallahassee, USA). The effect of PGRs, electric current, genotype or storage period on observed parameters were assessed within ANOVA. Fisher's least significant differences (LSD) were used following a significant ($P < 0.05$) F-test.

RESULTS AND DISCUSSION

***Initial screening trial*:** There were significant differences in length of tuber dormancy among the studied 22 genotypes evaluated in the screening trial (Table 1). Mean dormancy in these genotypes ranged from 36 to 85 days. From these, three distinct groups were chosen based on their tuber sprouting behaviour (Fig. 1).

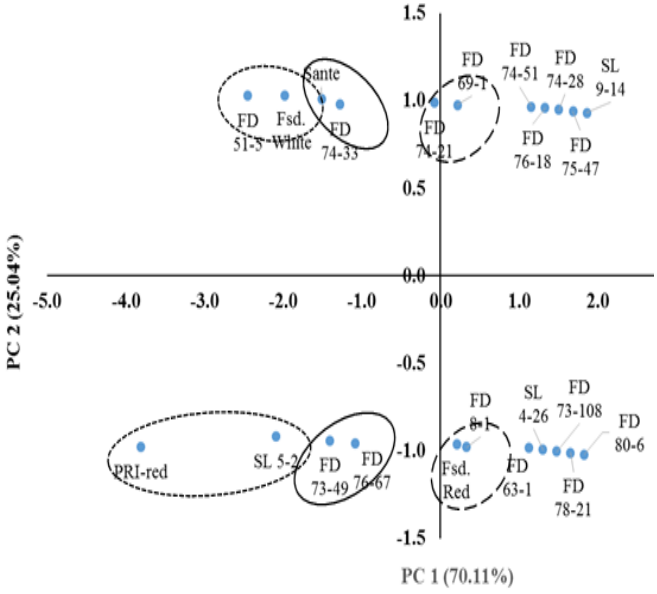

Figure 1. Biplot of principal component one (PC1) and principal component two (PC2) in experimental potato genotypes.

Table 1. Screening of potato genotypes based on dormancy duration and sprout length of tubers.

Genotypes		Dormancy duration (Days)	Sprout length (mm)
White skin genotypes (WSG)	FD51-5	41.75g	3.32a
	Faisalabad White	44.25f	2.69a
	Sante	52.25e	2.63a
	FD74-33	58.25d	3.06a
	SL9-14	85.00a	0.00c
	FD75-47	85.00a	0.00c
	FD74-28	85.00a	0.00c
	FD76-18	85.00a	0.00c
	FD74-51	85.00a	0.00c
	FD74-21	69.50c	1.08b
	FD69-1	72.75b	1.05b
LSD WSG ($P \leq 0.05$)		2.106	0.814
Red skin genotypes (RSG)	PRI Red	36.00f	6.77a
	FD76-67	58.50c	2.40b
	SL5-2	41.75e	2.69b
	FD73-49	53.50d	2.35b
	Faisalabad Red	74.25b	0.62cd
	FD8-1	76.00b	0.95c
	FD63-1	85.00a	0.00d
	SL4-26	85.00a	0.00d
	FD73-108	85.00a	0.00d
	FD78-21	85.00a	0.00d
	FD80-6	85.00a	0.00d
LSD RSG ($P \leq 0.05$)		2.548	0.640

Treatment means sharing same letter differ non-significantly. LSD = Least significant difference.

Short-term dormancy genotypes included FD51-5 (41.75 days) and PRI Red (36.00 days); genotypes with medium-term dormancy included Sante (52.25 days) and FD73-49 (53.50 days), while FD69-1 (72.75 days) and FD8-1 (76.00 days) were classified as long-term dormancy genotypes. The short-term and medium- term dormant genotypes produced >2 mm sprout length till the end of experiment. While long-term genotypes displayed <2mm length of sprout (Table 1). The variations in potato tuber dormancy are important to fit potato genotypes into cropping calendars of farmers (Fuglie, 2007; Helen, 2012). The variations in tuber dormancy and sprout length were in agreement with Suttle (2004b) who reported that length of tuber dormancy is a genetic character. Carli *et al.* (2012) found significant differences between cultivars for dormancy duration and concluded that short dormant genotypes exhibit greatest sprout length. The findings are further supported by Van Ittersum (1992), who found that dormancy duration varies greatly within the lot of seed tubers, and is cultivar specific.

Effect of PGRs on tuber dormancy:

***Dormancy duration and sprout length*:** Individual effects of PGRs and genotypes and their interaction (PGRs ×

genotypes) were significant ($P \leq 0.05$) for dormancy duration and sprout length (Table 2).

Table 2. Mean comparison of dormancy duration and sprout length of potato under different PGRs and genotypes.

Factors		Dormancy duration (days)	Sprout length (mm)
Treatment (T)	Control	55.66a	2.83f
	30 mgL^{-1} BAP	48.87b	3.09e
	60 mgL^{-1} BAP	36.58f	3.54c
	90 mgL^{-1} BAP	42.79d	3.31d
	10 mgL^{-1} GA_3	40.50e	3.87b
	20 mgL^{-1} GA_3	45.46c	4.25a
	30 mgL^{-1} GA_3	45.96c	3.67c
LSD T ($P \leq 0.05$)		0.974	0.151
Genotype (G)	FD 51-5	34.11e	3.47b
	PRI red	27.11f	6.90a
	Sante	45.21c	3.30c
	FD 73-49	43.00d	3.39bc
	FD 69-1	59.54b	2.20d
	FD 8-1	61.75a	1.80e
LSD G ($P \leq 0.05$)		0.902	0.140
LSD T × G ($P \leq 0.05$)		2.386	0.370

Treatment means sharing same letter differ non-significantly. BAP = Benzylaminopurine. GA_3 = Gibberellic acid. LSD = least significant difference.

Among PGRs, BAP performed better than GA_3 in lowering dormancy duration and vice versa for sprout length. 60 mg L^{-1} and 20 mg L^{-1} were the most effective levels of BAP and GA_3 for dormancy break and sprout length, respectively, in all genotypes. Dormancy break and sprout length with GA_3 was significantly different from the control but did not differ within any GA_3 treatment. The interaction was more pronounced in the short (FD51-5 and PRI Red) and medium (Sante and FD73-49) dormancy genotypes and less pronounced in the long (FD69-1 and FD8-1) dormancy genotypes (Tables 3 and 4).

Cytokinins and gibberellins are proposed to break tuber dormancy and promote sprouting (Suttle, 1996; Carrera *et al.*, 2000). Results of present study are comparable with the findings of Shibairo *et al.* (2006) who reported that visible sign of sprouting in benzyl adenine (BA) + GA_3 treated tubers appeared 10 days after application. However, sprouting commenced after the 2nd week after treatment with GA_3 alone. Our findings are also in agreement with those of Muchiri *et al.* (2015) who found that tubers treated with GA_3 alone or in combination with BA exhibited faster dormancy termination and sprout growth compared to control. Together, these results suggested that cytokinins have stimulating role for breaking tuber dormancy and GA_3 for sprout development.

***Antioxidant enzymes and H_2O_2 contents*:** The main effects of PGRs, genotypes and storage periods and the two-way interaction of genotype × storage period were significant ($P \leq 0.05$) for all antioxidant enzymes. The rest of interactions

Table 3. Mean comparison of dormancy duration (days) of potato tubers under genotype × PGR interactive effect.

Treatments (T)	Genotypes (G)					
	FD 51-5	PRI Red	Sante	FD 73-49	FD 69-1	FD 8-1
Control	41.00op	35.25stu	57.50fgh	55.25hij	71.00b	74.00a
30 mgL^{-1} BAP	38.25qr	30.50w	48.75l	48.00l	62.00de	65.75c
60 mgL^{-1} BAP	26.25xy	21.00z	33.75tuv	33.25uv	51.75k	53.50jk
90 mgL^{-1} BAP	32.50vw	25.00xy	43.25no	40.25pq	57.75fg	58.00fg
10 mgL^{-1} GA_3	30.75w	24.00y	39.75pq	36.75rs	55.00ij	56.75ght
20 mgL^{-1} GA_3	34.25tuv	27.25x	46.75lm	42.75no	59.50f	62.25d
30 mgL^{-1} GA_3	35.75st	26.75x	46.75lm	44.75mn	59.75ef	62.00de

LSD T × G ($P \leq 0.05$) = 2.386. Treatment means sharing same letter differ non-significantly. BAP = Benzylaminopurine. GA_3 = Gibberellic acid. LSD = least significant difference.

Table 4. Mean comparison of sprout length (mm) of potato tubers under genotype × PGR interactive effect.

Treatments (T)	Genotypes					
	FD 51-5	PRI Red	Sante	FD 73-49	FD 69-1	FD 8-1
Control	3.15ijk	6.47c	2.96jklm	3.48nopq	1.03tu	0.93u
30 mgL^{-1} BAP	2.90jklm	6.63c	3.01jkl	3.01jkl	1.68s	1.30t
60 mgL^{-1} BAP	3.50fghi	6.73bc	3.12jk	3.52fgh	2.46opq	1.88rs
90 mgL^{-1} BAP	3.19hijk	6.68c	3.05jkl	3.25ghij	2.00rs	1.72s
10 mgL^{-1} GA_3	3.75ef	7.08b	3.6fg	3.81ef	2.75lmno	2.24pqr
20 mgL^{-1} GA_3	4.20d	7.94a	4.10de	4.03de	2.84klmn	2.40opq
30 mgL^{-1} GA_3	3.61fg	6.81bc	3.25ghij	3.61fg	2.60mnop	2.15qr

LSD T × G ($P \leq 0.05$) = 0.370. Treatment means sharing same letter differ non-significantly. BAP = Benzylaminopurine. GA_3 = Gibberellic acid. LSD = least significant difference.

Table 5. The periodic change in tuber antioxidative activities and H_2O_2 contents in different potato genotypes under the effect of plant growth regulators.

Factors		APX (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	SOD (U mg^{-1} protein)	H_2O_2 (μmol g^{-1} FW)
	Control	13.61f	13.09a	20.75a	16.83e	43.86g
	30 mgL^{-1} BAP	13.82e	12.68b	20.31b	17.16d	44.31f
	60 mgL^{-1} BAP	15.38a	10.50e	17.41e	18.79a	47.93a
Treatment (T)	90 mgL^{-1} BAP	14.83c	11.08d	18.26d	18.05b	45.51c
	10 mgL^{-1} GA3	15.15b	10.73e	17.62e	18.74a	46.19b
	20 mgL^{-1} GA3	14.04d	12.24c	19.64c	17.48c	45.16d
	30 mgL^{-1} GA3	13.86e	12.55b	19.99b	17.38c	44.79e
LSD T ($P \leq 0.05$)		0.137	0.239	0.332	0.182	0.281
	FD51-5	13.96d	10.50d	18.05d	13.69e	43.59e
	PRI Red	14.32c	13.97b	21.58b	15.21d	46.90b
Genotype (G)	Sante	15.47a	11.33c	12.80f	19.69c	47.85a
	FD73-49	13.80e	10.39d	20.86c	13.10f	43.34e
	FD69-1	15.11b	14.44a	24.47a	21.65b	44.93d
	FD8-1	13.63f	10.42d	17.07e	23.32a	45.74c
LSD G ($P \leq 0.05$)		0.127	0.222	0.307	0.168	0.259
Storage period (SP)	1 Week	13.32 b	13.12a	22.83a	16.45b	14.51b
	3 Weeks	15.45a	10.55b	15.45b	19.10a	76.27a
LSD SP ($P \leq 0.05$)		0.073	0.128	0.177	0.097	0.150
LSD T × G ($P \leq 0.05$)		NS	NS	NS	0.445	0.688
LSD T × SP ($P \leq 0.05$)		NS	NS	NS	0.257	0.397
LSD G × SP ($P \leq 0.05$)		0.180	0.314	0.434	0.238	0.367
LSD T × G × SP ($P \leq 0.05$)		NS	NS	NS	0.630	0.972

NS=Non-significant at $P \leq 0.05$. Treatment means sharing same letter differ non-significantly. BAP = Benzylaminopurine. GA_3 = Gibberellic acid. APX = Ascorbate peroxidase. CAT = Catalase. POD = Peroxidase. SOD = Superoxide dismutase. H_2O_2 = Hydrogen peroxide. LSD = least significant difference.

were significant for only H_2O_2 and SOD (Table 5). All PGRs treatments significantly lowered the CAT and POD while increased the APX, SOD and H_2O_2 concentrations than that of untreated control (Table 5). From the first to third week of storage, CAT and POD activities reduced and vice versa for APX, SOD and H_2O_2 contents (Table 5).

CAT activity was the highest in FD69-1 (14.43 U mg^{-1} protein) and the lowest in FD73-49 (10.38 U mg^{-1} protein), FD8-1 (10.42 U mg^{-1} protein) and FD51-5 (10.50 U mg^{-1} protein) (Table 5). The smallest reduction in CAT activity was observed in FD69-1 (15.68 U mg^{-1} protein) between the first and third week of storage, while the largest reduction was observed in FD51-5 (8.95 U mg^{-1} protein), FD8-1 (8.97 U mg^{-1} protein) and FD73-49 (9.10 U mg^{-1} protein) (Figs. 2A and 2B). APX activity was the highest in Sante (15.47 U mg^{-1} protein) and the lowest in FD8-1 (13.64 U mg^{-1} protein) (Table 5). The smallest increase in APX activity was observed in FD73-49 (12.47 U mg^{-1} protein) between the first and third week of storage, while the largest increase was observed in FD69-1 (16.71 U mg^{-1} protein) and Sante (16.61 U mg^{-1} protein) (Figs. 2C and 2D).

H_2O_2 contents were the highest in Sante (47.85 μmol g^{-1} FW) and the lowest in FD73-49 (43.34 μmol g^{-1} FW) and FD51-5 (43.59 μmol g^{-1} FW) (Table 5). The smallest increase in H_2O_2 contents was observed in FD51-5 (12.83 μmol g^{-1} FW) and FD73-49 (13.02 μmol g^{-1} FW) between the first and third week of storage, while the largest increase was observed in Sante (78.05 μmol g^{-1} FW), PRI Red (77.94 μmol g^{-1} FW) and FD8-1 (7.94 μmol g^{-1} FW) (Figs. 3A and 3B). SOD activity was highest in FD8-1 (23.32 U mg^{-1} protein) and lowest in FD73-49 (13.10 U mg^{-1} protein) (Table 5). The smallest increase in SOD activity was observed in FD73-49 (12.16 U mg^{-1} protein) and FD51-5 (12.21 U mg^{-1} protein) between the first and third week of storage, while the largest increase was observed in FD8-1 (25.17 U mg^{-1} protein) (Figs. 3C and 3D). POD activity was the highest in FD69-1 (24.47 U mg^{-1} protein) and the lowest in Sante (12.80 U mg^{-1} protein) (Table 5). The smallest reduction in POD activity was observed in FD69-1 (29.82 U mg^{-1} protein) between the first and third week of storage, while the largest reduction was observed in Sante (9.35 U mg^{-1} protein) (Figs. 4A and 4B).

POD and CAT activities in this study decreased with progression of storage period after application of PGRs is in agreement with the results reported by Petit-Paly (1999) while H_2O_2, SOD and APX concentrations increased. The results indicate that H_2O_2 accumulation with the advancement in

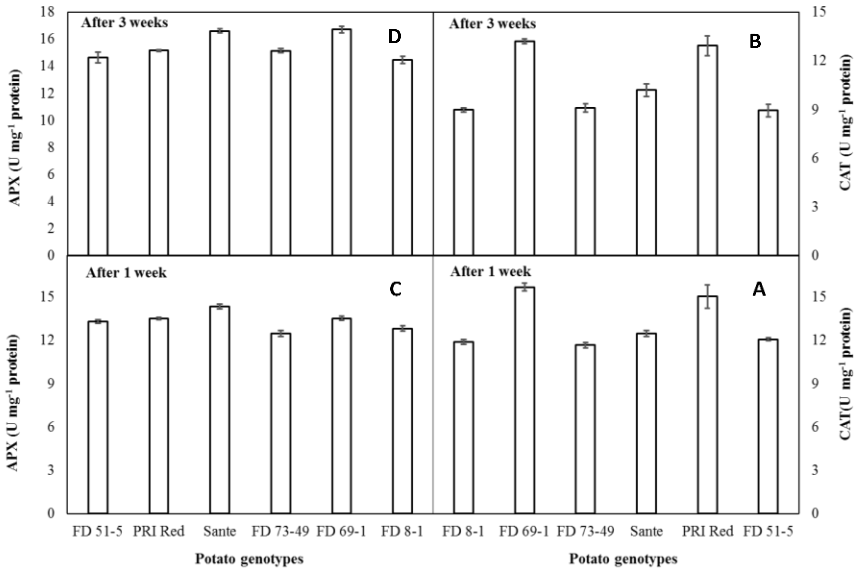

Figure 2. The activity of CAT (A and B) and APX (C and D) in tubers of different potato genotypes at two storage periods under the effect of PGRs. Vertical error bars represent standard error of means.

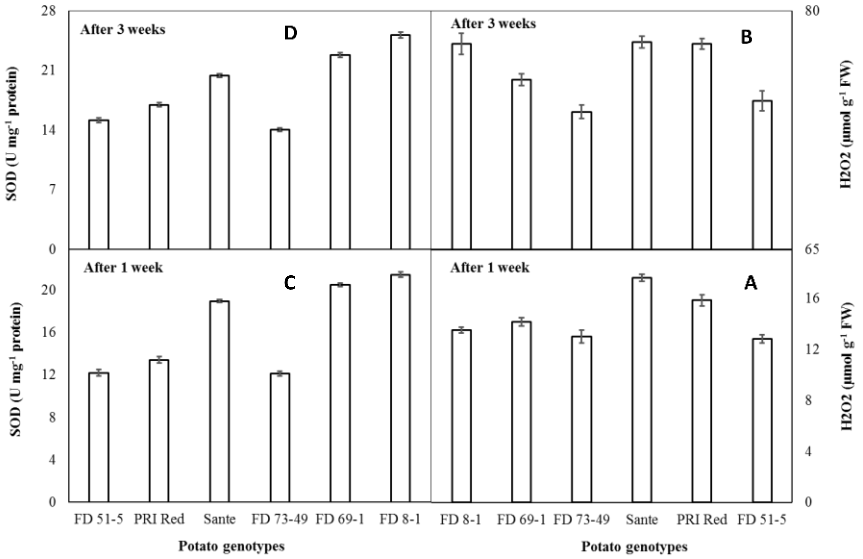

Figure 3. H_2O_2 (A and B) and SOD (C and D) concentration in tubers of different potato genotypes at two storage periods under the effect of PGRs. Vertical error bars represent standard error of means.

storage period resulted in dormancy break, as reported by previous findings (Delaplace *et al.* 2008). H_2O_2 also regulates the expression of a number of genes whose expression products are involved in dormancy (Kazushiro *et al.*, 2004). A relationship was established by Kloosterman *et al.* (2007) between endogenous H_2O_2 level and activation of genes (ox GA_1, ox GA_2 and ox GA_3) involved in the biosynthesis of gibberellins. The increase in H_2O_2 contents results in reduction of endogenous abscisic acid. This happened due to deactivation of protein phosphatases 1 and ABI 1, ABI 2 and 2C involved in the biosynthesis of abscisic acid (Nicolas *et al.*, 2003).

Effect of electric current on tuber dormancy:

***Dormancy duration and sprout length*:** Individual effect of electric current and genotype was significant ($P \leq 0.05$) for dormancy duration and sprout length (Table 6). However, the interaction was non-significant. With every increment in voltage, electric current lowered the number of dormancy days and increased the length of sprout. Therefore, 80 volt was the most effective voltage for breaking dormancy in all

genotypes. The dormancy duration was the highest in FD8-1 and the lowest in PRI Red. The sprout length was maximum in PRI Red and minimum in FD8-1 and FD69-1.

Table 6. Mean comparison of dormancy duration and sprout length of potato tubers under electric current and genotype main effects

Factors		Dormancy duration (days)	Sprout length (mm)
Electric current (Ec)	0 volts	54.04a	2.75b
	20 volts	49.71b	2.79b
	40 volts	48.16c	2.79b
	60 volts	46.46d	2.84b
	80 volts	41.08e	3.52a
LSD Ec ($P \leq 0.05$)		1.369	0.179
Genotype (G)	FD 51-5	36.90d	3.19b
	PRI red	30.75e	6.46a
	Sante	48.05c	2.98c
	FD 73-49	48.25c	2.64d
	FD 69-1	60.20b	1.26e
	FD 8-1	63.20a	1.09e
LSD G ($P \leq 0.05$)		1.500	0.196
LSD Ec × G ($P \leq 0.05$)		NS	NS

NS=Non-significant at $P \leq 0.05$. Treatment means sharing same letter differ non-significantly. LSD = least significant difference.

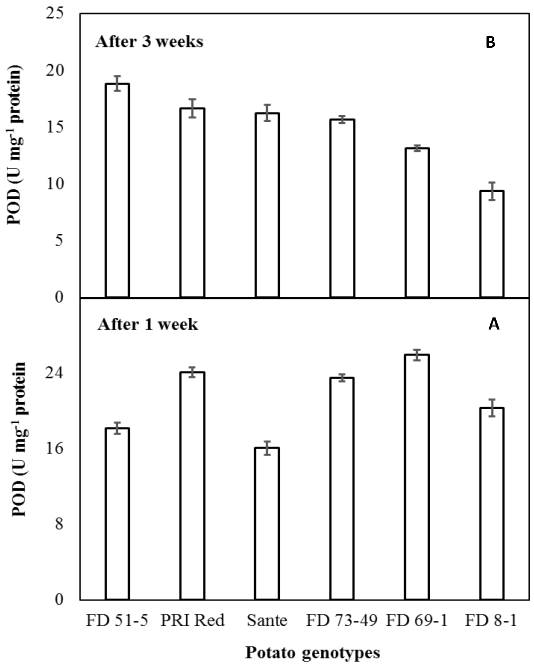

Figure 4. The activity of POD in tubers of different potato genotypes at (A) one week and (B) three week storage period under the effect of PGRs. Vertical error bars represent standard error of means.

To date, the effect of chemicals have been reported for breaking the tuber dormancy (Muthoni *et al.*, 2014), but only report of Kocacaliskan *et al.* (1989) is found on the effect of electric current. The variations observed in dormancy duration and sprout length of different genotypes under the effect of electric current are in line with the findings of Kocacaliskan *et al.* (1989) who observed the increase in sprout length with the passage of storage period following application of electric current however the interaction, voltages × days was non-significant. This encouragement of sprouting by electric current might be the consequence of stimulation of GA_3 synthesis.

***Antioxidant enzymes and H_2O_2 contents*:** The main effects of electric current, genotype and storage period and the two-way interaction of genotype × storage period were significant ($P \leq 0.05$) for all antioxidant enzymes (Table 7). Moreover, the interactions of electric current × genotype and electric current × storage period was also significant for POD and H_2O_2, respectively (Table 7). Application of all electric current treatments resulted in significant decrease in CAT and POD and increase in APX, SOD and H_2O_2 concentrations than the untreated control. From first to third week of storage, CAT and POD activities decreased and vice versa for APX, SOD and H_2O_2 contents was observed (Table 7).

CAT activity was the highest in genotype FD69-1 (14.15 U mg^{-1} protein) and the lowest in FD8-1 (9.50 U mg^{-1} protein) (Table 7). The smallest reduction in CAT activity was observed in FD69-1 (15.56 U mg^{-1} protein) between the first and third week of storage, while the largest reductions was observed in FD8-1 (8.25 U mg^{-1} protein) and FD51-5 (8.35 U mg^{-1} protein) (Figs. 5A and 5B). APX activity was the highest in FD73-49 (14.75 U mg^{-1} protein) and the lowest in FD8-1 (12.49 U mg^{-1} protein) (Table 7). The smallest increase in APX activity was observed in FD8-1 (11.78 U mg^{-1} protein) between the first and third week of storage, while the largest increase was observed in FD73-49 (12.54 U mg^{-1} protein) (Figs. 5C and 5D).

H_2O_2 contents were the highest in FD73-49 (39.56 µmol g^{-1} FW) and the lowest in Sante (35.26 µmol g^{-1} FW) and FD8-1 (35.36 µmol g^{-1} FW) (Table 7). The smallest increase in H_2O_2 contents was observed in FD8-1 (11.93 µmol g^{-1} FW) and Sante (12.26 µmol g^{-1} FW) between the first and third week of storage, while the largest increase was observed in FD73-49 (64.98 µmol g^{-1} FW) (Figs. 6A and 6B). SOD activity was the highest in FD69-1 (21.31 U mg^{-1} protein) and the lowest in FD51-5 (13.78 U mg^{-1} protein) and FD73-49 (13.88 U mg^{-1} protein) (Table 7). The smallest increase in SOD activity was observed in FD51-5 (12.05 U mg^{-1} protein) between the first and third week of storage, while the largest increase was observed in FD69-1 (23.21 U mg^{-1} protein) (Figs. 6C and 6D). POD activity was highest in FD69-1 (22.99 U mg^{-1} protein) and lowest in Sante (13.14 U mg^{-1} protein) (Table 7).

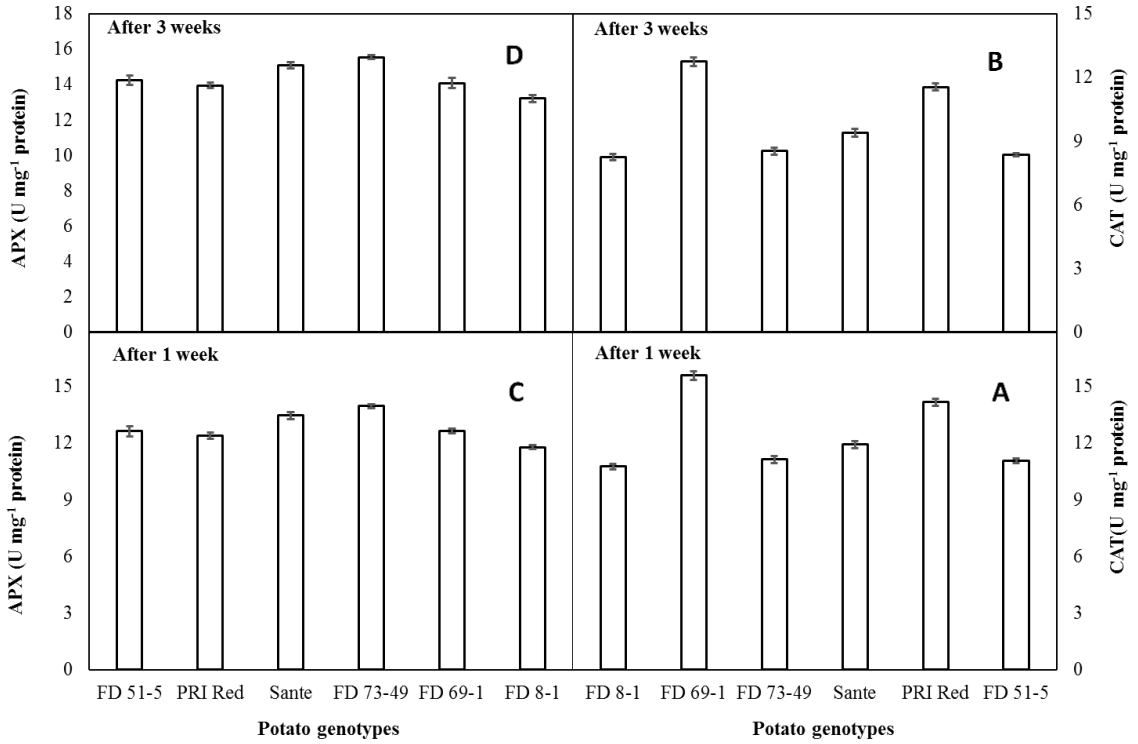

Figure 5. The activity of CAT (A and B) and APX (C and D) in tubers of different potato genotypes at two storage periods in response to electric current. Vertical error bars represent standard error of means.

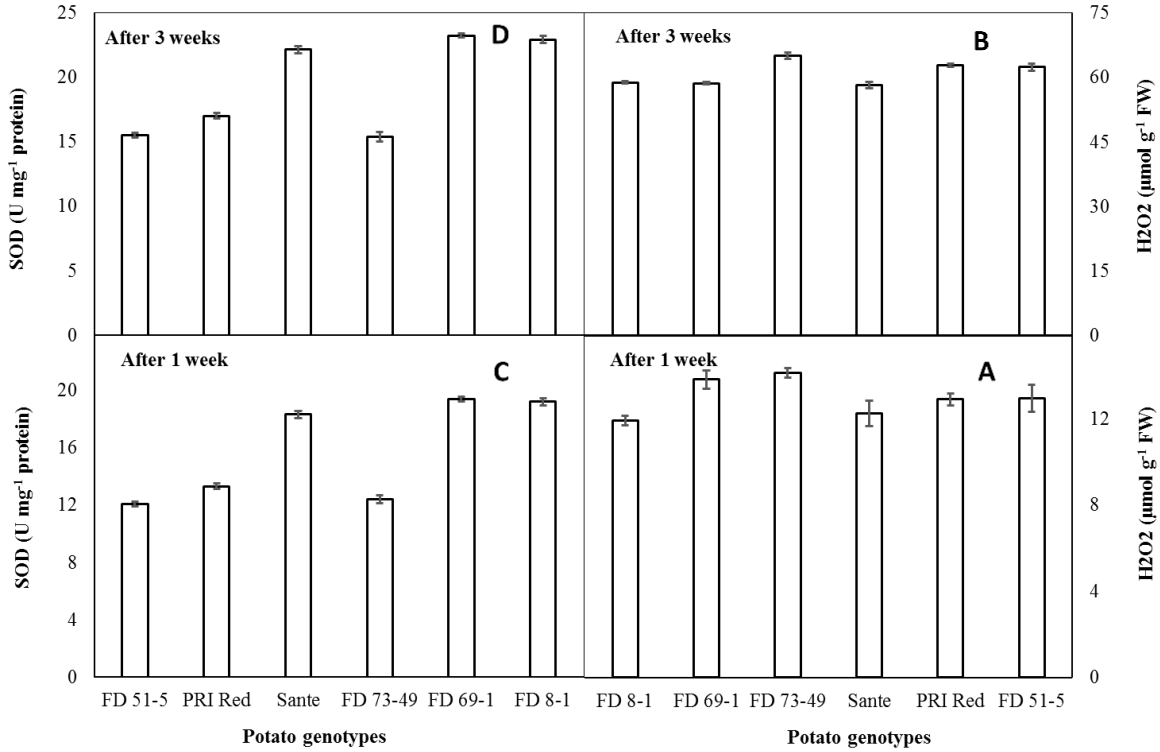

Figure 6. H_2O_2 (A and B) and SOD (C and D) concentration in tubers of different potato genotypes at two storage periods in response to electric current. Vertical error bars represent standard error of means.

Table 7. The periodic change in tuber antioxidative activities and H_2O_2 contents in different potato genotypes in response to electric current application

Factors		APX (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	SOD (U mg^{-1} protein)	H_2O_2 (µmol g^{-1} FW)
	0 volt	13.11e	11.62a	19.65a	17.04e	35.62e
	20 volts	13.29d	11.41b	19.48b	17.25d	35.93d
Electric current (Ec)	40 volts	13.52c	11.16c	19.28c	17.50c	36.31c
	60 volts	13.74b	10.92d	19.06d	17.74b	36.99b
	80 volts	14.23a	10.47e	16.93e	18.31a	40.06a
LSD C (P≤0.05)		0.058	0.102	0.129	0.121	0.229
	FD51-5	13.44c	9.71e	16.01e	13.78e	37.67b
	PRI Red	13.16e	12.84b	22.75b	15.16d	37.84b
Genotype (G)	Sante	14.28b	10.66c	13.14f	20.22c	35.26d
	FD73-49	14.75a	9.82d	20.61c	13.88e	39.56a
	FD69-1	13.35d	14.15a	22.99a	21.31a	36.21c
	FD8-1	12.49f	9.50f	17.75d	21.06b	35.36d
LSD G (P≤0.05)		0.063	0.111	0.142	0.132	0.251
Storage period (SP)	1 Week	12.80b	12.42a	21.34a	15.79b	12.99b
	3 Weeks	14.35a	9.80b	16.42b	19.359a	60.97a
LSD SP (P≤0.05)		0.037	0.064	0.082	0.076	0.145
LSD Ec × G (P≤0.05)		NS	NS	0.316	NS	NS
LSD Ec × SP (P≤0.05)		NS	NS	NS	NS	0.324
LSD G × SP (P≤0.05)		0.089	0.157	0.200	0.187	0.355
LSD Ec × G × SP (P≤0.05)		NS	NS	NS	NS	NS

NS=Non-significant at P≤0.05. Treatment means sharing same letter differ non-significantly. APX = Ascorbate peroxidase. CAT = Catalase. POD = Peroxidase. SOD = Superoxide dismutase. H_2O_2 = Hydrogen peroxide. LSD = least significant difference.

The smallest reduction in POD activity was observed in FD69-1 (25.91 U mg^{-1} protein) between the first and third week of storage, while the largest reduction was observed in Sante (10.22 U mg^{-1} protein) (Figs. 7A and 7B).

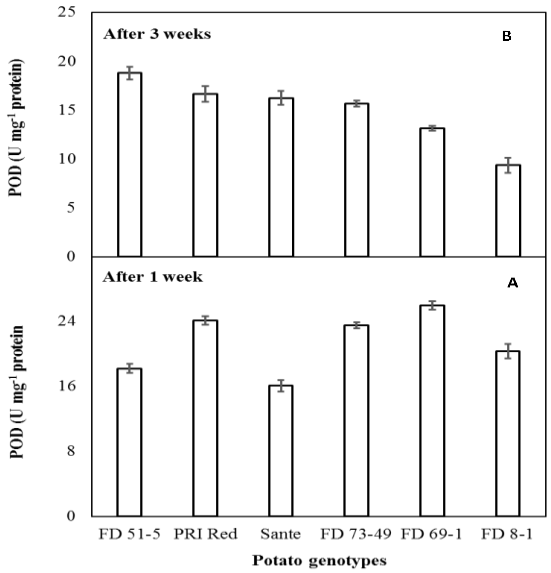

Figure 7. The activity of POD in tubers of different potato genotypes at (A) one week and (B) two week storage period in response to electric current. Vertical error bars represent standard error of means.

Following electric current application, the changes in POD and CAT activities during the storage were the slightest and reached their lowest point at the end of storage time and vice versa for H_2O_2, SOD and APX. A study carried out by Macheix *et al.* (2005) suggests that H_2O_2 oxidises sprouting inhibitors (not studied in our experiments) such as chlorogenic acid and caffeic acid, to stimulate sprouting. H_2O_2 is also proposed to favour the oxidative pentose phosphate pathway or might yield oxygen for respiration and the monooxygenases are implicated in gibberellin biosynthesis (Fontaine-Roux *et al.*, 1997).

***Conclusion*:** The results showed that main effects of PGRs, electric current and selected genotypes had significant effect on tuber dormancy and sprout length as they went through marked antioxidative changes when analysed at two stages. For dormancy break, BAP (60 mgL^{-1}) performed superior to any level of GA_3. Development of sprout length was maximum in GA_3 (20 mgL^{-1}) treated tubers. The antioxidative activities in different genotypes changed considerably from first week to third week of storage.

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