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Role of Growth Promoting Substances in Breaking Potato (*Solanum tuberosum* L.) Tuber Dormancy

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ABSTRACT

The present investigation was conducted to determine the effect of benzyl adenine (BA), spermine (Spr) and cycocel (CCC) alone and in combination in different concentrations for breaking dormancy, growth and tuberization of two potato cultivars Diamant and Desiree. The experiment was conducted in pots under natural condition. Fresh harvested potato tubers were collected from National Agriculture Research Centre, Islamabad and soaked in aqueous solution of BA, Spr and CCC prior to sowing. BA and Spr used alone significantly enhanced sprouting and germination in both the cultivars, whilst CCC inhibited it. The magnitude of sprouting was less in combined treatments of BA, Spr and CCC. The response of BA and Spr was more pronounced in cv. Diamant. The combined treatments of BA, Spr and CCC showed significant increase in potato stolon and tuber formation in both the cultivars. Although all the treatments increased chlorophyll contents, BA alone excelled other treatments in this respect. Nodal parts of tubers showed comparatively greater IAA and GA contents than that of parts without eyes in both the cultivars. All the treatments showed an increase in endogenous GA and IAA contents of leaves as well as of tubers but Spr treatment in particular showed maximum response. These data suggest that changes in IAA/GA ratio might have a role in stolon and tuber formation in potato.

Key Words: Growth promoters; Dormancy; Potato

INTRODUCTION

Potato crop faces certain limitations during its sprouting and subsequent development. After harvesting, the eyes on the potato tubers remain dormant for 8 - 10 weeks and do not sprout regardless of favorable growth conditions. Endogenous hormones have been proposed to play a significant role in potato tuber bud (eye) dormancy regulation (Suttle, 2000; Coleman *et al*., 2001). An increase in cytokinin content is the principal factors leading to the loss of dormancy (Suttle, 2004). The nodes are the sites of synthesis of gibberellins and cytokinins. When the dormant period ceases, the increase in gibberellic acid (GA) concentration is higher as compared to indole ascetic acid (IAA). Therefore, relative ratio of GA/IAA was postulated as an important criterion in determining the dormancy and sprout growth. Suttle (2004) critically assessed the involvement of endogenous gibberellins in release of potato tuber dormancy and early sprout growth and found the role of GAs in the regulation of sprout growth. It was found that dormancy of whole potato tuber was effectively broken with benzyladenine (BA) at a concentration of 20 ppm used for 24 h (Suttle, 2004). In his previous studies Suttle (1998) concluded that application of cytokinins resulted in the termination of dormancy and enhanced sprouting of potato tuber. Many physiological studies support the involvement of polyamines in several plant processes (Tiburcio *et al*., 2002) including tuber development (Rafart-Pedrós *et al*., 1999), ripening (Mehta *et al*., 2002) and abiotic stresses (Roy & Nu, 2001). Plant growth hormones have been suggested to play a prominent role in the control of tuberization also (Xu *et al*., 1998).

With this background the present investigation was conducted to determine the effect of plant growth hormones viz., benzyl adenine (BA), spermine (Spr) and cycocel (CCC) alone and in combination in different concentrations on breaking dormancy, growth and tuberization of two potato cultivars Diamant and Desiree.

MATERIALS AND METHODS

Plant material and growing conditions. Potato tuber (*Solanum tuberosum* L.) cvs. Diamant and cv. Desiree were obtained from National Agricultural Research Centre (NARC), Islamabad. The pot experiment was conducted in the green house of Quaid-I-Azam University Islamabad, under natural conditions. One tuber was sown per pot after being soaked with plant growth regulators as per plan (Table I). After the completion of sprouting, plants of both the varieties were sown in earthen pots measuring 30 x 40 cm, containing garden soil (sterilized) and farmyard manure in 3:1 ratio. Three pots per treatment were used. The plants were watered weekly and harvesting of mature crop was done 90 days after sowing. Experimental design was completely randomized with three replications.

Sprouting of potato tubers. Tubers were obtained from NARC and soaked in the aqueous solution of Spr, BA and CCC used in different concentrations alone and in

combinations (distilled water was used in case of control). The treated and un-treated (control) tubers were incubated in darkness in growth room maintained at 25/10°C day and night temperature, respectively. Observation was made after every 24 h till sprouting.

Extraction and quantification of endogenous GA and IAA contents in potato tubers and leaves. Extraction and purification of endogenous GA and IAA were made from tuber following the method of Leclerc *et al.* (1994) and Kettner and Doerffling (1995) method was used for leaves. Each sample was analyzed for the presence of GA and IAA using HPLC (Shimadzu Japan) following the method of Li *et al.* (1994). The HPLC was equipped with C18 cartridge column measuring 3.9 x 150 mm and was operated at 30°C with Methanol and 1% Acetic Acid (2:3) as mobile phase at flow rate of 1 mL min^{-1}. The detection was made with a variable wavelength detector set at 254 nm and 278 nm for IAA and GA, respectively.

Chlorophyll contents of leaves. The chlorophyll contents of leaves (at 70 DAS) were determined by the method of Arnon (1949) as modified by Kirk (1968).

RESULTS AND DISCUSSION

Apical and lateral sprouting in potato tubers. Earlier apical and lateral sprouting of potato tubers in both the varieties were obtained by BA alone as well as in combination with Spr as compared to control and this effect was more pronounced in cv. Diamant as compared to cv. Desiree (Table II). This verified the possible involvement of cytokinins and polyamines in breaking dormancy and enhancement of sprouting of potato tubers (Berangernovat *et al.*, 1994; Suttle, 1998a). The presoaking treatment particularly of cytokinins and polyamines might reduces the time required for sprouting by altering the endogenous GA contents in potato tubers, which have a role in the regulation of sprout growth (Suttle, 2004).

Stolon formation and tuberization. The effect of CCC alone (T_3 & T_6) and in combination with BA and Spr (T_{11}, T_{17}, T_{20} & T_{21}) on potato stolon formation of cv. Diamant was more pronounced as compared to Desiree (Table III). Both BA and polyamines induced cell division in the tissues of stolon tip leading to increase in tuberization (Peterson *et al.*, 1985; Salisbury & Ross, 1985; Mader, 1997)). The cv. Diamant formed more tubers as compared to cv. Desiree. Tubers formed in cv. Desiree were rudimentary.

Chlorophyll contents of potato leaves. The leaves of cv. Diamant had more chlorophyll contents then that of cv. Desiree (Table IV). Earlier reports indicate that polyamines induced retention of chlorophyll contents, for example the one by Krishnamurthy (1991). BA stimulation of chlorophyll synthesis has also been observed (Mansoor *et al.*, 1994). Shang *et al*, (1995) and Borrell *et al.* (1996) reported similar findings.

Changes in endogenous contents of GA and IAA in potato tubers and leaves. The endogenous GA contents were increased in eye parts and parts without eyes of potato tuber after application of BA and Spr in both the varieties but maximum endogenous GA contents were observed in eye parts of potato tubers receiving BA 40 ppm soaking treatment (T_8). Suttle (1998b) reported that after harvesting of potato crop exogenous cytokinin have little effect on sprouting but efficiency increases with storage time thereby

Table I. Treatment plan showing concentration of plant growth regulators applied alone and in combination to potato tubers

Treatments	Concentration (ppm)	Denoted as	Treatment duration (Hours)
Control		T_1	24
Benzyladenine	20	T_2	24
Cycocel	10	T_3	24
Spermine	30	T_4	24
Benzyladenine	30	T_5	2
Cycocel	15	T_6	2
Spermine	45	T_7	24
Benzyladenine	40	T_8	24
Spermine	60	T_9	24
Benzyladenine + Spermine	20+30	T_{10}	24
Benzyladenine + Spermine+Cycocel	20+30+10	T_{11}	22+2
Benzyladenine + Spermine+Cycocel	20+45+10	T_{12}	22+2
Benzyladenine + Spermine+Cycocel	20+60+10	T_{13}	22+2
Benzyladenine + Spermine	30+30	T_{14}	24
Benzyladenine + Spermine+Cycocel	30+30+10	T_{15}	22+2
Benzyladenine + Spermine+Cycocel	30+45+10	T_{16}	22+2
Benzyladenine + Spermine+Cycocel	30+60+10	T_{17}	22+2
Benzyladenine + Spermine	40+30	T_{18}	24
Benzyladenine + Spermine+Cycocel	40+30+10	T_{19}	22+2
Benzyladenine + Spermine+Cycocel	40+45+10	T_{20}	22+2
Benzyladenine + Spermine+Cycocel	40+60+10	T_{21}	22+2

Table II. Duration (days) taken by potato tubers of both the varieties for sprouting in different treatment. Means differing in letters are significantly different with each other at 5% level of probability

Treatments	Diamant		Desiree	
	Apical Sprouting	Lateral Sprouting	Apical Sprouting	Lateral Sprouting
T_1	35 a	44 a	51 a	54 a
T_2	23 b	31 e	36 bc	44 de
T_3	26 a	36 ab	46 a	50 bc
T_4	21 bc	30 ef	35 de	44 de
T_5	21 bc	32 d	33 e	44 de
T_6	24 ab	31 e	44 ab	46 d
T_7	21 bc	39 a	36 bc	42 ef
T_8	17 e	32 d	31 f	42 ef
T_9	18 e	31 e	32 ef	47 c
T_{10}	22 b	35 b	41 b	50 bc
T_{11}	21 bc	36 ab	35 de	50 bc
T_{12}	21 bc	33 c	36 bc	46 d
T_{13}	21 bc	35 b	39 b	52 ab
T_{14}	21 bc	36 ab	36 bc	50 bc
T_{15}	21 bc	32 d	37 bc	53 a
T_{16}	21 bc	32 d	41 ab	46 d
T_{17}	26 a	33 c	36 bc	51 c
T_{18}	17 e	31 e	33 e	44 de
T_{19}	19 e	33 c	35 de	51 b
T_{20}	21 bc	34 bc	36 bc	50 bc
T_{21}	21 bc	34 bc	36 bc	52 ab

Means with same alphabets differ non-significantly

Table III. Effect of plant growth regulators on number of stolon and tubers per plant. Means differing in letters are significantly different with each other at 5% level of probability

Treatment	Number of stolon		Number of tubers	
	Diamant	Desiree	Diamant	Desiree
T_1	-	-	-	-
T_2	-	-	0.33 de	0.33 ab
T_3	0.33 de	0.66 ab	0.66 c	-
T_4	1.33 ab	0.33 cd	0.33 de	-
T_5	0.33 de	0.66 ab	-	-
T_6	0.33 de	0.66 ab	0.33 de	0.33 ab
T_7	2.00 a	0.66 ab	0.66 c	1.00 a
T_8	0.66 c	-	1.33 ab	-
T_9	1.00 ab	-	0.33 de	1.00 a
T_{10}	0.66 c	-	0.66 c	-
T_{11}	1.66 a	0.66 ab	1.00 b	-
T_{12}	1.00 ab	-	-	-
T_{13}	1.00 ab	0.33 cd	0.66 c	0.33 ab
T_{14}	1.00 ab	-	1.33 ab	-
T_{15}	0.33 de	-	1.66 ab	-
T_{16}	1.00 ab	-	2.00 a	-
T_{17}	0.66 c	1.33 a	1.00 b	0.33 ab
T_{18}	0.66 c	1.66 a	1.00 b	1.33 a
T_{19}	0.66 c	0.33 cd	2.00 a	0.33 ab
T_{20}	0.66 c	0.66 ab	0.66 c	-
T_{21}	0.33 de	0.66 ab	0.33 de	-

Means with same alphabets differ non-significantly

reducing the potato tuber dormancy. Hemberg (1985) observed that GA contents increased prior to onset of dormancy. Diamant variety showed relatively more GA content in potato eye parts (PEP) in response to BA, Spr and CCC as compared to cv. Desiree (Table Va & b). The endogenous IAA contents were increased in eye parts and parts without eyes of potato tuber after application of BA and Spr in both the varieties but the response was greater in eye parts. The endogenous IAA contents increased in tuber eyes prior to onset of sprout growth (Sorce *et al*., 1996). Diamant variety showed more IAA contents in PEP as compared to cv. Desiree. The cv. Desiree accumulated more endogenous GA contents in leaves of the plant emerged from tuber and receiving soaking treatment with BA and Spr (Table VI). BA and Spm either alone or in combination with CCC exhibited more endogenous content of IAA in cv. Desiree except treatmentT_{15} and T_{19}. The auxin level was reported to increase at the stolon tips during the early stages of tuber initiation and decreases as the tuber grows (Marschner *et al.*, 1984).

IAA/GA ratio and stolon and tuber formation. The maximum IAA/GA ratio (Table Vb) was observed in the treatment T_9 (Spr) both in PEP and potato parts without eyes (PPWE) of cv. Desiree and PPWE of cv. Diamant, while control of cv. Diamant exhibited the minimum IAA/GA ratio. The IAA/GA ratio was low in tubers as well as in the leaves of cv. Diamant (Table VI). This change in IAA/GA ratio may be responsible for potato stolon and tuber formation. However, the role of other hormones viz. ABA and cytokinins as well as factors other than hormones can not be overlooked. Highly significant correlation (r =

Table IV. Chlorophyll contents in leaves of potato plant as affected by the application of plant growth regulators. Means differing in letters are significantly different with each other at 5% level of probability

Treatment	Diamant			Desiree		
	Chlorophyll a	Chlorophyll b	Total	Chlorophyll a	Chlorophyll b	Total
T_1	3.992	1.827	5.827 b	2.706	1.269	3.980 b
T_8	5.431	2.144	7.827 a	4.928	2.165	7.097 a
T_9	4.545	2.333	6.878 a	3.573	2.078	5.624 a
T_{18}	4.201	2.266	6.467 ab	3.232	1.972	5.204 ab
T_{19}	4.161	2.109	6.207 ab	2.908	1.243	4.151 ab

Table V. Effect of plant growth regulators on endogenous level of IAA and GA contents in potato tubers (A) and IAA/GA ratio (B). Means differing in letters are significantly different with each other at 5% level of probability

(A)

	IAA (ng/g)				GA (ng/g)			
	Diamant		Desiree		Diamant		Desiree	
Treatment	PEP	PPWE	PEP	PPWE	PEP	PPWE	PEP	PPWE
T_1	70 ab	27 ab	83 ab	50 ab	893 ab	814 ab	653 ab	594 ab
T_6	124 ab	102 ab	115 ab	89 ab	1398 ab	1260 ab	1235 ab	1190 ab
T_8	252 a	234 a	235 a	210 a	2982 a	2444 a	2318 a	2165 a
T_9	301 a	270 a	335 a	280 a	1924 a	1722 a	1535 a	1432 a

Means with same alphabets differ non-significantly

(B)

Treatments	Diamant		Desiree	
	PEP	PPWE	PEP	PPWE
T_1	0.0784	0.0332	0.0127	0.0831
T_6	0.0885	0.0820	0.0923	0.0752
T_8	0.0845	0.0945	0.1012	0.0963
T_9	0.0156	0.0156	0.2181	0.1952

Table VI. Effect of plant growth regulators on endogenous level of IAA and GA content and their ratio in leaves of potato plant. Means differing in letters are significantly different with each other at 5% level of probability

Treatment	IAA (n/g)		GA (ng/g)		IAA/GA ratio	
	Diamant	Desiree	Diamant	Desiree	Diamant	Desiree
T_1	430 bc	481 b	18 bc	24 bc	24	20
T_6	378 bc	378 bc	14 bc	13 bc	20	29
T_8	862 ab	1038 ab	44 ab	46 ab	19	22
T_9	1172 a	1231 a	92 a	82 a	12	14
T_{14}	664 ab	727 ab	30 b	38 ab	21	19
T_{15}	622 b	457 bc	28 b	27 b	21	16
T_{18}	978 a	1228 a	46 a	53 a	21	23
T_{19}	643 b	631 b	35 ab	34 b	17	18

Means with same alphabets differ non-significantly

0.95) existed between GA content of tubers having PEP and PPWE with tuberization of cv. Diamant. The same cultivar also showed a positive correlation for (r = 0.73) and (r = 0.68) tuberization with IAA level measured form PPWE and PEP.

CONCLUSION

In crux BA and Spr, as seed soaking treatments, can be implicated for breaking dormancy and to enhance sprouting and germination in both the cultivars. The cv. Diamant proved to be better in sprouting and germination than cv. Desiree. The IAA/GA ratio in leaves appear to have a possible role in potato stolon and tuber formation and endogenous GA contents of eye parts may be taken as an indicators for early sprouting of potato tuber.

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