International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 9, Issue 30, 2017, pp.-4408-4411.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

EFFECT OF GROWTH REGULATORS ON POTATO MICROTUBER FORMATION AND STORAGE EFFECT ON MICROTUBER DORMANCY

MACWAN S.J.[1*], UPADHYAY N.V. [2], SHUKLA Y.M.[3] AND VAISHNAV P.R.[4]

[1]Department of Plant Physiology Bansilal Amrutlal College of Agriculture, Anand Agricultural University, Anand, Gujarat 388110
[2]Main Vegetable Research Station, Anand, Anand Agricultural University, Anand, Gujarat 388110
[3]College of Polytechnique and Agriculture, Vaso, 387380, Anand Agricultural University, Anand, Gujarat 388110
[4]Department of Agricultural Statistics, Bansilal Amrutlal College of Agriculture, Anand Agricultural University, Anand, Gujarat 388110
*Corresponding Author: Email-sjmacwan@aau.in

Received: May 23, 2017; Revised: June 04, 2017; Accepted: June 05, 2017; Published: June 30, 2017

Abstract- Effect of microtuber formation in long term keeping with and without growth retardants under dark conditions revealed in presence of BAP and CCC, the period of microtuber formation was reduced from120day to 90 days. The optimum level of 5 mgl^{-1} BA and 500 mgl^{-1} CCC obtained maximum weight and size of microtuber. The varietal differences were significant and Kufri Pukhraj found best for the weight and size of microtuber. The level 500 mgl^{-1} CCC favours the increase microtuber weight irrespective of the presence of cytokinin.CCC at both lower and higher level than this reduced the microtuber weight. Effect of storage temperature and duration of storage on microtuber dormancy were evaluated and find out that the best dormancy breaking treatment was 25 °C. Among different temperature 4° C temperature reported no germination and may be used for storing the microtuber up to six month period.

Keywords- Growth Regulators, Potato, Microtuber, Storage Effect, Dormancy

Citation: Macwan S.J., et al., (2017) Effect of Growth Regulators on Potato Microtuber Formation and Storage Effect on Microtuber Dormancy. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 9, Issue 30, pp.-4408-4411.

Introduction

The potato is the first among major food crops, which has been extensively subjected to tissue culture manipulations. In India, systemic work on potato tissue and cell culture was initiated at Central Potato Research Institute, Shimla in 1972 [1]. During the 21st Century, microtuber may emerge as the propagate of choice in the nuclear production phase of seed potato industry and as an essential research tool in wide range of tuber related studies [2].India now ranks number three both in area as well as production of potato in the world and is poised to achieve the maximum annual growth rate in the coming decade. The physiological quality and safety of seed tubers are one the most important factors influencing potato yield [3]. Different growth regulators for in vitro induction of microtubers in potato were studied by Tugrul and Samanci [4] and Hossain [5]. Various research has revealed that, in vitro tuberization is controlled by different factors like hormonal combination, nutrient compositions were reported by El-Sawy et al., [6], Anoop Badoni and Chauhan [7] and El Dessoky et al,[8]. Microtubers are the first generation of potato seed from tissue culture being used to solve the problem of transplanting the plantlets from in vitro to in vivo conditions [9].

Materials and Methods

Effect of BA and growth retardants (CCC) under dark

Effect of microtuber formation in long term keeping with and without growth retardants under dark condition were observed in the following treatments.

M_1. MS + 2.5 mgl^{-1} BA + 250 mgl^{-1} CCC + 8% sucrose + 0.8 % Agar
M_2. MS + 2.5 mgl^{-1} BA + 500 CCC + 8% S + 0.8 % Agar
M_3. MS + 2.5 mgl^{-1} BA + 750 CCC + 8% S + 0.8 % Agar
M_4. MS + 5.0 mgl^{-1} BA + 250 CCC + 8% S + 0.8 % Agar
M_5. MS + 5.0 mgl^{-1} BA + 500 CCC + 8% S + 0.8 % Agar
M_6. MS + 5.0 mgl^{-1} BA + 750 CCC + 8% S + 0.8 % Agar
M_7. MS +10.0 mgl^{-1} BA + 250 CCC + 8% S + 0.8 % Agar
M_8. MS + 10.0 mgl^{-1} BA + 500 CCC + 8% S + 0.8 % Agar
M_9. MS + 10.0 mgl^{-1} BA + 750 mgl^{-1} CCC + 8% Sucrose + 0.8 % Agar
M_{10}. MS + 2.5 mgl^{-1} BA + 8% S + 0.8% Agar
M_{11}. MS + 5.0 mgl^{-1} BA + 8% S + 0.8% Agar
M_{12}. MS + 10.0 mgl^{-1} BA + 8% S + 0.8% Agar
M_{13}. MS + 8% Sucrose + 0.8% Agar

Effect of storage temperature and duration of storage on microtuber dormancy

Effect of storage temperature and duration of storage on microtuber dormancy were studied. The microtuber were stored under three temperature *viz.*, 4 ºC, 20 °C and 25 °C temperature in case of all the three varieties (Kufri Badshah, Kufri Pukhraj and Kufri Chipsona-1). The microtubers produced in dark conditions were used in these experiments. Each petriplates contain five microtubers and it was repeated two times. The microtubers were stored under the following storage temperature after harvesting and germination count was taken at different durations of storage.

(1)Stored in 4 °C temperature (T3)
(2)Kept in 20 °C temperature (T2)
(3)25 °C temperature storage (T1)

Result and Discussion:

Effect of BA and growth retardants (CCC) under dark

Weight of microtuber

The results from [Table-1] denotes that the highest (0.108 gm) average mean weight of microtuber in the M_5 (MS + 5BA + 500 CCC + 8% S + 0.8% Agar) treatment and the lowest (0.024 gm) average mean weight of microtuber were in M_{13} (MS + 8 % sucrose). Microtuber of Kufri Pukhraj reported significantly the highest (0.095 gm) average mean weight followed by Kufri Chipsona-1 (0.058 gm) and Kufri Badshah (0.055 gm). It was recorded that the cytokinin (BAP) and growth retardants (CCC) were incorporated then the treatment M_2, M_5, M_8 and M_{11} shows the higher mean weight of the microtuber i.e. 0.079gm, 0.108gm, 0.064gm and 0.099gm respectively where 500 mgl^{-1} CCC were found the same in all the treatments while cytokinin level were different i.e 2.5 mgl^{-1}, 5.0 mgl^{-1} and 10.0 mgl^{-1} BAP were reported. Further, it was reported that in all the treatments (M_1 M_4, M_7) where reduced levels of (250 mgl^{-1}) CCC while in the treatments (M_3 M_6 and M_7) where increase level (750 mgl^{-1}) of CCC were used then average mean weight were found less as compared to the treatments (M_2, M_5 and M_8) where 500 mgl^{-1} CCC were used. Further, it was observed that when the levels of cytokinin were consider the M_{11} treatment where 5.0 mgl^{-1} BA found to be best to obtained higher weight of microtuber. So it was concluded that CCC at 500 mgl^{-1}CCC level was best and amoung the different levels of (2.5, 5.0 and 10.0 mgl^{-1}) BA, the best level was 5.0 mgl^{-1} BA.

Size of microtuber

The results from the [Table-2] it was reported that variety, media and its interaction found to be significant for the size of microtuber. The highest (0.667 cm) size of microtuber was reported in M_5 (MS + 5 mgl^{-1} BA + 500 mgl^{-1} CCC + 8 % sucrose + 8 % Agar) and the lowest (0.275 cm) size of microtuber in the treatment M_7 were higher (10.0 mgl^{-1}) dose of cytokinin and the lowest (250 mgl^{-1}) levels of growth _retardants. The Kufri Pukhraj reported significantly the highest (0.544 cm) average mean size of microtuber followed by Kufri Chipsona-1 (0.449 cm) and Kufri Badshah (0.365 cm). In general days to microtuber formation were reported in 90 days but the treatment M_5 (MS + 5.0 mgl^{-1} BA + 500 CCC + 8% S + 0.8 % Agar) reported early and its size were also found maximum while the treatment M_{13} (MS + 8% Sucrose + 0.8% Agar) where absence of growth regulators reported late formation of microtuber, it was found 120 day to microtuber formation. The response of microtuberisation was overall 100% except the treatment no. 13 (V_1 83%, V_2 53% and V_3 66%) and treatment 7 has 50% microtuberisation. The colour of microtuber was whitish yellow. From the above discussion it was concluded that M_5 treatment where optimum levels of 5 $mgl^{-1}$of BA and 500 mgl^{-1}CCC obtained maximum weight and size of microtuber followed by M_{11} treatment where the growth retardants CCC was absent but in presence of cytokenin of 5 mgl^{-1}BA. The varietal difference was significantly higher in Kufri Pukhraj. The lower levels and higher levels of cytokinin as well as growth retardants reduces the weight and size of microtuber. At the very same time it was reported that in absence of growth regulators lowest weight of microtuber(0.024 gm) and minimum size (0.32cm) which was at par with the treatment M_7 (0.28) and M_9(0.31cm) where highest level (10 mgl^{-1})of cytokinin were incorporated. This indicates that higher level of BAP with combination of growth retardants reduce the size of microtuber. Our result was akin with the results of Palmar and Smith [10] where the promotive effect of cytokinin on tuber formation was observed. In our results the optimum level of BAP and CCC influence the positive effect as compared to BAP alone, which indicates that CCC accelerates the tuberisation. Yamamoto and Nakata [11] showed that CCC, which prevent biosynthesis of GA_3 enhance the tuberisation and reinforce the promoting effect of cytokinin. The best result obtained on the medium supplemented with 5 mgl^{-1} BA and 500 mgl^{-1}CCC, which was accordance with Yamamoto and Nakata [11] and many other workers *viz.,* Hussey and Stacey [12] and Estrada *et al*., [13].

Effect of storage temperature and duration of storage on microtuber dormancy

Number of germinated microtuber

The numbers of germinated microtubers were recorded and the results were tabulated in [Table-3]. First initiations of germination of microtuber were observed after 6 days. After that one day interval observations were recorded second week duration.

Table-1 *Effect of cytokinin and growth retardants on(a) Average weight of microtuber (gm)*

Treatment	Variety			
	V_1 Kufri Badshah	V_2 Kufri Pukhraj	V_3 Kufri Chipsona-1	Mean (M) gm
M_1	0.033	0.064	0.064	0.054
M_2	0.047	0.095	0.096	0.079
M_3	0.042	0.075	0.088	0.066
M_4	0.076	0.046	0.124	0.083
M_5	0.083	0.076	0.164	0.108
M_6	0.062	0.045	0.149	0.085
M_7	0.033	0.046	0.071	0.048
M_8	0.065	0.049	0.078	0.064
M_9	0.062	0.027	0.058	0.050
M_{10}	0.048	0.065	0.117	0.077
M_{11}	0.092	0.078	0.125	0.099
M_{12}	0.043	0.068	0.073	0.061
M_{13}	0.029	0.020	0.024	0.024
Mean (V)	0.055	0.058	0.095	
		S.Em. ±	C.D.	
V		0.0001	0.001	
M		0.001	0.002	
V x M		0.002	0.004	
CV %		5.46		

Table-2 *Effect of cytokinin and growth retardants on (b)size of microtuber (cm)*

Treatment	Variety			
	V_1 Kufri Badshah	V_2 Kufri Pukhraj	V_3 Kufri Chipsona-1	Mean (M) (cm)
M_1	0.423	0.492	0.542	0.486
M_2	0.360	0.502	0.615	0.494
M_3	0.343	0.427	0.467	0.412
M_4	0.443	0.440	0.662	0.522
M_5	0.538	0.616	0.543	0.667
M_6	0.365	0.362	0.640	0.469
M_7	0.277	0.318	0.230	0.275
M_8	0.445	0.345	0.442	0.411
M_9	0.298	0.275	0.352	0.308
M_{10}	0.285	0.590	0.620	0.498
M_{11}	0.520	0.650	0.665	0.612
M_{12}	0.390	0.550	0.540	0.493
M_{13}	0.288	0.240	0.430	0.319
Mean (V)	0.364	0.448	0.544	
		S.Em. ±	C.D.	
V		0.006	0.017	
M		0.012	0.0034	
V x M		0.022	0.060	
CV %		11.51		

First observation was taken at 7th day and treatment was found to be significant while the varieties and their interaction were not significant. The same results were reported during 9th and 10 day after the plumule was first observed. In that the maximum number of germinated microtuber in Kufri Pukhraj followed by Kufri Chipsona-1 and Kufri Badshah. Among the three temperature treatment, the treatment T_1 - 25 °C temperature reported maximum and best responsive for the germination and best responsive for the germination of microtuber. No response in treatment No. 3 where 4 °C temperature were maintained. Same trend was found at the 20th day and 25th day observation where the treatment T_1 (25 °C temperature) and V_3 Kufri Pukhraj found to be significantly higher number of germinated microtuber. The treatment T_3 (4 °C temperature) responded negative i.e. still there was no germination. From these results it was noted that germination process starts from 7th day onwards and it was highly variable which

reflects in the CV % during initiation phase. During 15th and 20th day observation lower the CV % value found to be lower indicate that the days passes the variation became reduced.

Number of sprouts per microtuber

Number of sprouts per microtuber was significantly respond in the treatments of temperature but non-significant in variety and its interactions. 25 °C temperature gave the maximum sprouting during the period of experiment i.e. 7th day to 25th day observation. Result found the same trend and no response in treatment of 4 °C temperature. After the 20th day number of sprouts per microtuber found to be reduced or it may not observe due to the decay. [Table-4]

Percentage of germination of the microtuber

Germination percentage was found to be significant in treatment of temperature during whole storage period of experiment while the variety found significant during 10th day onwards. When interaction between variety and temperature were recorded at to be significant at 10th day and 25th day observation. Treatment T_1 (25°C temperature) reported maximum 71.83% while Kufri Pukhraj gave maximum (50.01%) germination percentage.

The results, [Table-5] 7th and 8th day observation of treatment T_1 (25 °C temperature) express higher percentage and Kufri Pukhraj shows higher value of percentage followed by Kufri Chipsona-1 and Kufri Badshah. Further the results from 10th, 15th and 25th days observation was recorded that higher percentage of germination microtuber in the 25 °C temperature treatment while least germination (1.28) percentage were recorded in the treatment of 4 °C temperature.

Sprout length

When the sprout length was observed the result in [Table-6] indicates that varietal responses were non-significant while the temperature treatment effects were reported to be significant. The sprout length were maximum in T_2 (20 ºC temperature) treatment but the 4 ºC temperature treatments effects was not responding and remain dormant. The length of sprouts growth more in low temperature (20 ºC), which supports the conditions of growing of potato, potato growers in cool condition with low temperature time.

Table-3 *Effect of storage temperature and duration of storage on microtuber germination*

Treatment	Number of germinated microtuber					
	7th day	9th day	10th day	15th day	20th day	25th day
V_1	0.965 (0.4312)	1.052 (0.606)	1.111 (0.7343)	1.230 (1.0129)	1.327 (1.2609)	1.386 (1.4209)
V_2	1.025 (0.5506)	1.230 (1.0129)	1.278 (1.1333)	1.434 (1.5563)	1.476 (1.6785)	1.645 (2.206)
V_3	1.717 (0.8712)	1.327 (1.2609)	1.386 (1.4209)	1.518 (1.8043)	1.608 (2.0856)	1.682 (2.329)
S.Em.±(V)	0.098	0.076	0.068	0.052	0.056	0.040
C.D.	NS	NS	NS	0.167	0.179	0.129
T_1	1.257 (1.0800)	1.511 (1.782)	1.558 (1.9273)	1.857 (2.9484)	(1.996 (3.484)	2.150 (4.1225)
T_2	1.197 (0.9328)	1.392 (1.4376)	1.501 (1.7530)	1.618 (2.1179)	(1.708 (2.4172)	1.857 (2.9484)
T_3	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)
S.Em.±(T)	0.098	0.076	0.068	0.052	0.056	0.040
C.D.	0.313	0.244	0.219	0.167	0.179	0.129
S.Em. ± (V x T)	0.169	0.132	0.119	0.091	0.097	0.070
C.D.	NS	NS	NS	NS	NS	0.0223
CV%	22.71	15.33	13.32	9.18	9.30	6.29

* Value in the parenthesis is retransformed value.

Table-4 *Effect of storage temperature and duration of storage on number of sprouts per microtuber in different cultivar of potato*

Treatment	Number of sprouts per microtuber					
	7th day	9th day	10th day	15th day	20th day	25th day
V1	0.966 (0.4331)	1.052 (0.6067)	1.059 (0.6215)	1.052 (0.6067)	1.095 (0.6990)	1.052 (0.6067)
V2	1.085 (0.6772)	1.230 (1.0129)	1.267 (1.1053)	1.211 (0.9665)	0.144 (0.8087)	1.148 (0.8179)
V3	1.112 (0.7365)	1.112 (0.7365)	1.153 (0.8294)	1.187 (0.9089)	1.163 (0.8525)	1.111 (0.7343)
S.Em.+(V)	0.078	0.048	0.055	0.062	0.011	0.036
C.D.	NS	NS	NS	NS	0.036	NS
T1	1.257 (1.080)	1.343 (1.3036)	1.407 (1.4796)	1.339 (1.2929)	1.451 (1.6054)	1.299 (1.1874)
T2	1.197 (0.9328)	1.343 (1.3036)	1.366 (1.3659)	1.404 (1.4712)	1.244 (1.0475)	(1.305 (1.2030)
T3	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)	0.707 (0.000)
S.Em.+(T)	0.078	0.048	0.055	0.062	0.011	0.036
C.D.	0.251	0.155	0.177	0.200	0.036	0.117
S.Em. + (V x T)	0.136	0.084	0.096	0.108	0.019	0.063
C.D.	NS	NS	NS	NS	0.062	NS
CV%	18.21	10.50	11.68	13.29	2.43	8.08

* Value in the parenthesis is retransformed value.

Table-5 *Effect of storage temperature and time three different cultivar for the percentage of germination*

Treatment	% of germination				
	7th day	9th day	10th day	15th day	25th day
V_1	18.13	20.24	24.460	28.30	30.42
V_2	24.46	26.38	30.4149	34.47	44.80
V_3	26.38	30.41	35.596	40.58	50.01
S.Em.±(V)	2.628	2.581	1.339	2.384	2.794
C.D.	NS	NS	4.283	7.627	8.938
T_1	34.99	38.83	51.124	57.07	71.83
T_2	32.69	36.91	38.066	44.98	52.12
T_3	1.28	1.28	1.28	1.28	1.28
S.Em.±(T)	2.628	2.581	1.339	2.384	2.794
C.D.	8.405	8.257	4.283	7.627	8.28
S.Em. ±(V x T)	4.551	4.471	2.319	4.130	4.840
C.D.	NS	NS	7.418	NS	15.482
CV%	27.9	24.6	10.87	16.9	16.40

Table-6 *Storage temperature and storage duration effect on length of sprouts in three cultivar of potato*

Treatment	Length of sprouts		
	7th day	10th day	25th day
V_1	0.20	0.31	1.58
V_2	0.13	0.28	2.27
V_3	0.66	0.78	2.35
S.Em.± (V)	0.077	0.114	0.061
C.D.	NS	NS	0.197
T_1	0.17	0.26	2.63
T_2	0.23	0.50	3.53
T_3	0.00	0.00	0.00
S.Em.± (T)	0.077	0.114	0.061
C.D.	NS	0.365	0.197
S.Em. ± (VxT)	0.133	0.198	0.107
C.D.	NS	NS	0.341
CV%	14.12	11.07	7.34

[Plate-1] shows the effect of temperature on germination. The 4 °C temperature effect found no germination while 25 °C temperature found best maximum dormancy breaking and its growth. From the above results it was concluded that the best dormancy breaking treatment was 25 °C temperature. The intiation of the germination took place seventh day duration while maximum germination obtained within 20-25 days. Among the different treatment of temperature, 4 °C temperature reported no germination, so it may be used for storing microtuber upto (24 week during) six months. It was observed that the stored microtuber in 4 °C get break its dormancy after 9 months storage. Desire *et al.* [14] supported our results and they were also did not reported germination up to 22 weeks in cold storage condition at 4 °C. Further, they reported germination after transferring at higher temperature of 19 °C for three weeks showed a sharp increase from 8% to 80% between 14 and 22 weeks, respectively. Further, they found out that between 4 to 9 weeks (i.e. 28 to 36 days) duration accumulation of polypeptides and a protein set of the patatin family were increased while after that duration (after 9 weeks), these polypeptides decreased in amount or were not longer detected, at 10th week no protein changes were found. It suggests that this accumulation of protein play important role in dormancy breaking.

T1 **T2** **T3**
25 ºC **20 ºC** **4 ºC**

Plate 1: Effect of temperature on germination

Conclusion

Microtuber formation in the presence of BAP and CCC was associated with the early and positive response for the microtuberization. Production of microtuber was affected by growth regulating substances. The best dormancy breaking treatment was 25 °C temperature and 4 °C temperature inhibits the germination. Dormancy duration was about six month in 4 °C temperature.

Acknowledgment

Author acknowledges Emeritus Scientist, CSIR, Dr. A.R. Mehta for his valuable remarks and suggestions for research work.

Author Contributions: All author equally contributed

Abbreviations

MS- Murashige and Skoog
BAP- 6-Benzylaminopurine, benzyl adenine
CCC-Chlorocholine Chloride
V- variety
M-media
% -percentage, gm-gram, mg- milligram, ml-milliliters, cm- centimetre,

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors

Conflict of Interest: None declared

References

[1] Chandra R. and Naik S.P. (1993) Potato tissue and cell culture.In Advances in horticulture Vol. 7.Potato eds. by K.L. Chadha and J.S. Grewal, Pub.by Malhotra Publishing House, New Delhi.
[2] Coleman W.K., Donnelly D.J. and Coleman S.E. (2001) *Amer. J. of Potato Res.,* 78, 47-55.
[3] Wiersema S.G. (1986) *Am. Potato J.,* 63, p. 465–472
[4] Tugrul S. and Samanci B. (2001) *Potato Abstr.,* 26,86.
[5] Hossain M.J. (2005) *Plant Tissue Cult. & Biotech.,* 15(2), 157-166.
[6] El-Sawy A., Bekheet S. and Aly U.I. (2007) *In J Agri Biol.,* 9(5), 675-680.
[7] Anoop B., Chauhan J.S. (2009) *Nature and Science*, 7(9),31-34.
[8] El Dessoky S. Dessoky, Attia O. Attia, Ismail A. Ismail and Ehab I. El-Hallous (2016) *International Journal of Advanced*, 4(1), 684– 689
[9] Chankya M.S., Maurya K.A. and Hargobind W.F. (2015) *Afr. J. Crop Sci.*, 3(5), 176-186.
[10] Palmer C.E. and Smith O.E. (1969) *Nature*, 221, 279-280
[11] Yamamoto T. and Nakata K. (1997) *Jpn. J. Crop Sci.,* 66 (4), 663-668.
[12] Hussey G. and Stacey N.J.(1984) *Annals of Botany,* 53, 565-578.
[13] Estrada R., Tovar P. and Dodds J.H. (1986) *Plant Cell Tissue Organ Cult.,* 7, 3-10.
[14] Desire S., Couillerot J.P. and Vasseur J. (1995) *Acta bot Gallica,* 142, 371-378.