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Optimization of stripping system of stem tips in burbank potato breed

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ABSTRACT

Viruses that infect and accumulate in potatoes during the planting process cause the low productivity and low quality of potatoes. The rapid stem tip culture with good quality can earn time and efficiency for the production of detoxified plantlets and potatoes. Therefore, it is necessary to optimize the detoxification culture method of potatoes' stem tips. We carried out experiments on the buds of Burbank's pre-basic, basic, level 1 and commodity potatoes and on the test-tube plantlets by placing stripped stem tips on MS culture media with hormone in different densities, stripping the growing points on the stem tips and observing in the culture room. We calculated the pollution rate, survival rate and seeding rate and had ELISA virus detection on the acquired detoxified plantlets. The results showed that the growing points of all kinds of Burbank's potatoes' stem tips on MS+6-BA0.5mg/L+NAA0.01mg/L+GA30.1mg/L culture medium had higher survival rate and seeding rate (both above 80%) and detoxification rate (over 90%)than others. Compared with the control groups, the density of hormone in this culture medium was more conducive to the stripping and seeding of all kinds of Burbank's potatoes' stem tips.

Key words: Potato (solanum tuberosum); Burbank; Stem tip; Virus detection

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INTRODUCTION

Potato, (solanum tuberosum, 2n=48), a dicotyledon, is an annual herbaceous plant in Solanceae Solanum. It conducts asexual reproduction with the tuber. The breeds include Kexin NO.1, Zaodabai, Favorita, Atlantic, Shepody, Yushu NO.1, Burbank and etc. Burbank is an American breed, introduced in China by the Seeds Bureau of China Ministry of Agriculture. The plant type is up-right, with thick stalk, spotted purpurin and the tuber is oval. The tuber is big and tidy. The skin is brown with overlapping curves. The inside is white, with few and shallow bud eyes. The tuberization is concentrated [1-3]. This breed has demanding requirements on the planting conditions as it is neither drought-resisting nor flood-resisting. So it is suitable to plant in the arid and semi-arid areas in the north and the northwest areas. As it is especially suitable for frying, it is the ideal breed for processing potato chips.

The distribution of viruses in potatoes is very uneven. The stem tip contains the least or no viruses; and the closer to the tip, the lower the virus concentration. According to this feature, strip the shoot apical meristem by using anatomical lens under aseptic condition and inoculate it to the culture bottle which contains specific culture medium to cultivate and then can get the detoxified plantlet [4-5]. Cut and cultivate the plantlet and then use substrate culture to produce a large number of detoxified potato seeds [6-7]. The production quality and stress resistance of the detoxified potato seeds are greatly enhanced and the tissue culture technique has been an important method to improve the potato productivity. Therefore, the use of detoxified tuber tip plantlet to produce virus-free potato seeds is the fundamental measure to solve the problem of viral degradation of potatoes and to improve the productivity.

1. EXPERIMENTAL SEEDS AND CULTURE MEDIA

The experimental breeds include the pre-basic seeds, basic seeds, level 1 seeds and commodity seeds in Burbank

breed, provided by Yulin Municipal Academy of Agricultural Sciences. The test-tube plantlets, the major reagents and equipments were provided by plant tissue culture lab in College of Life Science. The culture media were strictly made and sterilized according to the requirements.

2. RESEARCH METHODOLOGIES

3.1 Accelerating germination

Accelerate germination for the pre-basic seeds, basic seeds, level 1 seeds and commodity seeds in Burbank breed [8]. Dice the potatoes in accordance with the bud eyes and soak the dices in 6ppmGA3 for 30 minutes. Then dry in the air and accelerate germination with wet sand respectively with temperature always under 20℃ [9-10].

3.2 Separate stem and tip

Wrap the buds of the pre-basic seeds' tubers with gauze and wash with water for 30 minutes to remove the mud and other big particles. In sterile interior, sterilize the buds with 75% ethyl alcohol for 20 seconds, then sterile water for 3 times, and then use 0.1%$HgCl_2$ and 10% NaCLO to sterilize the surface of the buds for 2,4,6,8,10,12 minutes respectively (table 1). Calculate the optimal disinfectant and disinfection time.

Table1 Different Disinfectants and Disinfection Time

Disinfection Times (min)	2	4	6	8	10	12
0.1%$HgCl_2$	S1	S2	S3	S4	S5	S6
10%NaClO	C1	C2	C3	C4	C5	C6

3.3 Differentiation culture of stem tips

Put the stripped stem tips of various potato seeds on the cultivation media of 3 kinds of hormone with different densities: 6-BA, NAA and GA3. The code names are A1,A2,A3,A4,A5,A6,A7,A8,A9,A10,A11,A12,CK1,CK2 (table 2). Among them, CK1 (MS+6-BA0.5mg/L+NAA0.1mg/L-GA3 0.1mg /L) and CK2 are the control group.

Table 2 Cultivation Media with Growth Regulator of Different Densities

Code of cultivation medium	Growth Regulator /(mg /L)		
	6-BA	NAA	GA3
A1	0.05	0.1	0.1
A2	0.1	0.1	0.1
A3	0.2	0.1	0.1
A4	0.4	0.1	0.2
A5	0.7	0.1	0.2
A6	1.0	0.1	0.2
A7	0.5	0.01	0.1
A8	0.5	0.02	0.1
A9	0.5	0.05	0.1
A10	0.5	0.08	0.2
A11	0.5	0.2	0.2
A12	0.5	0.5	0.2
CK1	0.5	0.1	0.1
CK2	0	0	0

Inoculated culture bottles should be cultivated in the culture room, with temperature of 20~ 25℃, 16 hours of illumination with 2000~ 3000lx illumination intensity. Under normal circumstances, new buds (1~ 3cm) come out after inoculation of 7 days and reach 6cm on the 20th day. Detect the viruses when the buds reach 8~ 10cm. Calculate the pollution rate, survival rate and seeding rate of various potato seeds. The buds with viruses should be immediately eliminated and those with same code in the culture room as well.

3.4 Virus detection

Virus detection should be carried out only when the buds reach 10cm [11]. The most common method is enzyme-linked immunosorbent assay (ELISA) [12].

RESULTS AND ANALYSIS

Table 3 the Pollution Rate with Different Disinfectants and Disinfection Time

Method	S1	S2	S3	S4	S5	S6	C1	C2	C3	C4	C5	C6
No. of seeds	20	20	20	20	20	20	20	20	20	20	20	20
No. of pollution	5	3	2	1	0	0	9	7	6	4	3	2
Pollution rate (%)	25	15	10	5	0	0	45	35	30	20	15	10

4.1 The impact of different disinfectants and disinfection time on the pollution rate of stem tip
The calculation of the pollution rate of the sterilized buds with different disinfectants, 0.1% $HgCl_2$ and 10% NaClO, is shown in table 3.

As can be seen from the table 3, the longer the disinfection time, the lower the pollution rate under the same disinfectant. But if the disinfection time is too long, the stem tips would be harmed or dead. Within 10 minutes disinfection time, 0.1%$HgCl_2$ has better disinfection result than 10%NaClO.

4.2 The impact of different inducing culture media on the seeding
4.2.1 The impact of different inducing culture media on the seeding of pre-basic seeds
Put stripped pre-basic seeds' stem tips on the hormone culture-medium with different densities. Put culture bottles in the culture room with temperature of 20~ 25℃, 16 hours of illumination with 2000~ 3000lx illumination intensity. Calculate the pollution rate, survival rate and seeding rate (table 4).

Table 4 the Impact of Different Inducing Culture Media on the Seeding of Burbank Pre-basic Seeds' Stem Tips

Method	No. of Inoculated Stem Tips	No. of Pollution	Pollution Rate (%)	No. of Survivors	Survival Rate (%)	No. of Seeding	Sprout Rate (%)
A1	120	3	2.5	98	83.8	79	80.6
A2	120	5	4.2	95	82.6	78	82.1
A3	120	3	2.5	98	83.8	80	81.6
A4	120	6	5.0	95	83.3	76	80.0
A5	120	7	5.8	93	82.3	75	80.6
A6	120	4	3.3	95	81.9	76	80.0
A7	120	2	1.7	101	85.6	88	87.1
A8	120	9	7.5	90	81.1	74	82.2
A9	120	5	4.2	92	80.0	73	79.3
A10	120	8	6.7	89	79.5	70	78.7
A11	120	4	3.3	92	79.3	72	78.3
A12	120	7	5.8	90	79.6	70	77.8
CK1	120	3	2.5	99	84.6	85	85.9
CK2	120	5	4.2	87	75.7	69	79.3

As can be seen from table 4, the stem tip growing points of Burbank pre-basic seeds on A7(MS+6-BA 0.5mg /L+NAA 0.01mg/L +GA3 0.1mg /L) culture medium have a higher survival rate (85.6%) and seeding rate (87.1%), and pollution rate of 1.7%. Compared with the control group, A7 culture medium is more in conducive to the seeding of Burbank pre-basic seeds.

4.2.2 The impact of different inducing culture media on the seeding of basic seeds
Put stripped basic seeds' stem tips on the hormone culture-medium with different densities. Put culture bottles in the culture room. The culture method is the same as with the pre-basic seeds. Calculate the pollution rate, survival rate and seeding rate (table 5).

Table 5 the Impact of Different Inducing Culture Media on the Seeding of Burbank Basic Seeds' Stem Tips

Method	No. of Inoculated Stem Tips	No. of Pollution	Pollution Rate (%)	No. of Survivors	Survival Rate (%)	No. of Seeding	Sprout Rate (%)
A1	120	9	7.5	99	85.3	80	80.8
A2	120	11	9.1	95	82.6	78	82.1
A3	120	10	8.3	98	83.8	82	83.7
A4	120	15	12.5	94	82.5	76	80.9
A5	120	14	11.6	93	81.6	75	80.6
A6	120	11	9.1	95	81.9	76	80.0
A7	120	10	8.3	100	84.7	89	89.0
A8	120	15	12.5	90	79.6	74	82.2
A9	120	14	11.6	92	80.0	73	79.3
A10	120	11	9.l	90	80.4	72	80.0
A11	120	12	10.0	92	82.9	72	78.3
A12	120	10	8.3	90	79.6	70	77.8
CK1	120	11	9.1	99	84.6	85	85.9
CK2	120	15	12.5	56	49.6	36	64.3

As can be seen from table 5, the stem tip growing points of Burbank basic seeds on A7 (MS+6- BA0.5mg /L+NAA 0.01mg/L+GA30.1mg/L) culture medium have a higher survival rate (84.7%) and seeding rate (89.0%), and pollution rate of 8.3% . Compared with other groups, the survival rate and seeding rate of A3 and CK1 are also relatively high, but the culture medium of A7 is the most conducive to the seeding of Burbank basic seeds. The survival rate and seeding rate of those without hormone in culture media are low.

4.2.3 The impact of different inducing culture media on the seeding of level 1 seeds

Put stripped level 1 seeds' stem tips on the hormone culture-medium with different densities. Put culture bottles in the culture room. The culture method is the same as with the pre-basic seeds. Calculate the pollution rate, survival rate and seeding rate (table 6).

Table 6 the Impact of Different Inducing Culture Media on the Seeding of Burbank Level 1 Seeds' Stem Tips

Method	No. of Inoculated Stem Tips	No. of Pollution	Pollution Rate (%)	No. of Survivors	Survival Rate (%)	No. of Seeding	Sprout Rate (%)
A1	120	12	10.0	99	85.3	79	79.8
A2	120	14	11.7	94	81.0	78	83.0
A3	120	15	12.5	98	83.8	80	81.6
A4	120	13	10.88	96	85.0	77	80.2
A5	120	12	10.0	93	81.6	75	80.6
A6	120	16	13.3	95	82.6	79	83.2
A7	120	11	9.2	99	83.9	90	90.9
A8	120	16	13.3	90	78.9	74	82.2
A9	120	17	14.2	90	78.3	78	86.7
A10	120	19	15.8	90	79.6	72	80.0
A11	120	15	12.5	92	82.9	72	78.3
A12	120	13	10.8	90	78.9	76	84.4
CK1	120	17	14.2	97	82.9	85	87.6
CK2	120	16	13.3	50	44.2	30	60.0

As can be seen in table 6, though the stem tip growing points of Burbank level 1 seeds on CK1 (MS+6-BA0.5mg/L+NAA 0.01mg/L+GA30.1mg /L) culture medium have a higher survival rate and seeding rate, both rates on A7 culture medium are respectively 83.9% and 90.9%, with pollution rate of 9.2%. Therefore, the A7 culture medium is more conducive for the seeding of Burbank level 1 seeds.

4.2.4 The impact of different inducing culture media on the seeding of commodity seeds

Put stripped commodity seeds' stem tips on the hormone culture-medium with different densities. Put culture bottles in the culture room. The culture method is the same as with the pre-basic seeds. Calculate the pollution rate, survival rate and seeding rate (table 7).

Table 7 the Impact of Different Inducing Culture Media on the Seeding of Burbank Level 1 Seeds' Stem Tips

Method	No. of Inoculated Stem Tips	No. of Pollution	Pollution Rate (%)	No. of Survivors	Survival Rate (%)	No. of Seeding	Sprout Rate (%)
A1	120	19	15.8	97	83.6	80	82.5
A2	120	20	16.7	94	81.0	77	81.9
A3	120	23	19.2	95	81.2	80	84.2
A4	120	22	18.3	96	85.7	77	80.2
A5	120	21	17.5	96	84.2	75	78.1
A6	120	22	18.3	95	81.9	80	84.2
A7	120	17	14.2	99	84.6	92	92.9
A8	120	21	17.5	89	78.1	74	83.1
A9	120	24	20	90	78.3	78	86.7
A10	120	25	20.8	87	76.3	74	85.1
A11	120	23	19.2	94	83.2	72	76.6
A12	120	20	16.7	92	80.7	76	82.6
CK1	120	17	14.2	97	82.9	87	89.7
CK2	120	22	18.3	49	42.6	28	57.1

As can be seen in table 7, the stem tip growing points of Burbank level 1 seeds on A7(MS+6-BA 0.5mg/L+ NAA 0.01mg/L+GA3 0.1mg/L) culture medium have a pollution rate, survival rate and seeding rate respectively 4.2%,84.6% and 92.9%. Therefore, the A7 culture medium is more conducive to the seeding of Burbank commodity seeds.

4.2.5 The impact of different inducing culture media on the seeding of test-tube plantlets

Put stripped test-tube plantlets' stem tips on the hormone culture-medium with different densities. Put culture bottles in the culture room. The culture method is the same as with the pre-basic seeds. Calculate the pollution rate, survival rate and seeding rate (table 8).

As can be seen in table 8, the stem tip growing points of test-tube plantlets on A7(MS+ 6-BA 0.5mg /L +NAA 0.01mg /L+GA3 0.1mg/L) culture medium have a higher survival rate (87. 2%) and seeding rate (93.1%) respectively, with a pollution rate of 0.8%, than other groups. Therefore, the A7 culture medium is more conducive for the seeding of test-tube plantlets.

Table 8 the Impact of Different Inducing Culture Media on the Seeding of Test-tube Plantlets' Stem Tips

Method	No. of Inoculated Stem Tips	No. of Pollution	Pollution Rate (%)	No. of Survivors	Survival Rate (%)	No. of Seeding	Sprout Rate (%)
A1	120	3	2.5	95	82.6	82	86.3
A2	120	4	3.3	96	82.8	79	82.3
A3	120	2	21.7	95	81.2	84	88.4
A4	120	5	4.2	93	82.3	80	86.0
A5	120	3	2.5	96	84.2	79	82.3
A6	120	3	2.5	95	82.6	82	86.3
A7	120	1	0.8	102	87.2	95	93.1
A8	120	4	3.3	92	82.1	78	84.8
A9	120	3	2.5	92	80.0	79	85.9
A10	120	4	3.3	90	78.9	75	83.3
A11	120	3	2.5	94	81.7	79	84.0
A12	120	5	4.2	92	80.7	80	87.0
CK1	120	4	3.3	97	82.9	89	91.8
CK2	120	4	3.3	52	46.0	28	53.8

4.3 Virus detection

The major viruses that seriously harm the potatoes are Potato Leaf Roll Virus (PLRV), Potato virus Y (PVY), Potato virus X (PVX), Potato virus S (PVS), Potato virus M (PVM), Potato virus A (PVA), etc [13-15]. With ELISA virus detection, it is known from table 9 that the detoxification rate of Burbank's pre-basic, basic, level 1, commodity potatoes and test-tube plantlets are 94.5%,92.7%,91.9%,90.5% and 95.4% respectively. The test-tube plantlets have the highest 95.4% while the commodity potatoes the lowest 90.5%. Through the stem tip stripping, the detoxification rate is significantly increased, thus ensuring the quality of seed potatoes.

Table 9 Virus Detection

Virus		PVA	PLRV	PVM	PVS	PVX	PVY	Detoxification rate
Level	No. in test	130	130	130	130	130	130	
Pre-basic Basic Level 1 Commodity Test-tube	No. of Detoxification	126	127	122	118	120	124	94.5
		123	125	120	118	115	122	92.7
		122	124	118	115	118	120	91.9
		120	122	117	113	115	119	90.5
		127	129	123	120	120	125	95.4

CONCLUSION

The experiment shows that proper hormone can promote the stripping of stem tips. As compared with control groups, A7 (MS+6-BA 0.5mg/L+NAA 0.01mg/L+GA3 0.1mg/L) culture medium is more conducive to the seeding of stem tips of various Burbank's seed potatoes. On this culture medium, the survival rate and seeding rate of seed potatoes of all levels reach over 80%, and the detoxification rate 90%. So an appropriate ratio of auxin and cytokinin is very helpful to the seeding of stem tip.

Under the most suitable culture conditions, the size of the stem tips decides the survival rate of them. Generally speaking, the bigger the stem tips, the easier for them to survive. Yet too big stem tips also seriously influence the detoxification rate. Therefore, the selection of stem tips of appropriate size is very important to the tissue culture. In the culture of stem tips, the smaller the stem tips, the more difficult to survive. But the detoxification efficiency is higher as there is basically no detoxification effect on too big stem tips. The detoxification of stem tips with 2 phyllopodium is the more ideal.

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