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Establishment of Regeneration System of Potato Variety Xuanshu 2

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Abstract. In this study, the cultivated potato variety Xuanshu 2 was used as plant material, and MS was used as the basic medium, the in vitro regeneration culture of stem segments was carried out to study the optimal combination of phytohormone concentrations for callus induction and bud differentiation. The results indicated that the optimal medium for callus induction in stem segments was MS + 2.0 mg·L⁻¹ 6-BA + 0.2 mg·L⁻¹ NAA, with 100% induction rate. The optimal medium for bud differentiation was MS + 2.0 mg·L⁻¹ 6-BA + 2.0 mg·L⁻¹ ZT + 0.5 mg·L⁻¹ GA₃, with 88% differentiation rate. In this study, the regeneration system of potato variety Xuanshu 2 was established, laying the foundation for the subsequent genetic transformation research of this variety.

1. Introduction

Potato (*Solanum tuberosum* L.) is a perennial herb of the Solanaceae family, and its tuber is the edible part. It is the fourth most important food crop in the world after wheat, rice and corn [1]. Xuanshu 2 is a mid-late maturing cultivated potato variety selected by the Agricultural Technology Promotion Center of Xuanwei City, Yunnan Province, China. The tuber of this variety is large and tastes good, which is deeply popular in consumers of southwest China. The field shows moderate growth period, strong resistance and high yield. In 2009, it began to be widely promoted and achieved significant yield-increasing benefits.

Plant tissue culture has the advantages of short incubation period, no seasonal restriction, and ability to maintain the original good traits of the parents. Therefore, the establishment of potato regeneration system used for tissue culture and rapid propagation can provide high-quality seedlings for production and has a great significance for agricultural production. The types of explants commonly use leaves [2], stem segments [3], protoplasts [4], anthers [5], tubers [6], etc. The callus regeneration rate of stem segments is high. Studies have shown that the regeneration of potato is limited by the different genotypes and factors of phytohormones [7]. So that, different phytohormone combinations need to be studied for different potato varieties. At present, researchers have successfully established the regeneration system of potato varieties such as 'Longshu 10' [8], 'Longshu 5' [9], 'Longshu 11' [10], but there are no reports on the establishment of the regeneration system of



Xuanshu 2. In this experiment, Xuanshu 2 was used as the plant material to screen the best concentrations of phytohormone. The aim was to establish a regeneration system for inducing callus and adventitious bud differentiation by plant tissue culture technique, laying a foundation for the genetic transformation of this variety.

2. Materials and methods

2.1. Plant materials

In this study, the virus-free tube seedlings of potato variety Xuanshu 2 were used as the plant material.

2.2. Medium and culture conditions

Based on MS medium, 3% sucrose and 7 g/L agar were added, the pH was adjusted to 5.8. Culture conditions: temperature was 25 °C, illumination time was 16 h/d, and light intensity was 3000 lx.

2.3. Callus induction

Under the clean bench, the stem segments of the virus-free tube seedlings were cut to 0.5cm (excluding axillary buds) and flat inoculated in MS solid medium. The MS medium was added with different kinds and concentrations of phytohormone. Each culture dish was inoculated with 15 explants. Among them, the phytohormones used 6-BA ($1.0 \text{ mg}\cdot\text{L}^{-1}$) + 2, 4-D ($0.1 \text{ mg}\cdot\text{L}^{-1}$, $0.3 \text{ mg}\cdot\text{L}^{-1}$, $0.5 \text{ mg}\cdot\text{L}^{-1}$, $1.0 \text{ mg}\cdot\text{L}^{-1}$, $2.0 \text{ mg}\cdot\text{L}^{-1}$), a total of 5 treated groups numbered from A1 to A5. And 6-BA ($2.0 \text{ mg}\cdot\text{L}^{-1}$) + NAA ($0.1 \text{ mg}\cdot\text{L}^{-1}$, $0.2 \text{ mg}\cdot\text{L}^{-1}$, $0.5 \text{ mg}\cdot\text{L}^{-1}$, $2.0 \text{ mg}\cdot\text{L}^{-1}$, $5.0 \text{ mg}\cdot\text{L}^{-1}$), a total of 5 treated groups numbered from B1 to B5. After 15 days, the callus induction rate, callus color and volume of each treatment were observed and analyzed to determine the optimal medium combination for callus induction.

2.4. Bud differentiation

The stem segment with callus induced on the best medium was transferred to bud differentiation medium, and the adventitious bud differentiation was induced. Each treatment was transferred to 50 stem segments. Among them, the phytohormones used 6-BA ($1.0 \text{ mg}\cdot\text{L}^{-1}$) + ZT ($0 \text{ mg}\cdot\text{L}^{-1}$, $2.0 \text{ mg}\cdot\text{L}^{-1}$) + GA₃ ($0.5 \text{ mg}\cdot\text{L}^{-1}$, $1.0 \text{ mg}\cdot\text{L}^{-1}$, $2.0 \text{ mg}\cdot\text{L}^{-1}$, $3.0 \text{ mg}\cdot\text{L}^{-1}$, $5.0 \text{ mg}\cdot\text{L}^{-1}$), a total of 10 treated groups numbered from C1 to C10. After 15 days, the number of adventitious buds in each treatment was observed and the bud differentiation rate was calculated to determine the optimal medium combination for bud differentiation.

3. Results

3.1. The results of Callus induction

The stem segments of the potato variety Xuanshu 2 inoculated for 15 days were observed in Fig. 1, and the callus inductions were counted. The results showed that in spite of the different concentrations of 2, 4-D and NAA, the induction rates both were 100%. But the callus color and callus growth were significantly different. Among them, under the condition that $1.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA and $0.5 \text{ mg}\cdot\text{L}^{-1}$ 2, 4-D, the callus was green and small; when the concentration of 2, 4-D increased to $1.0 \text{ mg}\cdot\text{L}^{-1}$ and above, the callus was dark yellow and large, but no regenerative capacity (Table 1). While, under the condition that $2.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA and $0.2 \text{ mg}\cdot\text{L}^{-1}$ NAA, the callus was green and large; when the concentration of NAA increased to $0.5 \text{ mg}\cdot\text{L}^{-1}$ and above, the callus was dark yellow and large, but no regenerative capacity (Table 2). In summary, comparing the results of callus induction by 2, 4-D and NAA, we found that $0.5 \text{ mg}\cdot\text{L}^{-1}$ 2, 4-D and $0.2 \text{ mg}\cdot\text{L}^{-1}$ NAA both have good effects on the callus induction of Xuanshu 2, but from the growth of the respective callus, $2.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA + $0.2 \text{ mg}\cdot\text{L}^{-1}$ NAA combination has a greater growth (Fig. 1 B, C). Therefore, the optimal medium combination for callus induction was selected as MS + $2.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA + $0.2 \text{ mg}\cdot\text{L}^{-1}$ NAA.

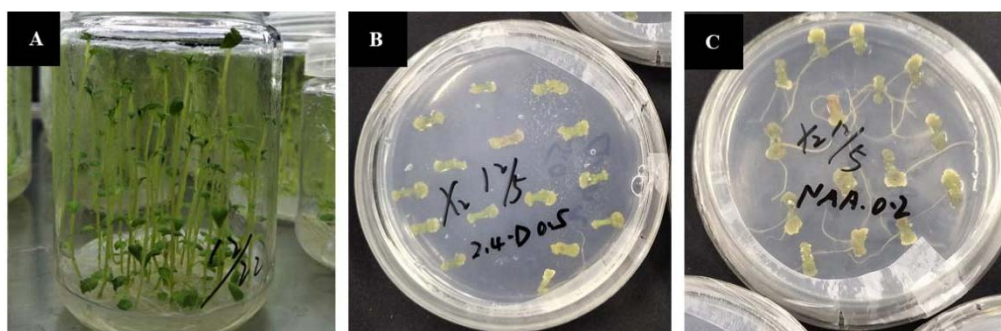


Fig 1. The optimal concentration of 2, 4-D and NAA on callus induction

Note: A: potato virus-free tube seedling. B: the results of $0.5 \text{ mg} \cdot \text{L}^{-1}$ 2, 4-D on callus induction. C: the results of $0.2 \text{ mg} \cdot \text{L}^{-1}$ NAA on callus induction.

Tab 1. Effects of different concentrations of 2, 4-D on callus induction of potato variety Xuanshu 2

No.	6-BA / ($\text{mg} \cdot \text{L}^{-1}$)	2,4-D / ($\text{mg} \cdot \text{L}^{-1}$)	Induction rate / %	Callus color	Callus volume
A1	1.0	0.1	100	green	minimum
A2		0.3	100	green	small
A3		0.5	100	green	small
A4		1.0	100	dark yellow	large
A5		2.0	100	dark yellow	maximum

Tab 2. Effects of different concentrations of NAA on callus induction of potato variety Xuanshu 2

No.	6-BA / ($\text{mg} \cdot \text{L}^{-1}$)	NAA / ($\text{mg} \cdot \text{L}^{-1}$)	Induction rate / %	Callus color	Callus volume
B1	2.0	0.1	100	green	minimum
B2		0.2	100	green	medium
B3		0.5	100	dark yellow	large
B4		2.0	100	dark yellow	maximum
B5		5.0	100	dark yellow	large

3.2. The results of bud differentiation

The callus segments transferred for 15 days were observed (Fig. 2), and the number of buds was counted. The results showed that without ZT addition, when the concentration of 6-BA was $2.0 \text{ mg} \cdot \text{L}^{-1}$ and GA_3 was $1.0 \text{ mg} \cdot \text{L}^{-1}$, the number of bud differentiation was the highest, and the rate was 34.5%. While, under the condition of adding ZT, when the concentration of 6-BA was $2.0 \text{ mg} \cdot \text{L}^{-1}$ and GA_3 was $0.5 \text{ mg} \cdot \text{L}^{-1}$, the number of bud differentiation was the highest, and the rate was 88%. Adventitious buds were green and well grown. By comparison, it can be found that when ZT was added, the bud differentiation rate was significantly higher than that without ZT. Therefore, the optimal medium combination for bud differentiation was MS + $2.0 \text{ mg} \cdot \text{L}^{-1}$ 6-BA + $2.0 \text{ mg} \cdot \text{L}^{-1}$ ZT + $0.5 \text{ mg} \cdot \text{L}^{-1}$ GA_3 (Table 3).

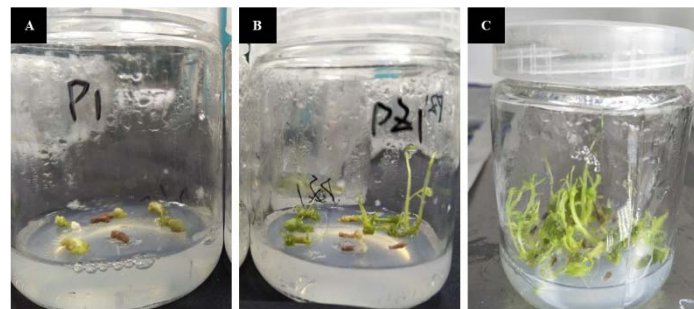


Fig 2. The results of bud differentiation

Note: A: Bud differentiation results without adding ZT. B: Bud differentiation results with adding ZT. C: Subculture of adventitious buds induced by MS + 2.0 mg·L⁻¹ 6-BA + 2.0 mg·L⁻¹ ZT + 0.5 mg·L⁻¹ GA₃.

Tab 3. Effects of different concentrations of ZT and GA₃ on bud differentiation of potato variety Xuanshu 2

No.	6-BA/ (mg·L ⁻¹)	ZT/ (mg·L ⁻¹)	GA ₃ / (mg·L ⁻¹)	Transfer number	Bud differentiation number	Bud differentiation rate / %
C1	2.0	0	0.5	50	3	6.0
C2		0	1.0		17	34.0
C3		0	2.0		3	6.0
C4		0	3.0		5	10.0
C5		0	5.0		8	16.0
C6		2.0	0.5		44	88.0
C7		2.0	1.0		22	44.0
C8		2.0	2.0		10	20.0
C9		2.0	3.0		7	14.0
C10		2.0	5.0		14	28.0

4. Discussion

MS is commonly used as the basic medium in the report of potato regeneration culture. A large number of studies have shown that different varieties of potato require different types and concentrations of phytohormone for callus induction and bud differentiation [11]. The researchers optimized the hormone types and concentrations during the culture process and obtained the optimal combination for different potato varieties. Jia et al. [8] screened out that the optimal medium combination for the callus induction of stem segments of Longsu 10 was MS + 2.0 mg·L⁻¹ 6-BA + 0.2 mg·L⁻¹ NAA + 3.0 mg·L⁻¹ GA₃ + 0.5 mg·L⁻¹ 2,4-D; the optimal medium combination for adventitious bud differentiation was MS + 2.0 mg·L⁻¹ 6-BA + 2.0 mg·L⁻¹ ZT + 3.0 mg·L⁻¹ GA₃. Bao et al. [9] screened out that the optimal medium combination for the callus induction of stem segments of Longsu 5 was MS + 1.0 mg·L⁻¹ 2,4-D + 1.0 mg·L⁻¹ NAA; the optimal medium combination for adventitious bud differentiation was MS + 0.5 mg·L⁻¹ NAA + 2.0 mg·L⁻¹ KT + 0.5 mg·L⁻¹ GA₃. Chen et al. [10] screened out that the optimal medium combination for the callus induction of stem segments of Longsu 11 was MS + 7.5 mg·L⁻¹ GA₃ + 2.5 mg·L⁻¹ 6-BA + 0.5 mg·L⁻¹ 2,4-D; the optimal medium combination for adventitious bud differentiation was MS + 0.25 mg·L⁻¹ NAA + 0.1 mg·L⁻¹ IAA + 3.0 mg·L⁻¹ KT. However, in this experiment, the optimal medium combination for stem callus induction was MS + 2.0 mg·L⁻¹ 6-BA + 0.2 mg·L⁻¹ NAA; the optimal medium combination for adventitious bud differentiation was MS + 2.0 mg·L⁻¹ 6-BA + 1.0 mg·L⁻¹ ZT + 0.5 mg·L⁻¹ GA₃. The reason for this

difference may be due to the different potato varieties have different genotypes, which require different types and concentrations of phytohormone.

In this study, different plant hormone combinations were screened, and the optimal hormone concentration of callus and adventitious bud differentiation was determined. The reason why the rooting system of potato was not performed is that potato is easy to root. Rooting can be achieved by using MS or 1/2 MS for potato culture [12]. In the results of callus induction, we can find that 2, 4-D and NAA can induce callus within a certain concentration range; outside the range, although the callus can be induced, the explants have no regeneration ability. Comparing the effects of 2, 4-D and NAA on callus induction, it can be seen that the induction effect of NAA is better than 2, 4-D. In the results of bud differentiation, the differentiation rate of stems in the ZT phase was significantly higher than that in the ZT-free phase. Therefore, it can be inferred that ZT is a good phytohormone for inducing adventitious bud differentiation of potato, which is consistent with the findings of predecessors [13].

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